Copper Binding to the PrP Isoforms: a Putative Marker of Their Conformation and Function

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We show here that PrP^C, the normal isoform of the prion protein (PrP^{Sc}), could be retained by a Cu²⁺-loaded resin through two different binding sites. Contrarily, PrP^{Sc} was not retained at all by such resin. This constitutes a new prion-specific property of PrP^{Sc}, which in addition to protease resistance and β -sheet content, may result from its aberrant conformation.

PrP^C is the normal isoform of PrP^{Sc}, the protein component of the prion. Prions cause transmissible neurodegenerative diseases such as scrapie and bovine spongiform encephalopathy as well as Creutzfeldt-Jakob disease in humans (12). The two PrP isoforms have the same amino acid sequence, but in contrast to PrP^C, PrP^{Sc} comprises a protease-resistant core peptide denominated PrP²⁷⁻³⁰. The function of PrP^C is still unknown, despite the creation and investigation of several lines of PrP^{0/0} mice (2). The only clue for the function of PrP^C is the finding that it binds copper specifically (14, 16).

The established copper binding site on PrP was shown to be comprised in its N-terminal eight tandem repeats (octarepeats) (3). However, Pan et al. (11) showed that when PrP^C was purified on a Cu²⁺-loaded immobilized metal affinity chromatography (IMAC) resin, an N-terminally truncated metabolite of PrP^C (called PrP^{II}) was also enriched. This suggests the presence of a second copper binding site on PrP, downstream from the N-terminal repeats. This conclusion, however, should be considered with caution, since in the case of a Cu²⁺-loaded IMAC resin, the Cu²⁺ ions are ligated to both the immobilized chelator on the resin and the protein simultaneously, not to the protein only.

In this work, we examined the elution profiles of the PrP isoforms (PrP^C and PrP^{Sc}) as well as of their metabolites from a Cu²⁺-loaded IMAC resin at increasing imidazole concentrations. Our results show that as opposed to PrP^C and denatured PrP^{Sc}, both native PrP^{Sc} and PrP²⁷⁻³⁰ were not retained by the Cu²⁺-loaded resin. This constitutes a new prion-specific property of PrP^{Sc}, which in addition to protease resistance and β -sheet content, probably results from its aberrant conformation.

MATERIALS AND METHODS

Fifty-microgram aliquots of brain or cells membranes were extracted with 2.5 ml of cold 1% Triton X-100 in phosphate-buffered saline (Triton-PBS) and subsequently mixed for 1 h at room temperature with an IMAC resin which was previously loaded with copper ions as described elsewhere (11). The resin was precipitated by centrifugation, and the nonbound extract was designated the flowthrough. The IMAC resin was washed four times with 2.5 ml of Triton-PBS.

The attached proteins were eluted from the resin by the addition of 2.5 ml of imidazole at increasing concentrations (0.05 M twice, 0.1 M twice, and 0.2 M twice). Finally, 2.5 ml of 50 mM EDTA was used (twice) to release the copper ions from the IMAC resin. Other scrapie extracts were denatured with 3 M (final concentration) guanidium isothiocyanate (GuSCN) for 30 min and precipitated with 4 volumes of methanol before resuspension in Triton-PBS and application to the resin. All eluted fractions were recovered by centrifugation of the resin and subsequently immunoblotted with the appropriate antibodies.

RESULTS AND DISCUSSION

A 2% Triton X-100 extract of normal hamster brain membranes was incubated with a Cu²⁺-loaded IMAC resin and subsequently eluted as described in Materials and Methods. When the eluted fractions were immunoblotted with anti-PrP monoclonal antibody (MAb)3F4 (Fig. 1a), which reacts with residues 108 to 111 in hamster or human PrP (5), only full-length PrP was detected, mostly in the fractions eluted with high imidazole concentrations or EDTA. When the same fractions were immunoblotted with MAb 6H4 (Prionics, Zurich, Switzerland), which reacts with PrP residues 144 to 152 (Fig. 1b), a shorter PrP peptide was also detected, but at the fractions eluted with low imidazole concentrations. These results suggest that this shorter PrP, which did not react with MAb 3F4 and thereby must be truncated at its N terminus, still binds to a Cu²⁺-loaded IMAC resin, albeit with a lower affinity than full-length PrP. Since, like the PrP^{II} described by Pan et al. (11), it lacks the putative PrP copper binding site present in the N-terminal octarepeats (IMAC1), it must comprise another Cu²⁺ IMAC retention site (IMAC2).

