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Protein Aggregates Probed

Inclusion bodies may have more diverse structures than anticipated

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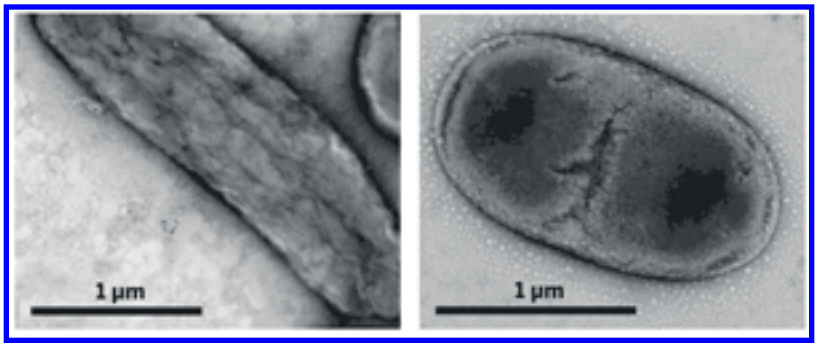
RESEARCHERS AND COMPANIES frequently turn to *Escherichia coli* to provide an ample supply of proteins for experimental or therapeutic use. But proteins overexpressed in bacteria are frequently unusable because they become trapped in large insoluble aggregates known as inclusion bodies. A pair of papers now provides new data on the structure of inclusion bodies, perhaps offering insights that could lead to new ways to extract active protein from the aggregates or prevent their formation entirely.



David Weliky/Michigan State

View Enlarged Image

Aggregates Normal *E. coli* cells (left) can become filled with inclusion bodies (right, large dark spots) when induced to overexpress recombinant protein.

Inclusion bodies form when protein- sequence changes or high local concentrations of protein bolster an aggregation pathway that is in kinetic competition with the correct protein folding pathway, says Ronald Wetzel, a professor of structural biology at the University of Pittsburgh School of Medicine. "This can also happen in vitro in the refolding of a denatured protein. Proteins that have the greatest tendency to make inclusion bodies in cells are therefore often the ones most problematic to refold in vitro," he adds.

Current methods to extract proteins from inclusion bodies typically involve denaturing the aggregates and refolding the proteins, an approach that is inefficient and may not yield active protein in the end. A better understanding of inclusion-body protein structure and of protein-protein interactions could provide new ways to dissolve inclusion bodies and yield active protein, says David P. Weliky, a chemistry professor at Michigan State University. "If we can understand what makes them stick together, maybe we can come up with better ways to solubilize them," he says.

Previous research on dehydrated inclusion bodies using infrared spectroscopy indicated that some proteins enmeshed in inclusion bodies have a greater degree of β-sheet structure than in their native forms. These results led researchers to propose that inclusion bodies form from intermolecular β-sheet interactions. One of the new studies, led by Roland Riek, a chemistry professor at the Swiss Federal Institute of Technology, Zurich, and David Eisenberg, a professor of molecular biology at the University of California, