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α -Synuclein in α -helical conformation at air–water interface: implication of conformation and orientation changes during its accumulation/aggregation†

Chengshan Wang^a, Nilam Shah^a, Garima Thakur^b, Feimeng Zhou^{*,a}, and Roger M. Leblanc^{*,b}

^a5151 State University Drive, California State University, Los Angeles, Los Angeles, CA 90032, USA. Fax: +1 323-343-6490; Tel: +1 323-343-2390

^b1301 Memorial Drive, Department of Chemistry, University of Miami, Coral Gables, FL 33146, USA. Fax: +1 305-284-4571; Tel: +1 305-284-2194

Abstract

α -Synuclein, a natively unstructured protein important in the neuropathology of Parkinson's disease, was found to form a Langmuir monolayer in an α -helical conformation with its helical axis parallel to the air–water interface. This study sheds light on the role of vesicles in neuronal cells in the accumulation/aggregation of α -synuclein.

Neurodegenerative diseases are manifested by the aggregation of the amyloidogenic proteins or peptides (*e.g.*, α -synuclein (α -syn) in Parkinson's disease (PD) and β -amyloid or A β in Alzheimer's disease (AD)).¹ In general, the amyloidogenic proteins are natively unstructured in aqueous solution, whereas the aggregates found in amyloid plaques in postmortem tissues comprise significant amounts of pleated β -sheets.¹ α -Syn, a protein of 140 amino acids whose two termini sandwich a hydrophobic mid-segment (*i.e.*, the non-amyloid component), is the major component of Lewy bodies (the hallmark of PD) inside dopaminergic cells of PD patients.² Because the structure of the various aggregates produced *in vitro* from the unstructured monomers resembled those extracted from Lewy bodies, the misfolding and subsequent aggregation of the α -syn monomers have been suggested to play an important role in the etiology of PD.²

Among the various factors affecting the misfolding and aggregation of α -syn, interfaces have been noted to be important,^{2,3} especially those of amphiphilic nature (*e.g.*, cell membrane, micelle, and vesicle interfaces).^{2,3} α -Syn *in vivo* was found to enrich near the presynaptic terminals (PST) of neuronal cells.³ Because each PST contains abundant vesicles,⁴ it is of interest to investigate whether the presence of these heterogeneous interfaces enhance the adsorption and accumulation of α -syn. Although it has been suggested that α -syn in the presence of vesicles adopted an α -helical conformation,^{5,6} there is still a lack of information about the orientation of the helices at these interfaces. Determination of the molecular orientation should therefore yield a fundamental understanding of the accumulation and conformational change of α -syn at interfaces.

†Electronic supplementary information (ESI) available: Expression of α -synuclein, Langmuir monolayer technique, IRRAS measurements and CD spectroscopy. See DOI: 10.1039/c0cc02098b

*fzhou@calstatela.edu, rml@miami.edu .

On the basis that an aqueous layer of a few nanometre thickness exists at the interface of the cell membrane and its physicochemical properties are different to the bulk aqueous phase in the cytoplasm,^{7,8} the air–water interface has been accepted as a simple model to mimic the interface of the cell membranes.⁹ Furthermore, the Langmuir monolayer technique provides a unique and accurate methodology to determine the chemical and physical properties (*e.g.*, stability or aggregation) of amphiphile biomacromolecules at the air–water interface.^{9–11} We recently observed that A β molecules accumulated at the air–water interface were predominantly present in the α -helical conformation.⁹ A kinetic model was proposed to explain the A β adsorption behavior. Specifically, a conformational change from the natively unstructured A β to its α -helical counterpart occurs in the subphase prior to the adsorption step to the interface. Our finding, which is consistent with the theoretical prediction that A β near or inside lipid bilayer is in the α -helical conformation,¹² suggested that the α -helical conformation might be a stable intermediate during the transition from the unstructured monomer to the β -sheet-containing oligomers and larger aggregates. Although the orientation of the α -helical A β molecules was assumed to be parallel to the air–water interface, the circular dichroism and attenuated total reflection spectroscopic results did not yield information about the exact molecular orientation of A β molecules accumulated at the interface.

