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The Rich Electrochemistry and Redox Reactions of the Copper Sites in the Cellular Prion Protein

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Abstract

This paper reviews recent electrochemical studies of the copper complexes of prion protein (PrP) and its related peptides, and correlates their redox behavior to chemical and biologically relevant reactions. Particular emphasis is placed on the difference in redox properties between copper in the octarepeat (OR) and the non-OR domains of PrP, as well as differences between the high and low copper occupancy states in the OR domain. Several discrepancies in literature concerning these differences are discussed and reconciled. The PrP copper complexes, in comparison to copper complexes of other amyloidogenic proteins/peptides, display a more diverse and richer redox chemistry. The specific protocols and caveats that need to be considered in studying the electrochemistry and redox reactions of copper complexes of PrP, PrP-derived peptides, and other related amyloidogenic proteins are summarized.

Keywords

Prion protein; copper binding; electrochemistry; redox reactions; oxidative stress

1. Introduction

The cellular prion protein (PrP^C) is emerging as a significant player in the regulation of copper and perhaps other metal ions of the central nervous system. The prion protein was originally identified through its role in the transmissible spongiform encephalopathies (TSEs) where misfolding of the protein to PrP^{Sc} leads to the development of mad cow disease, scrapie in goats and sheep, and the human diseases kuru and Creutzfeldt-Jakob disease (CJD). A significant effort in recent research, however, now focuses on the inherent function of PrP^C, which remains poorly understood. PrP^C, a glycoprophosphatidylinositol (GPI)-anchored glycoprotein of approximately 210 residues, contains two primary domains, a structured, helical C-terminal region, and an unstructured N-terminus. The N-terminal segment takes up both copper and zinc, with dissociation constants that approximately match the respective extracellular concentrations of these species in cerebral spinal fluid

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(CSF). Recent X-ray fluorescence imaging demonstrates that brain tissue distributions of these metals are dramatically affected by PrP expression [1].

Copper and zinc bind to the PrP octarepeat (OR) domain, composed of four tandem segments of the repeating sequence PHGGGWGQ, with HGGGW providing the ligands responsible for metal binding [2, 3]. Zn^{2+} coordinates exclusively to the imidazole side chains of the four octarepeat histidine residues [4]. Copper binding to PrP^C is more complex. First, copper is found in two different oxidation states, Cu(I) and Cu(II), both of which can bind with high affinity to the OR domain. Second, the specific coordination mode for Cu(II) depends on the ratio of copper to protein [5]. As shown in Figure 1, at 1:1 [Cu(II)]:[OR], coordination is through the four imidazoles on the His residues, similar to that of Zn^{2+} . However, at higher Cu(II) occupancy, each HGGGW segment in the OR domain takes up a single equivalent of the metal ion. The coordination sphere is composed of the His imidazole, the backbone nitrogens from the two Gly residues that immediately follow the His, and a Gly carbonyl oxygen [6]. The Trp indole ring is positioned closely to the Cu(II) center and hydrogen bonds to an axial water molecule [6]. Right next to the OR domain is a segment with two His residues (His-96 and His-111 for human PrP; His-95 and His-110 for mouse PrP) that can also bind at least one Cu(II) ion. This so-called “fifth binding site” [2, 3] is also referred to as the non-OR in this review.

Studies over the last decade consistently find that PrP^C protects neurons against copper toxicity. Fibroblasts derived from PrP ablated mice display significantly enhanced sensitivity to copper [7]. Cell culture work also shows that PrP-deficient neurons are more susceptible to copper toxicity than normal neurons [8]. Moreover, copper added to the drinking water of Tg mice carrying an E199K mutation (analogous to E200K associated with CJD) exhibit an accelerated rate of spontaneous prion disease [7]. Insertional mutations leading to elongation of the OR domain, and consequent loss of high occupancy coordination, lead to inherited prion disease in humans [9].

The mechanism by which PrP^C carries out its neuroprotective function has yet to be established. One hypothesis, based on early electrochemical studies [10], is that coordination by PrP^C inhibits copper's intrinsic redox activity, which would otherwise generate reactive oxygen species through Fenton-like reactions. Recent experiments by our groups, however, suggest that the protective mechanisms may be much more complex, with redox activity dependent on the specific copper coordination mode.

The goal of this review is to take a critical look at the electrochemistry of copper coordinated to the prion protein, and its related peptides, under varying conditions. We also contrast results from these studies to those of several representative amyloidogenic proteins to illustrate the critical need of carrying out voltammetric studies in a rigorous manner. Other aspects of PrP-Cu interactions, such as coordination mode and affinity, have been reviewed and described elsewhere [2, 3, 11, 12] and will not be discussed in detail here. Instead, we focus on the methods required for a meaningful evaluation of copper redox activity relevant in biological tissues, specific findings for PrP^C and possible implications in neuroprotective schemes. We conclude this review by summarizing the specific protocols and caveats required for evaluating the electrochemistry and redox reactions of copper complexes of PrP, and the outlook of this field from the perspective of redox reactions involving the copper complexes of PrP.

2. Voltammetric studies of copper complexes of PrP peptides, PrP, and other amyloidogenic molecules

2.1. A brief review of voltammetry and some relevant concepts

Before reviewing correlation between the electrochemical results of the PrP-Cu complexes and their reported redox reactions, we briefly summarize how information about the redox property of a species is meaningfully obtained from voltammetric experiments. Incorrect extraction of these parameters or misinterpretation of them may lead to erroneous conclusions. In the context of studying metal complexes of amyloidogenic molecules, these parameters include redox potential, rate of electron transfer reactions, and binding constant between a given metal oxidation with an amyloidogenic species. Biochemists and biologists are quite familiar with concepts governed by equilibrium conditions (e.g., membrane potentials) and use of electrochemical techniques for measuring parameters under equilibrium conditions (e.g., determination of proton activity with a pH electrode and binding constant measurement employing potentiometric titration). However, care needs to be exercised when using electrochemical techniques to extract parameters under non-equilibrium conditions, especially for the study of reaction mechanisms. Potential scan techniques, with cyclic voltammetry (CV) being the most important variant, are frequently used under non-equilibrium conditions. Important thermodynamic and kinetic information can be gleaned from CV measurements.

A one-electron reduction of a species O to its reduced form R can be depicted as

where k_f and k_b denote the forward and backward (heterogeneous) electron transfer rate constants, respectively. When the electrode/solution interface is at equilibrium with a solution in which the bulk concentrations of O and R are the same, $k_f = k_b$ and their value is referred to as the standard heterogeneous electron transfer rate constant k^0 [13]. Reversibility, in this case, means that in eq. 1 an electron can be rapidly donated to O or extracted from R by the electrode. Figure 3A shows the overlay of two simulated cyclic voltammograms at two different scan rates. At 25 °C, the peak current (i_p) is governed by the following equation

$$i_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} C^* \nu^{1/2} \quad (2)$$

where n is the number of electron equivalents transferred, A is the area of the electrode, D is the diffusion coefficient, C^* is the bulk concentration of a species of interest, and ν is the scan rate. The voltammograms in Figure 3A were simulated with the following values: $n = 1$, $A = 0.071 \text{ cm}^2$, $D = 5 \times 10^{-6} \text{ cm}^2/\text{s}$, $C^* = 1 \text{ mM}$ and the standard heterogeneous electron transfer rate constant k^0 of 0.1 s^{-1} . Notice in Figure 3A, as the potential is scanned to a more negative value (more reducing power), the current actually decreases to produce a peak. The decrease is resulted from the fact that diffusion of O in the bulk is slower than the rate of electron transfer (i.e., the rate of the reaction shown by eq. 1 has become “diffusion controlled” [13]). Figure 3A also shows how the cathodic (forward) and anodic (backward) peak currents i_{pc} and i_{pa} and potentials E_{pc} and E_{pa} are determined or identified from a voltammogram. If a reaction is reversible, the following *four criteria* generally hold: $i_{pc}/i_{pa} \approx 1$, i_p values are proportional to $\nu^{1/2}$ (cf. eq. 2), E_p values are independent of the scan rate, and $\Delta E_p = E_{pa} - E_{pc} = 59.6 \text{ mV}/n$ [13]. For real experimental data, there exist slight

