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Transmembrane Proteins: Amyloids Hidden in Plain Sight?

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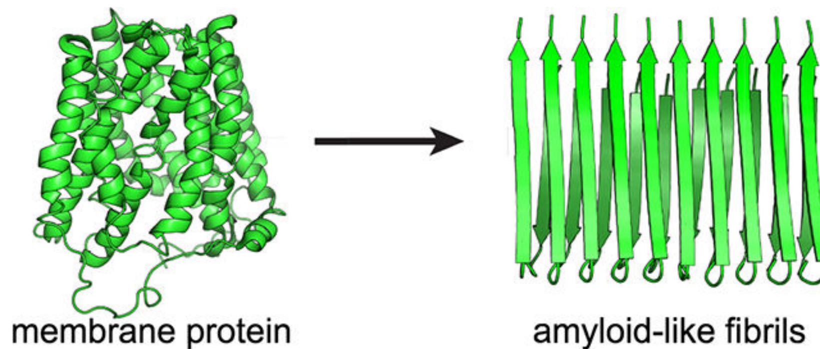
Graphical Abstract



VIEWPOINT

Amyloid formation is emerging as an important part of the protein energy landscape, and not simply a departure from normal protein folding. Amyloidogenesis marks the thermodynamic endpoint of protein aggregation and is characterized by the formation of insoluble fibrils. The fibrils typically consist of extended layered β-sheets with infinite networks of intermolecular hydrogen bonds and hydrophobic contacts. Soluble intermediates—called amyloid oligomers— can also form and consist of a heterogeneous mixture of smaller species that vary in size and aggregation state.

Identifying and characterizing new amyloids has become a fertile research area. Over 60 peptides or proteins that can form amyloid have been identified thus far.[1] Many of these amyloids are important in the pathology of amyloid diseases, such as Alzheimer's disease, Parkinson's disease, and type II diabetes. Other amyloids appear to have vital biological roles. A growing body of work suggests that amyloids are important in protein storage, cell-to-cell communication, and biofilm formation.[2] As additional new amyloids are identified and characterized, other important properties will undoubtedly be discovered.

Membrane proteins make up about 30% of the human proteome. These proteins are widely known to aggregate and often have substantial α-helical structure. Until recently, the structural features of the aggregates were unknown. Stroobants and coworkers recently shed light on the aggregates by examining a transmembrane protein, called LacY (Figure 1A).[3]

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LacY is well characterized and is known to aggregate. This protein consists of 12 transmembrane α-helices and transports β-galactosides across cell membranes.

The authors used a computational method called CamSol to analyze the solubility of LacY (Figure 1B). The PI (Vendruscolo) and coworkers had previously developed CamSol as a tool for analyzing protein solubility and designing proteins. CamSol accounts for the hydrophobicity or hydrophilicity of amino acids, as well as for the patterning of these amino acids within a protein. The method makes use of this information to determine the overall solubility profile of a protein and to suggest mutations that can improve solubility. The solubility profile of LacY is shown in Figure 1B, which indicates that large portions of LacY have low solubility and are thus prone to aggregate.

The authors subsequently analyzed the aggregation of LacY using several biophysical techniques. LacY transitioned from the folded protein to insoluble aggregates upon incubation for 48 h at 2 μM and 37 °C in phosphate buffer containing *n*-dodecyl β-D-maltoside micelles. At 0 h, circular dichroism (CD) spectroscopy shows a spectrum consistent with an α-helical structure, with characteristic minima at 208 and 223 nm. After incubation for 48 h, the CD signal diminished greatly, and the spectrum showed neither α-helical nor β-sheet character. UV-Vis absorption spectroscopy and SDS-PAGE also showed a decrease in signal over time. Monitoring LacY aggregation by a thioflavin-T (ThT) fluorescence assay showed an increase in fluorescence over time, suggesting the formation of amyloid-like fibrils.

The authors used FT-IR spectroscopy and X-ray fiber diffraction to gain further insights into the structure of the LacY aggregates. After incubation for 48 h, the FT-IR spectrum showed a decrease in IR bands associated with α-helical structure, and an increase in IR bands associated with β-sheet structure. X-ray fiber diffraction studies of the LacY aggregates showed a cross-β pattern consistent with that of a cross-β-sheet structure (Figure 2A and 2B). This pattern showed reflections at 4.6 Å and 10.8 Å, which are characteristic bands associated with the interstrand and intersheet spacing of amyloid fibrils. Transmission electron microscopy (TEM) studies showed that, upon treatment with guanidinum hydrochloride, the insoluble deposits formed by LacY resemble fibrils formed by other amyloidogenic peptides and proteins (Figure 2C and 2D). Collectively, these experiments established that the aggregates formed by LacY are amyloid-like fibrils. These studies provide the first example of amyloid formation by a helical membrane protein, but give only a glimpse of the molecular details.

This work sets the stage for future biophysical and biological studies on LacY aggregation, using techniques that are widely used for studying amyloidogenic peptides and proteins, such as Aβ, tau, and a-synuclein. It may lead to studies with high-resolution techniques, such as cryo-EM or solid state NMR, to further characterize the LacY fibrils and reveal the β- sheet structured regions.[4] A high-resolution fibril structure would then provide the basis for studying LacY aggregation using simulation-based approaches.[5] These studies would help build models for further understanding the β-sheet formation and aggregation of LacY. These studies may also prove broadly useful in understanding the aggregation of other transmembrane proteins.