Infrared reflection-absorption spectroscopy (IRRAS), capable of determining both the conformation and surface orientation of proteins, is amenable to *in situ* characterization of the protein Langmuir monolayer under different experimental conditions.^{10,13} It has already been shown that IRRAS was highly versatile to study the conformation of proteins at the air–water interface.^{13,14} Indeed, the behavior of α -syn at an interface has been shown to be different than that in the bulk.¹⁵ To gain insight into the structure of α -syn at the interface, we examined the surface pressure-area isotherm and the IRRAS of an α -syn Langmuir monolayer to characterize the conformation and orientation of α -syn in the Langmuir monolayer.

As shown in Fig. 1, the surface pressure has a lift-off point at 2450 Å²/molecule either on pure water or 0.15 M NaCl (pH 7.4) subphase. Further compression allows the observation of a kink point at 1560 Å²/molecule, which indicates a liquid expanded–liquid condensed phase transition in the surface pressure–area (π -A) isotherm. The extrapolation of the linear portion of the isotherms gives a limiting molecular area of about 1490 Å²/molecule. Considering the similarity of the two isotherms, the ionic strength of the subphase within our experimental conditions may not play any role in the surface properties of the α -syn in the Langmuir monolayer and the α -syn is stable at the air–water interface.

To further verify this stability, we compressed the α -syn Langmuir monolayer to a surface pressure of 10 mN/m and kept the surface pressure constant over an extended period of time (more than 100 min) and monitored the surface pressure (solid line curve) and area (dashed line curve) over the entire experiment (Fig. 2). When the α -syn Langmuir monolayer was compressed to 10 mN/m, the area decreased similarly to that in the π -A curve at 10 mN/m (Fig. 1). When the surface pressure was kept constant over 100 min, the molecular area decreased by less than 2%. The small decrease in the molecular area is indicative of the high stability of the α -syn Langmuir monolayer which assures that the protein did not denature during the compression. This stability may be explained by the possibility that the accessibility between the non-amyloid components of the neighboring α -syn molecules is hindered at the air–water interface.

To support this experimental observation, one Langmuir monolayer of α -syn was transferred onto both sides of a quartz slide using the Langmuir–Blodgett (LB) technique of deposition for circular dichroism (CD) measurements. The inset of Fig. 2 compares the CD spectra of

the α -syn protein in the LB films on quartz slides and in the aqueous phase. An unexpected result was observed regarding the conformation of α -syn in these two phases: the characteristic peaks of the α -helical conformation (two negative peaks (209 and 222 nm) and one positive peak (192 nm))¹⁶ were observed for an α -syn LB film, whereas a negative peak at 199 nm was observed for α -syn in solution, which can be attributed to the unstructured conformation.¹⁶ Particularly, the CD spectrum of the LB film of α -syn transferred after holding the surface pressure at 10 mN/m for two weeks was still identical to the solid line curve shown in the inset of Fig. 2. This demonstrates that the α -helical conformation of the α -syn Langmuir monolayer neither dissolved back into the subphase nor denature to form a pleated β -sheet conformation.

To confirm that the change to the α -helix conformation was not due to the transfer of the α -syn Langmuir monolayer to the quartz slides, we investigated the *in situ* s- and p-polarized IRRAS of the α -syn Langmuir monolayer on 0.15 M NaCl (pH 7.4) subphase. As shown in Fig. 3A, three peaks characteristic of the α -helix conformation were observed for the s-polarized spectra, namely 1655, 1545 and 1515 cm^{-1} .^{17,18} The absence of the characteristic peaks of the β -sheet conformation at 1690, 1630 and 1524 cm^{-1} ^{17,18} suggests that no β -sheet conformation was present in the α -syn Langmuir monolayer. In addition, D_2O was used as subphase to exclude that the peak at 1655 cm^{-1} is from H_2O deforming (results in ESI^+). Moreover, the peak positions remain unchanged when the surface pressure was varied. This correlates well with the CD data, which demonstrate the stability of the α -helix of α -syn at the air–water interface.