deviations from these relationships (especially the last relationship owing to solution resistance and peak broadening when charging current becomes high with respect to faradaic current) [13]. In Figure 3A, the i_p values of the voltammogram shown in black are doubled when the scan rate was increased by four fold (red curve), as predicted by eq. 2. From such a reversible voltammogram, E^0 (standard reduction potential of the O/R redox couple) can be approximated to be $E_{1/2}$, which is the average of E_{pa} and E_{pc} . However, for quasi-reversible voltammograms, E^0 cannot be equated to $E_{1/2}$, and the latter is generally given to reflect the (high or low) tendency of a given redox couple to undergo an electron transfer reaction. For an irreversible wave (i.e., only an oxidative or a reductive peak is observed), E_{pa} or E_{pc} is given to indicate the potential at which a species is oxidized or reduced. For an electroactive protein/peptide that is immobilized onto an electrode and undergoes reversible electron transfer, however, there is no hysteresis between the E_{pc} and E_{pa} (i.e., the CV waves in Figure 2A become symmetric, bell-shaped curves). The following equation describes the relationship between i_{pc} or i_{pa} value and the scan rate [13]:

$$i_p = (9.39 \times 10^5) n^2 \Gamma_o^* \nu \quad (3)$$

where Γ_o^* is the surface coverage of O on the electrode (or initial amount of O adsorbed onto the electrode). Thus, the immobilized molecule has i_p proportional to ν (instead of $\nu^{1/2}$ for a species in solution) and independent of diffusion coefficient.

Thus, by simulating the experimentally obtained voltammograms, CV can be used to determine the potential of a redox species and its k^0 value (or extent of reversibility). For detailed procedure of deducing the k^0 value for an electrochemical reaction from voltammetric experiments, interested readers can consult references [13–15]. In addition, it is also a powerful technique that affords information about reaction mechanisms at the metal/solution interface. By collecting voltammograms at different scan rates, varying the direction of potential scan, and changing the number of cycles, the chemical stability of the electrogenerated species, its lifetime, and how it undergoes follow-up chemical reactions, can all be determined. For example, if R in eq. 1 subsequently reacts with another species Y in the same solution to regenerate O and to produce Z as a co-product,

the mechanism is referred to as a catalytic reaction (or $E_r C_i'$ wherein E_r and C_i' respectively represent a reversible electron transfer and an irreversible chemical reaction that regenerates the original reactant O). The symbol k_c in eq. 4 is the rate constant of the follow-up chemical reaction. This mechanism is similar to an enzymatic reaction with O having the function equivalent to that of a redox enzyme. Notice that in Figure 3B, at a relatively slow scan rate, the typical peak-shaped voltammogram (black curve, caused by the abovementioned diffusion-controlled process), has changed to a sigmoid-shaped wave (red curve) when eq. 4 applies. A characteristic feature of the red curve is that i_{pc} is increased while i_{pa} largely diminishes. This takes place because O is hardly depleted near the electrode/solution interface due to the catalytic reaction (i.e., eq. 4).

Finally, determination of redox potential of a metal complex and comparison of it to the standard reduction potential of the free form of the same metal allows the binding constant of the complex be measured. Using a copper-protein(P) complex as an example, the following equilibria correspond to the formation of Cu(II)- and Cu(I)-containing complexes:

If the one-electron reduction of the Cu(II) center shown below is reversible,

the CV wave (or $E^0_{\text{Cu(II)-P/Cu(I)-P}}$) of this reduction might be different than that (or $E^0_{\text{Cu(II)/Cu(I)}}$) of the reduction of free Cu(II),

The shift of $E^0_{\text{P-Cu(II)/P-Cu(I)}}$ with respect to $E^0_{\text{Cu(II)/Cu(I)}}$ ($= 0.159$ V vs. NHE [13]) is dependent on the relative stabilization of Cu(II) by the protein over that of Cu(I). If the stabilization of Cu(II) is greater, the CV wave (or $E^0_{\text{Cu(II)-P/Cu(I)-P}}$) will be shifted in the negative (cathodic) direction and vice versa. The same applies to an organic ligand that binds to a metal ion. For example, EDTA binds Cu(II) strongly whereas bathocuproine disulfonate (BCS) ligates Cu(I) much more selectively. As a consequence, the EDTA-Cu(II) complexation will shift the reduction potential of eq. 8 in the negative direction, but formation of the 1:2 complex between Cu(II) and BCS does the opposite. In fact, owing to the extremely large formation constant between Cu(I) and BCS (6.3×10^{19}), the reduction potential of the Cu(II)/Cu(I) center in the Cu(BCS)₂ complex is considerably positive (0.844 V vs. NHE) [12]. Also noteworthy is that the shift is dependent on the protein concentration used [13]. While the above principle is not complicated to understand, as reviewed below, the correct interpretation of this shift is crucial to a good corroboration of the occurrence of related chemical redox reactions observed in vitro.

2.2. Voltammetric studies of copper complexes of PrP and PrP-derived peptides

In the absence of metal ions, both PrP and PrP-derived peptides are not reducible or oxidizable over a wide range of potential [16–18], suggesting that their amino acid residues remain stable under most circumstances. However, PrP and PrP peptides containing copper display rich electrochemistry, which is dependent on a variety of factors. Bonomo and coworkers were the first to use cyclic voltammetry, in conjunction with spectroscopic techniques such as circular dichroism (CD) and EPR, to investigate the binding modes of Cu(II) by peptides Ac-PHGGGWGQ-NH₂ and Ac-HGGG-NH₂ (Ac represents acetyl group) [10]. The former peptide has the octarepeat sequence (OP; see also Figure 1) and the latter corresponds to the key residues and the minimum peptide length to equatorially coordinate Cu(II). In their work, consistent with other spectroscopic studies [2, 3], Cu(II) formed

1:1 complexes with these two PrP-derived peptides. At pH 7 and 10, the Cu(II) center in both complexes appears to undergo quasi-reversible (almost irreversible; vide infra) electron transfer to produce Cu(I). Surprisingly, rather negative reduction peak potentials were reported, with the potential of Cu(II)-OP at ca. -0.311 V vs. NHE (or -0.507 vs. Ag/AgCl) and Cu(II)-HGGG at ca. -0.340 V vs. NHE (-0.536 vs. Ag/AgCl). Since the report did not show that CVs were collected at various scan rates [10], it appears that only two of the four criteria mentioned in section 2.1 were used to evaluate the reversibility of the CV wave. The CVs were also collected at a relative high scan rate (200 mV/s) at which high charging current broadens the CV wave [13], making the assessment of reversibility and determination of redox potentials difficult. It was concluded that Cu(I) remains stably coordinated by these complexes and the relatively low reversibility was attributed to a