Both PrP isoforms are associated with cholesterol-rich membrane microdomains called rafts (9, 15). To establish whether PrP^C binds directly to the Cu²⁺-loaded resin or is attached to it through a neighbor raft protein, the normal Triton X-100 extract was incubated with the anti-PrP antiserum (reacting against residues 142 to 172) (1) before being applied to the IMAC resin. In the presence of this antiserum (Fig. 1c), the N-terminally truncated PrP was eluted mostly in the flowthrough, while full-length PrP was eluted at considerable lower imidazole concentrations, suggesting that the anti-PrP antiserum lowered the affinity of PrP peptides to the Cu²⁺-loaded IMAC resin. These results indicate that PrP^C binds to the copper-loaded resin directly, but although suggestive, they do not prove that the IMAC2 retention site lies between res-

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FIG. 1. Two copper binding sites on PrP^C. (a to c) Triton X-100 extracts from normal hamster brains were applied to and eluted from a Cu²⁺-loaded IMAC resin as described in Materials and Methods. (a) Flowthrough samples, washes, and eluted samples immunoblotted with anti-PrP MAb 3F4; (b) same samples immunoblotted with MAb 6H4; (c) Triton X-100 extract incubated with the anti-P5 antiserum applied to the resin, eluted as above, and immunoblotted with MAb 6H4; (d) Triton X-100 extracts from mature sperm cells were applied to a Cu²⁺-loaded IMAC resin. Flowthrough samples, washes, and eluted samples were immunoblotted with anti-PrP MAb 6H4.

idues 142 and 174, since the antibody may also hinder the binding of copper to a nearby site of the protein. As expected from previous results (11), an IMAC resin loaded with zinc did not show any specific PrP retention (not shown).

We have shown recently that in mature sperm cells, PrP is truncated at its C terminus, probably between the two PrP N-glycosylation sites (residues 181 and 197) (13). Human ejaculates containing mature sperm cells were extracted in Triton X-100, applied to a Cu²⁺-loaded IMAC resin, and eluted as described above for brain extracts. All sperm PrP, which comprises the N-terminal but not the C-terminal part of PrP, was retained by the copper resin and eluted at low imidazole concentrations (Fig. 1d). These results indicate that the additional Cu²⁺ retention site may reside in the C-terminal part of PrP.

To determine whether PrP^{Sc} and PrP27-30 can be retained by a Cu²⁺-loaded IMAC resin, membranes from scrapie-infected hamster brains were extracted with Triton X-100 and applied to an IMAC resin as described for normal brain membranes. PrP^{Sc} molecules aggregate in the presence of detergents such as Sarkosyl (7), but this is not the case for scrapie-infected membranes extracted in cold Triton X-100, in which PrP^{Sc} is still incorporated into rafts and therefore does not aggregate (8).

Compared to normal brain membranes (Fig. 2a), most of the total PrP present in the extract (PrP^C and PrP^{Sc}) was found in the flowthrough and not in any of the retained fractions (Fig. 2b). Moreover, all PrP was detected in the flowthrough when the samples were digested with proteinase K (PK) before immunoblotting (Fig. 2c) or when the Triton X-100 scrapie-infected extract was digested with PK before application to the Cu²⁺-loaded IMAC resin (Fig. 2d). These results suggest that both PrP^{Sc} and PrP27-30 did not bind to a Cu²⁺-loaded IMAC resin. When the scrapie membranes were first denatured by 3 M GuSCN, the PrP protein was retained by the resin at the same fashion as PrP^C, suggesting the difference in retention of PrP^C and PrP^{Sc} by a Cu²⁺-loaded IMAC resin results from the

FIG. 2. PrP^{Sc} and PrP27-30 do not bind copper. Triton X-100 extracts from normal and scrapie-infected hamster brains were applied to and eluted from a Cu²⁺-loaded IMAC resin as described in Materials and Methods and the legend to Fig. 1. Before immunoblotting, the eluted scrapie brain samples were digested in the presence and absence of PK (50 µg/ml). Part of the scrapie extracts were digested with PK before the application to the resin. (a) Normal hamster brain; (b) scrapie-infected hamster brain; (c) scrapie samples digested with PK before immunoblotting; (d) scrapie samples digested with PK before application to the Cu²⁺-loaded IMAC resin; (e) scrapie samples after denaturation with 3 M GuSCN. Panels a and b were immunoblotted with MAb 3F4; panels c to e were immunoblotted with MAb 6H4.

aberrant conformation of PrP^{Sc} (Fig. 2e). Similar results were obtained previously for sodium dodecyl sulfate-denatured PrP^{Sc} (4). The fact that only a small fraction of total PrP molecules were retained by the Cu²⁺-loaded IMAC resin before PK digestion suggests that the concentration of functional PrP^C is very low in extracts from scrapie-infected brains. These results are consistent with those presented in a recent publication suggesting that specific anti-PrP^C antibodies react very poorly with scrapie-infected brain samples (17).

The fact that PrP^{Sc}, as opposed to PrP^C, does not bind to a Cu²⁺-loaded IMAC resin constitutes a newly identified difference between the two prion protein isoforms, which until now were distinguished mostly by their protease resistance and β -sheet content (6, 10). That full-length PrP^{Sc} is not retained by a Cu²⁺-loaded IMAC resin may be specifically important, since it suggests that also the N-terminal part of PrP^{Sc}, which is not protease resistant and does not present a β -sheet structure, may differ in conformation from the N-terminal part of PrP^C. It will be interesting to test the Cu²⁺-loaded IMAC resin retention properties of pathogenic mutant PrPs before they acquire protease resistance to determine whether they resemble PrP^C or PrP^{Sc}.

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