The p-polarized IRRAS can provide information about the molecular orientation of the protein in the Langmuir monolayer. For vibrations parallel to the interface, the peaks are initially negative and increase in intensity with an increase in the incident angle until the Brewster angle (*e.g.*, 54.2° for the IR light at 2850 cm^{-1}) is reached.¹⁴ Above the Brewster angle, there is an inversion of the peaks to positive values and the intensity decreases with a further increase in the incident angle. Regarding a vibration perpendicular to the interface, the opposite should be observed for both the direction and intensity of the peak when the angle of the incident light is varied. Fig. 3B shows the p-polarized IRRAS spectra of the α -syn Langmuir monolayer. The fingerprint of the spectra indicates that the axis of the α -helix is parallel to the interface.

From the above results, we conclude that α -syn, with its distinctively hydrophilic and hydrophobic domains, is “surface active” (*i.e.*, its amphiphilic nature prompts a preferential adsorption at an amphiphilic interface). The driving force for the transformation of the unstructured α -syn to its helical conformation at the air–water interface is to simultaneously maximize the hydrophilic interaction in the aqueous environment and the hydrophobic force in the air phase. As aforementioned, the amphiphilic air–water interface has been used to mimic the boundary dividing the cellular matrix and the cell membrane, at which the dielectric permittivity is much smaller than the bulk of the cell matrix.^{7–9} Consequently, the folding and accumulation of α -syn at the air–water interface render an α -syn concentration at the interface much greater than that in the bulk. In cellular milieu, there exists a high density of vesicles in presynaptic terminals (PSTs),⁴ and the amphiphilic interface associated with vesicles should also facilitate the accumulation of α -syn. In fact, enrichment of α -syn near PSTs has been reported,³ which contrasts to the very low concentration of α -syn in plasma (~20 nM).¹⁹ Another important finding of our study is the direct IRRAS observation of the previously postulated parallel orientation of the α -syn helices with respect to vesicle and micelle interfaces. Although it was suggested that α -syn may adopt a two-helix, antiparallel arrangement around small unilamellar vesicles²⁰ and becomes even more extended when it is in contact with less curved lipid bilayers,²¹ our work is the first unambiguously demonstrating that the orientation is parallel to the interface.

The high stability of the Langmuir monolayer of α -syn helices also has an important biological implication. Fibrillation of α -syn in the α -helical conformation in the presence of vesicles has been reported to be slow,^{6,22} suggesting that the α -helical conformation might be a rather stable intermediate during the transformation between the natively unstructured molecule to the β -sheet-containing aggregates. The accumulation of α -syn in the stable α -helical conformation should concurrently decrease the cytosolic α -syn concentration. It is widely known that the oligomerization/aggregation of α -syn decreases with the bulk concentration. Therefore, both of the aforementioned events collectively prolong the production of the β -sheet-pleated α -syn aggregates. Such a contention is in line with the clinical observation that the depletion of dopaminergic cells (which contain the α -syn aggregate-rich Lewy bodies) is a gradual process and late onset PD takes many years to develop.²

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Surface pressure-area isotherms of 0.3 mg/mL α -syn spread on the 0.15 M NaCl Tris buffer (pH 7.4) (black curve) and pure water (red curve) subphase.

Fig. 2.

Stability curves when a Langmuir monolayer of α -syn was compressed up to 10 mN/m and kept constant for more than 100 min on the Tris buffer (pH 7.4) subphase containing 0.15 M NaCl. Inset: CD spectra of 0.015 mg/mL α -syn dissolved in Tris buffer at pH 7.4 (dashed line curve), an α -syn Langmuir-Blodgett (LB) film on a quartz slide (solid line curve), and a clean quartz slide without an α -syn LB film attached (dotted line curve).

Fig. 3. S-polarized (A) and p-polarized (B) IRRAS spectra of the α -syn Langmuir monolayer at various surface pressures at an incident angle of 30° and at various incident angles at a surface pressure of 10 mN/m, respectively.