change in the coordination modes upon Cu(II) reduction to Cu(I). Specifically, it was suggested that these peptides render a coordination sphere that favors the square-planar (or elongated octahedral) binding of Cu(II), which does not accommodate well the tetrahedral coordination of Cu(I). While a stronger stabilization of the Cu(II) center explains well the negative shift of the Cu(II) reduction peak, the shift with respect to eq. 8 is rather dramatic (0.47 V for the OP–Cu(II) complex), implying that Cu(I) is bound rather weakly. At such a negative potential, Cu(I) would further be reduced to Cu(0) [13], which would be released from the complex and deposit onto the electrode [18]. The same group later extended their studies of the binding of Cu(II) by PEG (polyethylene glycol)-tagged tetrarepeats of OP (i.e., OP₄ or OR)[19] and the influence of nitric oxide (NO) on mono- and dinuclear Cu(II) complexes with OP and OP₂ [20]. The motivation behind the PEG tagging was to increase the solubility of the peptide while studying the redox reaction of PrP peptide in the presence of nitric oxide stems from the need to gain a better understanding of the putative role of PrP in modulating the activity and subcellular locations of nitric oxide synthase [21]. These papers also expanded on the studies of other binding stoichiometric ratios between Cu(II) and the respective peptide ligand. Using essentially the same approach, complexes with different binding stoichiometric ratios were all found to possess surprisingly negative reduction potentials. For example, the reduction potentials of OR–Cu(II)₄ and OR–Cu(II)₃ were ca. –0.530 and –0.500 V vs. NHE, respectively. Such values are even more negative than the aforementioned value of OP–Cu(II). Moreover, E_{pc} values of OR–Cu(II)₄ and OR–Cu(II)₃ were dependent on the scan rate, with negative shifts at higher scan rates. Thus, it appears that the Cu(II) centers in these complexes all undergo quasi-reversible electron transfer reactions [10, 19, 20]. The negative shift of E_{pc} at a higher scan rate could also suggest that there is a chemical reaction following the electron transfer. Indeed it was postulated that there could be a loss of weakly bound ligands such as NO or pyridine added into solution [20]. If this were the case (a non-catalytic follow-up reaction occurs after the electron transfer), E^0 cannot be deduced from the voltammogram from a single scan rate, as the peak potential is dependent on the scan rate used.[13] It is worth mentioning that all these potentials values are substantially more negative than a large number of copper complexes examined by the same group in 2002[22]. Interestingly, two of the peptides studied, cyclo(Gly-His)₄ and cyclo(Gly-His-Gly)₂ have similar amino acids to OR and OP₂, respectively. Yet the reduction potentials are more modest, with 0.195 V vs. NHE for the former and 0.219 V for the latter. Yamamoto and Kuwata carried out DFT (density function theory) calculations of the redox potentials of copper complexes with OP and non-OP regions of PrP[23]. The calculated standard reduction potential for the OR–Cu(II)/OR–Cu(I) couple, 0.38 V, is much closer to 0.195 V than to ~ –0.5 V.

Hureau et al. studied the pH-dependent Cu(II) coordination of the peptide GGGTH, which is the non-OR Cu(II) binding sequence (cf. Figure 1). At pH 6.7, a voltammogram with $\Delta E_p = 0.128$ V corresponds to a one-electron reduction. Well-defined reduction and oxidation peaks (i.e., with highly comparable i_{pc} and i_{pa} values) can be resolved, with $E_0 = 0.04$ V vs. Ag/AgCl (or 0.20 V vs. NHE). The modest E_0 value is much more comparable to 0.195 V measured_[10] for the 1:1 complex formed between Cu(II) and cyclo(Gly-His)₄ and what we measured recently[18] (vide infra).

Davies et al. attached wild-type (Wt) PrP fully loaded with Cu(II) (i.e., with four Cu(II) bound to the OR domain and another Cu(II) occupying the non-OR domain) onto a boron-doped diamond electrode and conducted a detailed voltammetric study [16]. As can be seen from Figure 3, a single and broad redox wave, with an $E_{1/2}$ value around 0.03 V vs. SCE (or 0.254 V vs. NHE), was observed. These researchers also performed CV studies on PrP with the OR and non-OR segments in the Wt-PrP deleted and observed again a single, broad wave. As shown by panel iv of Figure 3, i_{pa} and i_{pc} are proportional to the scan rate (see also eq. 3), suggesting that PrP is confined to the electrode surface. That the peak potentials are

dependent on the scan rate (cf. panel v in Figure 3) indicates that the electron transfer reaction between the electrode and the surface-confined PrP molecule is quasi-reversible. Indeed, the voltammograms shown in Figure 3 have different E_{pa} and E_{pc} values (i.e., large hysteresis for a surface-confined species), also implying that the electron transfer is quasi-reversible. Although it is not clear whether the adsorption process has disrupted the structure of PrP, especially for the non-OR or amyloidogenic region, it was suggested that PrP is immobilized onto the electrode in such a way (possibly through electrostatic interaction) that the N-terminus is close to the hydrophilic electrode surface. Since heterogeneous electron transfer at the metal/solution is distance dependent, the data suggest all the copper centers have comparable distances to the electrode. (Another possible interpretation of the data in Figure 3 could be that the five Cu(II) centers are all within close proximities to the electrode, yet at slightly different distances to yield redox waves that are too closely separated to be resolved by CV. As a result, the individual redox waves of the five Cu(II) centers merge into a broad peak. In a more recent work, the same group investigated the synergistic roles of individual histidines in PrP's binding to Cu(II) [17]. By systematically mutating the six histidines (His-60, 69, 76, and 84 in the OR domain and His-95 and 110 in the non-OR domain) and integrating the charges under the reduction peaks (from which the number of Cu(II) ions bound can be deduced according to the Faraday's law), it was demonstrated that CV is a powerful tool for gauging the contribution of individual histidines to PrP copper binding. Through comparison to isothermal titration calorimetric results, the authors concluded that the two highest binding events occur in the non-OR domain and the OR domain with a single or low Cu(II) occupancy. It was concluded that the first Cu(II) sequestered by PrP will first occupy the OR domain. The second Cu(II) then binds to the non-OR site and the rest enter the OR domain to yield the high Cu(II) loading or occupancy. An important point that can be extracted from these two publications is that PrP fully loaded with Cu(II) has a modest reduction potential ($E_{1/2}$), suggesting that copper complexation by PrP does not significantly shift the redox potential of eq. 8. In fact, the small positive potential shift suggests that the complexation reaction stabilizes Cu(I) slightly more than Cu(II). This point has an significant implication in assessing the possible roles of PrP plays in modulating Cu(II)-initiated redox reactions (vide infra).

Using PrP-derived peptides, our groups recently carried out a systematic voltammetric investigation of soluble complexes of Cu(II) respectively bound to a single OP, and the OR and non-OR domains [18]. Particular emphasis was placed on the elucidation of the possible differences in the electrochemical behaviors between OR-Cu(II) and OR-Cu(II)₄, the latter of which is equivalent to OP-Cu(II). We collected the voltammograms under a rigorously deoxygenated environment (in a glove box under N₂ with O₂ content less than 0.05 ppm; cf. black curves in panels A–C in Figure 4) and those in air-saturated solutions (red curves in Figure 4). We also collected the voltammograms of these complexes at different scan rates. On the basis that the binding constant of OR-Cu(II)₄ is in the sub- μ M range,[2, 3] the concentrations of the PrP-derived peptides were kept as low as possible to avoid having a large amount of free Cu(II) in solution. Under these experimental conditions, the free Cu(II) concentration should be at low μ M level, which is too low for cyclic voltammetry to detect (or to produce a discernible peak). Therefore, the peaks in Figure 4A–C are all attributable to the electron transfer involving these complexes. To gain a deeper insight into the modulations of electrochemical behaviors of the Cu(II) centers by the different domains and copper occupancies, we also compared their behaviors to free copper in aerobic and anaerobic solutions (red and black curves in panel D).