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The work of Stroobants and coworkers is part of a recent shift toward identifying new proteins that can form amyloid. This particular study is important, because transmembrane proteins make up such a large percentage of the human proteome. The study gives a reason to appreciate transmembrane protein aggregation, rather than to view it as a problem. It also provides a window for many thought-provoking questions: Is amyloid formation of the LacY transmembrane protein the exception or the rule? Is α-helix structure, rather than solubility, the critical component that prevents membrane protein aggregation? Do transmembrane proteins aggregate as part of "normal" processes or "pathological" processes? Do transmembrane proteins promote aggregation of other proteins? Addressing these questions and others will lay the groundwork for understanding the role of transmembrane protein aggregation in biological function.

ACKNOWLEDGMENTS

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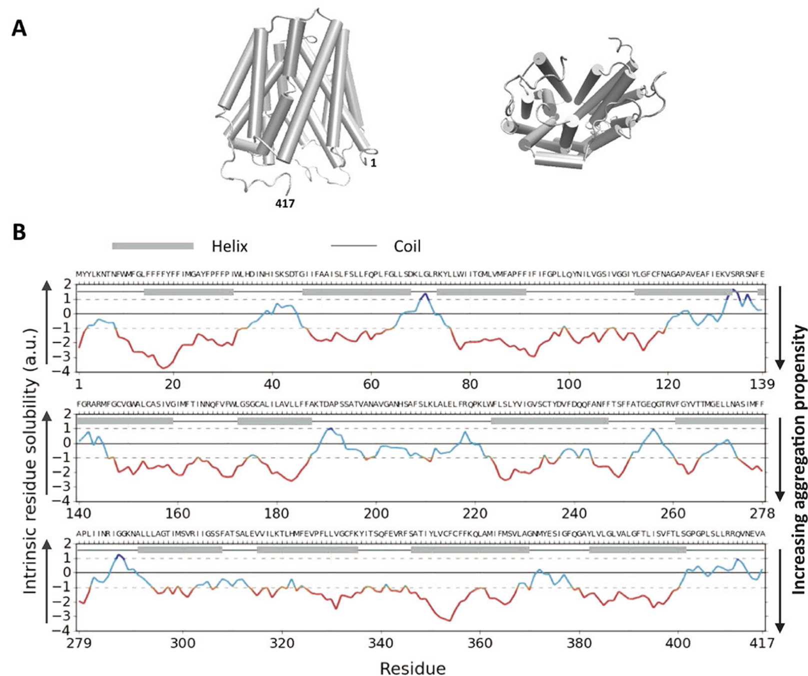

Figure 1.
(A) Structure of the LacY protein with α-helix regions shown as gray cylinders. (B) CamSol profile of the LacY protein, with regions in red indicating low solubility. Reproduced with permission (CC-BY) from Stroobants *et al.* (2017) Amyloid-like Fibrils from an a-Helical Transmembrane Protein, *Biochemistry 56,* 3225–3233.

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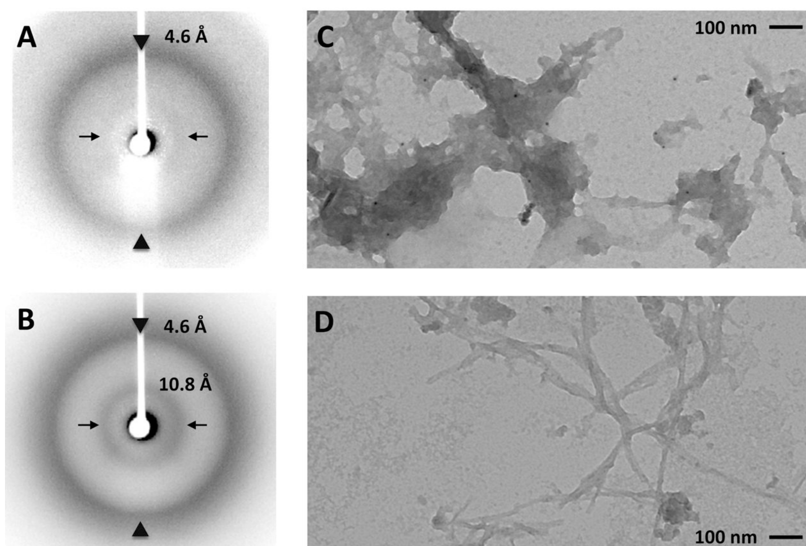

Figure 2.

X-ray fiber diffraction data of the LacY aggregates after (A) incubation in buffer and (B) after treatment with guanidinium hydrochloride. TEM data of the LacY aggregates after (C) incubation in buffer and (D) after treatment with guanidinium hydrochloride. Reproduced with permission (CC-BY) from Stroobants *et al.* (2017) Amyloid-like Fibrils from an a-Helical Transmembrane Protein, *Biochemistry 56*, 3225–3233.