Several points are noteworthy from Figure 4. First, similar to the copper-free Wt-PrP (cf. the dashed line in Figure 3), the PrP peptides without Cu(II) are not electroactive within the potential range studied. Second, in the absence of oxygen, the reversibility of OR-Cu(II) and OR-Cu(II)₄ [18], judged from the four criteria described in section 2.1 (i.e., the

dependence of peak currents and potentials on the scan rate), is high. Moreover, with respect to E^0 of eq. 8, only modest shifts were observed for the reduction potentials of OR–Cu(II), OR–Cu(II)₄ and non-OR–Cu(II), a point consistent with that observed by Davies et al. for the Wt–PrP Cu(II) complexes. E^0 values of OR–Cu(II)₄ (0.172 V vs. NHE) and non-OR–Cu(II) ($\approx$ 0.110 V) are in good agreement with that for the Wt–PrP reported by Davies et al. [16] and the GGGTH–Cu(II) studied by Hureau et al. [24], but much more positive than the values reported by Bonomo et al [10, 19, 20]. Third, by individually examining the electrochemical behaviors of the Cu(II) centers in the two domains (i.e., OR vs. non-OR) and two different stoichiometries (i.e., high vs. low occupancies) in solution, the influence of coordination chemistry and peptide sequence on the redox potential can be clearly resolved. The most striking difference is that E^0 of OR–Cu(II) is 0.323 V vs. NHE, which is 0.151 V more positive than that of OR–Cu(II)₄. The significance of this potential difference will become more obvious in the paragraph below and in section 3 wherein redox reactions of copper complexes of PrP or PrP peptides are discussed. It is also noteworthy that a small shoulder in the red curve of Figure 4B is discernible at ca. 0.075 V, which we attributed to the transitory binding mode in which the stoichiometric ratio between OR and Cu(II) is 1:2 or 1:3 [18]. At higher scan rates, the shoulder peak merges with the large peak of OR–Cu(II)₄ due to the peak broadening effect caused by the relatively high charging current associated with a higher scan rate.

O₂ and pH have profound influences on the electrochemical behavior of the Cu(II) centers in different domains or occupancies. In the presence of O₂ (red curves in panels A–C of Figure 4), the CV of OR–Cu(II) remains essentially the same as that in anaerobic solution (panel A). However, the voltammograms of OR–Cu(II)₄ and non-OR–Cu(II) evolved into sigmoid-shaped waves. As shown in Figure 2B, such a wave form is indicative of the E_rC_i' (catalytic reaction) mechanism, suggesting that O₂ in solution is capable of oxidizing the electrogenerated Cu(I) center(s). O₂ can be reduced to H₂O₂ via the following reaction:

whose E^0 is 0.296 V vs. NHE at neutral pH [25].

Thus, the different electrochemical behaviors between OR–Cu(II) and OR–Cu(II)₄ (or non-OR–Cu(II)) result from the shifts of the redox potentials, with E^0 value of OR–Cu(II) being higher and that of OR–Cu(II)₄ lower than the reduction potential the O₂/H₂O₂ couple. It is interesting to compare these behaviors to that of free Cu(II) in the absence and presence of O₂. As can be seen in Figure 4D, without O₂, Cu(I) electrogenerated from Cu(II) is not stable in aqueous solution and undergoes a disproportionation reaction to produce Cu(II) and Cu(0). Deposition (accumulation) of Cu(0) onto the electrode and the subsequent reoxidation leads to the appearance of the sharp oxidation peak in the black curve of Figure 4D. In the presence of O₂, however, the electrogenerated Cu(I) is quickly oxidized back to Cu(II), completing the electrocatalytic cycle shown by eqs. 1 and 3. The pronounced differences between the black and red curves in Figures 4B and 4C indicate the importance of completely eliminating O₂ in solution to obtain well defined redox waves for OR–Cu(II)₄ and non-OR–Cu(II).

The well-known pH dependence of PrP copper binding has been used to support the hypothesized function of PrP in copper trafficking. Once PrP–Cu(II) complexes are internalized and incorporated by endosomes, copper would be released from these complexes due to the acidic environment of the endosome. OR–Cu(II) remains quite stable at pH 6.5, but free Cu(II) is released from both OR–Cu(II)₄ and non-OR–Cu(II). Thus, the very first Cu(II) sequestered by PrP [17] is quite inert and the PrP complexation completely shuts down the generation of reactive oxygen species (ROS) by free Cu(II). For OR–Cu(II)₄

and non-OR-Cu(II), once the Cu(II) center(s) is(are) reduced to Cu(I), it(they) can be regenerated by O₂ in solution, producing H₂O₂ as a side product. Indeed, these predictions are consistent with other published reports and confirmed by our own chemical redox reactions [18, 26] (vide infra).

2.3. Voltammetric studies of copper complexes of other amyloidogenic proteins/peptides

Copper has been linked to pathologies of other neurological disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). The strongest evidence for copper's involvement in AD is the discovery that copper concentration in senile plaques of AD patients is remarkably high (~0.4 mM [27]), while that for the possible involvement of copper in PD is the elevated copper concentration found in the CSFs of PD patients [28]. In vitro studies have shown that Cu(II) can be complexed by amyloid- β (A β) peptides, whose aggregation is suggested to be causative to the AD etiology [29–33]. It has been suggested that in each A β peptide, the N-terminus, His-6, His-13 or 14, and an oxygen containing residue (possibly Glu-3) serve as the four ligands that bind one Cu(II) with nanomolar affinity [34–36]. A hallmark of PD is that the Lewy bodies contain filamentous inclusions are composed of the 140 amino acid residue α -synuclein (α -syn). Similar to A β , α -syn also binds copper and the binding mode is contributed by the first two amino acid residues on the N-terminal with Met-1 serving as an anchor. His-50 is also a possible binding site via wrapping around the Cu(II) center in the equatorial position [37]. Thus, A β , α -syn, and PrP are similar in that their hydrophilic N-terminal domains are all involved in copper binding. However, there are more copper binding domains in PrP. Consequently the different coordination chemistries alter the redox property of the copper centers, making PrP's electrochemistry and redox reactions more diverse and interesting.

Several voltammetric studies on the copper complexes of A β have been reported [38–43]. The first CV performed on the A β -copper complex was by Huang et al. who observed a CV wave with a formal potential between 0.50 and 0.55 V vs. Ag/AgCl [38]. They assigned this wave to the redox reaction involving the Cu(II) center in the complex. Such a large positive shift (with respect to E^0 of eq. 8) suggests that Cu(I) is stabilized by A β more substantially than Cu(II). This high potential was suggested to be responsible for the Met-35 oxidation observed in some studies [38, 44]. Through several control experiments and A β sequence-dependent studies, we showed that the correct assignment of this wave should be the redox reaction of the sole tyrosine residue (at position 10) in A β (cf. Figure 5A and its inset). The reduction of the Cu(II) center in the complex actually occurs at ~0.02 V vs. Ag/AgCl, again a value close to E^0 of the free Cu(II)/Cu(I) couple (cf. eq. 8). The voltammogram exhibits a quasi-reversible CV wave (thin solid curve in Figure 5A). Interestingly, in the presence of O₂, the peak-shaped CV wave morphs into a more sigmoid-like wave with a higher reduction wave and a lower oxidation current (dash-dot-dash curve in Figure 5A). Thus the electrogenerated Cu(I) center in the A β copper complex behaves much like those in OR-Cu(II)₄ and non-OR-Cu(II), via reaction with O₂ to produce H₂O₂. Indeed, we determined H₂O₂ as a product using spectrofluorimetry [39].

We later acquired voltammograms of α -syn and the α -syn(1–19) peptide with and without Cu(II) bound (cf. Figure 5B and its inset) [45]. Notice that the α -syn protein also exhibits the oxidation peak of Tyr residues (at positions 39 and 125), which are absent in α -syn(1–19). The Cu(II) reduction is again quasi-reversible, with $E_{1/2}$ of 0.018 V vs. Ag/AgCl (or 0.214 V vs. NHE). Recently Brown and coworkers have also reported voltammograms for the copper complexes of α -, β - and γ -synucleins, with $E_{1/2}$ values close to what we measured [46]. The $E_{1/2}$ values are 0.364 and 0.179 V vs. NHE for β - and γ -synucleins.

We tabulated the redox potentials of the copper complexes of the three amyloidogenic molecules (Table 1) and contrast them to the important biological redox couples implicated

to cause oxidative stress in neurological disorders. Species in Table 1 are ordered according to their respective reduction potentials, expressed in $E_{1/2}$ or E^0 . Thus, the oxidized form of a given species can oxidize the reduced forms of all the molecules below, whereas the reduced form of the same species can be oxidized by the oxidized form of the molecules listed above. For example, free Cu(II) can oxidize AA and GSH to DA and GSSH, while its reduced form Cu(I) can be oxidized by O_2 . Notice that potentials in Table 1 with respect to the NHE and Ag/AgCl reference electrodes are both given so that a better comparison to literature data can be made.

With the redox potentials accurately determined for the copper complexes of A β and α -syn, we were able to provide more reasonable explanations about some in vivo or cell-based findings. For example, both A β -Cu(II) and α -syn-Cu(II) have higher potentials than the four complexes in the respiratory chain (e.g., NADH in Table 1), which could explain why mitochondrial dysfunctions were found in both AD and PD [47, 48]. It is also evident that, both copper complexes are capable of oxidizing glutathione, whose concentration decreases in both AD and PD [49, 50]. On the other hand, these potentials are lower than that of dopamine ($E^0 = 0.370$ vs. NHE [51, 52]), suggesting why only iron has been linked to dopamine oxidation [53–55]. If the reduction of α -syn-Cu(II) occurred at the potential of the Tyr oxidation peak, dopamine would undergo oxidation in the presence of the α -syn-Cu(II) complex, a process we verified not to occur in vitro [45]. Similarly, it is conceivable why the reduced forms of A β -Cu(II), α -syn-Cu(II), OR-Cu(II)₄, and non-OR-Cu(II) can all react with O_2 to produce H_2O_2 . However, only OR-Cu(II) has a potential higher (more positive) than the O_2/H_2O_2 couple, suggesting any redox cycle involving regeneration of the Cu(I) center in the OR-Cu(II) binding mode would be completely quenched. This significant thermodynamic predication helps explain some key observations made by us and others (vide infra). The above examples highlight why it is imperative to determine the redox potential accurately and to assign it to the right redox reaction.

3. Redox reactions involving copper bound by PrP or PrP peptides

3.1 Inhibition/initiation of reactive oxygen species generation by the PrP-copper complexes

Long before the redox potentials of copper complexes of Wt-PrP and PrP peptides encompassing the key copper binding domains were measured, various chemical redox reactions were studied in solution to probe the possible role of PrP as an anti- or pro-oxidant [58, 59]. Such research activities have continued for more than a decade and show no signs of subsiding. The high level of interest originates from the fact that free Cu(II) in biological systems is capable of producing reactive oxygen species (ROS). Contributing to the dysfunction of copper homeostasis, free Cu(II) can participate in the following reactions to produce the highly toxic hydroxyl radical (OH•):

The highly reactive hydroxyl radicals are detrimental to the integrity of lipids, nucleic acids, and proteins. Eqs. 10 and 11 are referred to as the Harber-Weiss cycle, whereas Eq. 12 bears close resemblance to the Fenton reaction in which Fe(II) replaces Cu(II) as the reactant [60].

In organisms, free or complexed Cu(II) could initiate another redox cycle, which is dependent on the thermodynamic properties of the reactants and structures of the Cu(II) complexes. The cycle starts with the reduction of free or Cu(II) bound to a protein P by a biological reductant. The resultant Cu(I) can be subsequently oxidized by O₂, generating H₂O₂ as a product. Using AA as a representative biological reductant, this cycle can be described as [61–64]:

Notice that H₂O₂ produced in eq. 14 can in turn react with any remaining free or bound Cu(II) through the Harber-Weiss cycle to produce OH•.

As aforementioned, all of the copper complexes shown in Table 1 are predicted to oxidize AA or GSH. Indeed, in studying metal-catalyzed oxidation (MCO), Requena et al. showed that a recombinant SHa(29–231) PrP loaded with different amounts of Cu(II) can oxidize AA [65]. Consequently, Cu(I) generated via eq. 13 can react with H₂O₂ present in the solution to produce OH• through the Fenton-like reaction (i.e., eq. 12). The OH• radicals oxidize a number of amino acid residues of the PrP molecule, with His and Tyr residues most significantly modified [65, 66]. PrP precipitation was also observed, which presumably results from a conformational change accompanying the PrP oxidation that makes PrP more prone to aggregation [65]. Using three peptides derived from different copper binding domains of PrP, Srikanth et al. presented strong mass spectrometric evidence on the oxidations of His and Trp residues by OH• (produced again by MCO using AA, H₂O₂ and the copper complexes of these peptides) [66]. Interestingly, for the peptide containing both the OR and non-OR domains (PrP(23–28, 57–98)), significant Trp oxidation was also observed, but only when a higher peptide/Cu(II) concentration ratio (e.g., 1:3 or lower) was used. At [PrP peptide]:[Cu(II)] = 1:3 or lower, the first two Cu(II) ions are to occupy OR (in the OR–Cu(II) configuration) and non-OR. Additional Cu(II) ions, however, will lead to the formation of complexes with high copper occupancies (i.e., OR–Cu(II)_n with n ≥ 2). The data by Srikanth suggest that both the binding stoichiometry and coordination chemistry have strong effects on the amount of OH• radicals generated and the subsequent modification of residues. The abovementioned papers attributed the oxidation of His and/or Trp residues to their close vicinities to the copper centers (or the origins of the OH•). We believe that, in addition to the proximity effect, the higher tendency of His, Trp, and Try (in addition to Met and Cys) residues [67, 68] to oxidize, relative to other amino acids, is relevant.

Nishikimi and co-workers also studied the ascorbate oxidation at different [OR]:[Cu(II)] ratios and quantified the amount of OH• radicals produced [26]. They found that the ascorbate oxidation becomes more appreciable above the molar concentration ratio of 1:1. OR–Cu(II) is in a “redox-inactive” state, which produces little oxidized AA (i.e., DA) or OH• (cf. panels A and B in Figure 6). In contrast, the amounts of DA and OH• are substantially higher when the [OR]:[Cu(II)] ratio exceeds 1:1, with the ratio of 1:4 (i.e., OR–Cu(II)₄ as the predominate copper complex) producing the largest amounts of DA and OH•. By quickly acidifying the solution with trichloroacetic acid to quench the Fenton-like reaction and concurrently adding the copper chelator BCS into the solution, they also found that Cu(I) is quite stable at different [OR]:[Cu(II)] ratios. Similar findings were also obtained when AA was replaced with GSH [26]. We should emphasize that in this work that little AA consumed by the OR–Cu(II) complex is not contradictory to the predication from the difference in redox potentials (cf. Table 1). This is because the OR–Cu(II) concentration

used by Nishikimi (10–30 μM Cu(II) and 5 μM OR) and co-workers is much lower than the amount of AA used (500 μM). As stoichiometrically dictated by eq. 13, 490 μM AA should still remain in the solution if 10 μM Cu(II) is used. However, as the [OR]:[Cu(II)] ratio exceeds 1:1, OR–Cu(II)_n (with $n \geq 2$) will be formed, and the concurrent shifts in redox potentials will make the reaction shown by eq. 14 occur. The redox cycle shown by eqs. 13–14 and H₂O₂ production will continue until either AA or O₂ in solution is exhausted. In a sense, the small amount of OR–Cu(II)_n ($n \geq 2$) serves as a catalyst (or “enzyme”) that facilitates a rapid consumption of AA, as evidenced by data in Figure 6A. In our recent work, we reproduced the experiment performed by Nishikimi and coworkers. With a higher amount of OR–Cu(II), we showed that AA can also be oxidized appreciably, but little H₂O₂ is produced and Cu(I) cannot be reoxidized (as predicted by the redox potentials in Table 1).

The results from the abovementioned studies correlate well with the potentials listed in Table 1, demonstrating the usefulness of determining redox potentials of copper complexes of amyloidogenic species to make predictions and to rationalize the chemical redox reactions performed in solution. An advantage of using electrochemical techniques is that, rather than mixing an oxidant or reductant with the protein-metal complex, the oxidation (valence) state of the metal center can be altered via a precise control of the electrode potential. In doing so, possible side or unforeseen reactions can be avoided. In surveying the literature, we noticed that the observation by Nishikimi and co-workers and our results are in conflict with those reported by Ruiz et al.[69] and Miura et al.[70] whose results suggest that OR–Cu(II) is the most redox active stoichiometry among all the OR–Cu(II)_n ($n = 1–4$) complexes. Using BCS, they found that, at pH ranging from slightly acidic (e.g., 6.5) to physiological, the oxidation state of copper in the low occupancy (cf. Figure 1) is +1 and at least one Trp residue positioned in close proximity to the copper center is oxidized as soon as the complex is formed. This contention is also at odds with the results of Requena et al. [65] and Srikanth et al [66]. Because if Cu(II) upon complexation were reduced to Cu(I) by Trp on the protein, the complex would never be able to oxidize AA or GSH. Moreover, many EPR studies have unambiguously shown that the Cu(II) centers bound by OR in both low and high occupancies are stable [13, 22, 71, 72]. We believe that the discrepancy arises from the use of BCS in determining the oxidation state of the copper centers in the complexes. As described in section 2.1, with a strong binding affinity, BCS can sequester Cu(II) out from the PrP complexes. The high reduction potential of the (BCS)₂–Cu(II) complex (0.844 V vs. NHE) indicates that the Cu(II) center in the (BCS)₂Cu(II) complex possesses a much higher oxidation power than free Cu(II) [73]. Consequently, when oxidizable ligands are present in the solution (e.g., His, Tyr, Trp, Met and Cys residues on protein molecules), the (BCS)₂–Cu(II) complex can oxidize these redox-active residues, with (BCS)₂–Cu(I) produced simultaneously. The oxidation potentials of free Trp ranging from 0.60 to 0.98 V vs. NHE have been reported [68], which are all close from 0.844 V. It is likely that in protein molecules their oxidations could occur at lower potentials, as both the Trp and Tyr oxidation reactions are pH dependent and the local environment in a protein affects the pK_a values of these residues [68]. As shown by Figure 5, the Tyr residue(s) in A β and α -syn has(have) an oxidation potential of ca. 0.6 V vs. Ag/AgCl or ~0.8 V vs. NHE. Thus the shift of the copper redox potential through the BCS complexation explains well why Trp residues were oxidized in the studies conducted by Ruiz et al. and Miura et al.[70] Sayre[74] and Ivanov et al.[75], among others, have cautioned the use of BCS and its related analogs for studying reduction of Cu(II) bound by biomolecules. As for why Nishikimi did not observe the similar effect, we think that the acidification step to quench reactions shown by eqs. 13–14 before introducing BCS into the solution is essential. In a highly acidic solution, one or two of the nitrogen atoms on the phenanthroline moiety of BCS can be protonated, dramatically decreasing the binding affinity of BCS to Cu(II).

3.2. Roles of copper complexes of PrP in H₂O₂ generation and cell signaling

In our recent work, we demonstrated that H₂O₂ generation through eqs. 13–14 is both time- and pH-dependent. As shown by Figure 7A, over a period of 2 h, H₂O₂ was essentially undetectable when OR–Cu(II) was used. But H₂O₂ generated by OR–Cu(II)₄ or non-OR–Cu(II) increases gradually with time. Nevertheless, the amounts of H₂O₂ produced by OR–Cu(II)₄ and non-OR–Cu(II) are considerably lower than that by free Cu(II) (magenta curve in Figure 7A). Notice that in Figure 7A, the Cu(II) concentration was kept at low μ M and the PrP-derived peptides in large excess. In doing so, the amount of free Cu(II) is negligible and essentially all of the H₂O₂ measured is produced by the copper complexes of Prp peptides. We also chose to study the Cu(II)-glutamine complex (green curve) because glutamine is the most abundant amino acid in CSF. Representative results from the pH dependence study are shown in Figure 7B. At low pH values, the release of Cu(II) from OR–Cu(II) is substantial, which facilitates the catalytic H₂O₂ production (eqs. 13–14).

The initial sharp rises in the blue and magenta curves in Figure 7A were attributed to the rapid production of H₂O₂ via eqs. 13–14 by free Cu(II) and the glutamine-Cu(II) complex. The decay observed later on can be explained as follows. The net reaction of eqs. 10–12 (Harber-Weiss and Fenton-like reactions) is $3\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{OH}\cdot + 2\text{H}_2\text{O}$. Thus, as H₂O₂ builds up in the solution, the conversion to OH $\cdot$ accelerates. It is interesting to note that OR–Cu(II), OR–Cu(II)₄ and non-OR–Cu(II) exhibit different trends in Figure 7A. The rather slow rises suggest that OR–Cu(II), OR–Cu(II)₄ and non-OR–Cu(II) are all less active in facilitating the H₂O₂ production than free Cu(II) and other weakly bound copper. In fact, OR–Cu(II) completely quenching the redox cycle. Therefore, it is clear that these PrP-copper complexes all possess the property to cut down the generation of H₂O₂ and OH $\cdot$ that would otherwise be generated in the presence of free Cu(II). Regarding the relationship between OH $\cdot$ and H₂O₂, it is important to note that data by Nishikimi and coworkers [8], which clearly show that the OH $\cdot$ concentration (cf. Figure 6B) increases with the amount of H₂O₂ in solution (cf. Figure 6A). In principle, modification of proteins or other biomolecules by OH $\cdot$ generated by the MCO reaction should occur without externally introduced H₂O₂ [65, 66]. However, to be able to observe extensive damage caused by OH $\cdot$, externally introduced H₂O₂ is helpful in that a greater amount of OH $\cdot$ can be more rapidly produced through the Fenton-like reaction (i.e., eq. 12). It is also evident that both O₂ and a reductant (e.g., AA or GSH) play crucial roles in alternating the copper oxidation states between Cu(II) and Cu(I). Finally, as exhibited by the curves in Figure 7, it is evident that the amount of H₂O₂ in solution is not only dependent on reaction time, pH, and the amount of free or weakly bound Cu(II) in solution, but also on the type of metal complexes under investigation. Thus, meaningful comparisons between different studies can only be made when H₂O₂ or OH $\cdot$ is determined under the same experimental conditions. We also note that the choice of H₂O₂ detection method is important. In choosing commercial fluorescent and chemiluminescent detection kits or methods published in the literature, it is important to ensure that the components in the mixture that can reduce the copper center or any additives commonly employed as reductants (e.g., *tris*(2-carboxyethyl)phosphine [38] are absent.

There is significant literature on the relationship between PrP-copper complex activity and superoxide dismutase (SOD) [2, 3, 76]. There are some similarities between the two proteins (e.g., E^0 of OR–Cu(II) = 0.323 V vs. NHE is very close to that of SOD (0.32 V)[73] and Cu(I) is stable in both complexes). In addition, both molecules are involved in catalytic reactions with H₂O₂ produced. However, SOD dismutates superoxide radical to H₂O₂, but the PrP-copper complex helps convert molecular oxygen to H₂O₂. These are two completely different reactions. The most striking difference is perhaps that the “SOD-like activity” of PrP–Cu(II) is at least two orders of magnitude smaller than that of native SOD [76]. As can be seen from Table 1, A β –Cu(II) and α -syn–Cu(II) can also be switched between Cu(II) and Cu(I) to “catalyze” the reduction of O₂ to H₂O₂. Yet, the rate of H₂O₂ production is rather

sluggish. In fact, what we measured [64] for the H_2O_2 production from O_2 catalyzed by $\text{A}\beta$ - $\text{Cu}(\text{II})$, is 3–4 orders of magnitude slower than dismutation of superoxide radicals by SOD to produce H_2O_2 [77].

The findings described above are in general agreement that PrP may serve as a neuroprotectant by scavenging in vivo rogue $\text{Cu}(\text{II})$ or weakly complexed $\text{Cu}(\text{II})$, thereby ameliorating oxidative stress inherent in these species [9, 78]. The gradual generation of H_2O_2 by either $\text{OR-Cu}(\text{II})_4$ or non- $\text{OR-Cu}(\text{II})$ at the first glance may contradict the proposed neuroprotecting function of PrP, since H_2O_2 and any $\text{OH}\cdot$ produced from it are both ROS. Although a high concentration of H_2O_2 could cause oxidative stress/damage, we note that in vivo H_2O_2 is constantly produced as a by-product from many enzymatic reactions and can be readily decomposed by enzymes such as catalase and GSH peroxidase [18]. It was estimated that up to $250\text{ }\mu\text{M}$ H_2O_2 could be produced within brain neuropil every minute [79]. The H_2O_2 concentration depicted in Figure 7A (sub- μM levels produced) is only a small fraction of the known endogenous H_2O_2 concentration in the CNS. H_2O_2 can also react away with antioxidants such as AA and GSH, as shown from the aforementioned examples. Therefore in vivo a harmful buildup of H_2O_2 from eqs. 13–14 under normal circumstance should not occur. Moreover, as suggested in the literature, the capacity of brain homeostasis of endogenous H_2O_2 is rather high [79]. H_2O_2 is much less potent than the oxygen-containing radicals (e.g., $\text{OH}\cdot$), and does not readily cause as extensive oxidative damage as $\text{OH}\cdot$ [80–82]. This is supported by the work by Rice and coworkers who did not find obvious lipid peroxidation in cell membranes of dopaminergic neurons upon exposure to high dosage of H_2O_2 [79]. We have proposed that H_2O_2 generated at the PrP-residing membrane could act as an important signaling molecule, as H_2O_2 is reported membrane permeable, and its penetration through cell membrane can trigger a cascade of reactions in the cytosol [81–85]. A prominent mechanism for H_2O_2 -triggered cell signaling involves extracellular permeation of H_2O_2 into cytosol to enhance protein tyrosine phosphorylation via simultaneous inhibition of tyrosine phosphatases and activation of tyrosine kinases [83]. We noted that Mouillet-Richard and coworkers demonstrated that PrP's role in signal transduction that may be linked to the intracellular tyrosine kinase Fyn and suggests, therefore, that H_2O_2 might be the messenger [86].

4. Summary and Outlook

A clearer understanding of the relationship between copper and PrP is emerging out of intensive studies over the past decade on the coordination chemistry, redox reactions, electrochemistry, and cytotoxicity of copper complexes of PrP and PrP-derived peptides. Although the exact biological function of PrP is still unknown, it is evident that PrP is a copper binding protein with unique coordination chemistry and redox property that can change in response to different conditions in cellular milieu. If the primary role of PrP were to regulate the concentration and redox reactions of cellular copper, the toxicity inherent in free copper in cellular milieu would be significantly attenuated by copper complexation with PrP. Consequently, PrP would serve as an important biomolecule to counteract oxidative stress/damage in vivo (instead of facilitating the generation of ROS). The many observations reviewed here strongly suggest that productions of ROS such as H_2O_2 and $\text{OH}\cdot$ by copper-initiated redox cycles are significantly suppressed in the presence of PrP. In contrast to other copper-binding amyloidogenic molecules (e.g., $\text{A}\beta$ and α -syn) that generally bind copper strongly in a single domain or configuration, PrP has at least two copper-binding domains with varying binding stoichiometries in response to the cellular copper concentrations (cf. Figure 1). This interesting property confers to PrP a more diverse set of redox reactions with rich electrochemistry.

A major goal of this review is to clarify some of the misconceptions and inconsistencies in the literature on the use of electrochemistry and chemical reactions to study the redox properties of the copper-PrP complexes. The existence of these inconsistencies indicates a pressing need for chemists and biochemists/biologists alike to use electrochemical techniques more effectively for the studies of metal complexes of PrP and other amyloidogenic molecules. Below we summarize the general precautions that one must use in studying electrochemistry and redox reactions of metal complexes of amyloidogenic proteins:

1. Redox potentials, binding constants, and reaction mechanisms should be deduced from data obtained from carefully designed experiments and verified by data collected with different parameters (e.g., scan rates, potential ranges, and scan directions).
2. Criteria for assessing the reversibility of an electron transfer reaction of a metal-protein complex in solution are different than those for a complex confined to the electrode;
3. Certain experimental conditions (e.g., O₂ content in solution and concentrations of the protein or metal ions, etc.) need to be carefully chosen.
4. To detect products or intermediates of redox reactions initiated by free redox metal ions or their protein complexes, additives used to “tag” or “trap” these products/intermediates should not alter the redox properties of free metal ions or their complexes or initiate other unwanted redox reactions.
5. If all possible, the occurrence/inhibition of a specific chemical redox reaction should be interpreted on the basis of the order of the redox potentials of the reactants. On the other hand, one needs to pay special attention to the reliability of the redox potentials to formulate a rational explanation of the reactions.

We believe that many more exciting aspects about the role of PrP in regulating copper redox activity and copper transport are yet to be uncovered. The contribution of the non-OR–Cu(II) complex to MCO, as well as to generation/inhibition of ROS, awaits for further elucidation, especially in context with a similar role played by the complexes formed between Cu(II) and the OR domain. Moreover, how the different domains/copper occupancies and their suppression/facilitation of the ROS generation are related to PrP aggregation may be a topic of interest. Thus far, contradictory results have been reported regarding the effect of copper on the generation of PrP^{Sc} [58]. For instance, introducing copper into recombinant PrP solution retards the formation of amyloid [87, 88]. However, exposing purified mouse PrP^C accelerates its conversion to a PrP^{Sc}-like aggregate [89]. Similar controversies can be found from published cell-based assays and animal model studies [90, 91]. With a better defined role of PrP in inhibiting/ameliorating ROS production and the resultant oxidative damage, the effect of copper on PrP aggregation can now be investigated from a new perspective. Another interesting aspect of the PrP property is that a range of metal ions (zinc, nickel, manganese, and possibly iron) interact with PrP [78, 92, 93]. Among these metals, iron would be expected to behave very similarly to copper in terms of the redox reactions reviewed above. Although the general consensus is that iron is not bound by PrP specifically, PrP^C influences iron uptake and transport [94, 95]. Along this line, binding of Zn(II) by PrP and the important role of zinc in this field has been more convincingly demonstrated [58, 59, 78]. Although Zn(II) is not redox-active, it could compete with Cu(II) for PrP binding and consequently serve as a modulator of the overall redox activities, as nicely shown in a recent paper by Shearer and coworkers [96]. Finally, our hypothesis that the controlled production of H₂O₂ by OR–Cu(II)₄ might serve as a signaling process across cell membrane remains to be tested. The hypothesis would be verified if H₂O₂ permeated into the cytosol and its initiation of the downstream events (e.g.,

activation of tyrosine kinases and deactivation of tyrosine phosphatases) were monitored at the cellular level.

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Figure 1.

Structures of OR-Cu(II) (low Cu(II) occupancy), OR-Cu(II)₄ (high Cu(II) occupancy) and non-OR-Cu(II). OR = (PHGGGWGQ)₄ and the Cu(II) binding in OR-Cu(II)₄ is equivalent to OP-Cu(II) where OP = PHGGGWGQ. The non-OR copper binding segment has a sequence of GGGTH.

Figure 2.

(A) Simulated cyclic voltammograms (CVs) for a reversible reduction of O to R at 0.01 (black curve) and 0.04 V/s (red curve). Other parameters for the simulations were given in the text. (B) CV for a reversible electron transfer (black curve) and overlaid with that followed by a catalytic reaction (red curve) as shown in equation 4.

Figure 3. Cyclic voltammograms of Wt-PrP fully loaded with Cu(II) and immobilized onto a boron-doped diamond electrode acquired at (i) 0.05, (ii) 0.01, and (iii) 0.001 mV/s. The dashed line represents the background voltammogram without PrP immobilized. Panels (iv) and (v) indicate the plots (in logarithmic scale) of scan-rate dependences of the peak currents and potentials, respectively. (from Ref [16] with copyright permission from American Chemical Society).

Figure 4. Cyclic voltammograms of (A) OR-Cu(II), (B) OR-Cu(II)₄, (C) non-OR-Cu(II) and (D) free Cu(II) in N₂-saturated (black curve) and O₂-purged solutions (red curve), respectively. [Cu(II)] in all cases was 90 μM , while the OR and non-OR concentrations were 100, 25, and 100 μM in panels (A), (B) and (C), respectively. The dotted line curve in (A) corresponds to the CV of OR. The scan rate was 5 mV/s (from [18] with copyright permission from American Chemical Society).

Figure 5.

Cyclic voltammograms of (A) 200 μM $\text{A}\beta(1-16)$ (thick line), 100 μM Cu(II) (dashed curve), a mixture containing $\text{A}\beta(1-16)$ and 200 μM Cu(II) (thin solid curve), and an O_2 -saturated mixture of $\text{A}\beta(1-16)$ and Cu(II) (dash-dot-dash curve) and (B) 200 μM $\alpha\text{-syn}$ and 50 μM Cu(II) (solid line curve), 100 μM $\alpha\text{-syn}(1-19)$ and 50 μM Cu(II) (dashed line curve), and 50 μM free Cu(II) (dotted line curve). All solutions were prepared with 5 mM phosphate buffer (pH 7.4) containing 0.1 M Na_2SO_4 . The scan rate was 20 mV/s for (A) and 5 mV/s for (B) and the horizontal arrows indicate the initial scan direction. A voltammogram from a 50 μM tyrosine solution is shown in the inset of (A). A voltammogram of 100 μM $\alpha\text{-syn}$ only is shown in the inset of panel (B) with the vertical arrow indicating the irreversible tyrosine oxidation peak. (Adopted from Reference [39] for panel (A) and Reference [45] for panel (B) with permission from American Chemical Society).

Figure 6.

Suppression of Cu(II)-catalyzed ascorbate oxidation and production of OH• by the OR peptide. Ascorbate (500 μM) was allowed to react with varying concentrations of Cu(II) (5–80 μM) in 20 mM Mes buffer (pH 7.5) in the presence (solid circles) and absence (open circles) of 20 μM OR peptide, at 25 °C for 2 min, and the concentrations of remaining ascorbate (A) were measured spectrophotometrically. OH• formed during the Cu(II)-catalyzed oxidation of ascorbate (B) were measured with coumarin-3-carboxylic acid (CCA), which forms the product 7-OHCCA whose fluorescence emission can be measured at 450 nm. Adopted from Reference [26] with copyright permission).

Figure 7.

(A) Time-dependence of H_2O_2 generation in solutions of: OR-Cu(II) (black), OR-Cu(II)₄ (red), non-OR-Cu(II) (blue), Cu(II)/glutamine (green) and free Cu(II) (magenta). All solutions contained 5 μM Cu(II) and 200 μM AA and the ligand concentration was 100 μM except for the solution of OR-Cu(II)₄ wherein [OR] = 1.25 μM . (B) Variations of $[\text{H}_2\text{O}_2]$ generated by OR-Cu(II) in solutions of different pH values: 7.4 (black), 6.5 (red), 6.0 (green) and 5.5 (blue) (from Ref [18] with copyright permission from American Chemical Society).

Table 1

Redox potentials of Cu(II) complexes with select amyloidogenic molecules and redox couples.

Species	$E_{1/2}$ or E^0 (V) vs. NHE	References
Dopamine <i>o</i> -quinone/Dopamine	0.370 [#] (E^0)	[51]
OR–Cu(II)/OR–Cu(I)	0.272, 0.323 (both are $E_{1/2}$)	[16, 18]
O ₂ /H ₂ O ₂	0.296 (E^0)	[25]
A3–Cu(II)/A3–Cu(I)	0.696–0.746(E^0), 0.277 ($E_{1/2}$)	[38, 39]
PrP–Cu ₅ (II)/PrP–Cu(I) ₅	0.267 ($E_{1/2}$)	[16]
3-Syn–Cu(II)/3-Syn–Cu(I)	0.215, 0.102 ($E_{1/2}$)	[45, 46]
OR–Cu(II) ₄ /OR–Cu(I) ₄	30.305, 0.172 ($E_{1/2}$)	[10, 18]
Free Cu(II)/Cu(I)	0.159 (E^0)	[13]
non-OR–Cu(II)/non-OR–Cu(I)	0.222, 0.110 [*] ($E_{1/2}$)	[16, 18]
DA/AA	0.052 [#] (E^0)	[56]
GSSG/GSH	–0.228 [#] (E^0)	[57]
NADH/NAD ⁺	–0.320 ($E_{1/2}$)	[57]

[#] The oxidized forms of these redox couples are chemically unstable and the redox reactions are pH-dependent. The values listed here are standard reduction potentials, not peak potentials from the electron transfers that are followed by irreversible chemical reactions.

^{*} Accuracy is affected by the less reversible redox wave of non-OR–Cu(II) [18]. DA = dehydroascorbate, AA = ascorbate, GSH = glutathione, and GSSG = the oxidized form of GSH. NAD⁺ = nicotinamide adenine dinucleotide and NADH = reduced form of NAD⁺, both of which are involved in complex I of the respiratory chain.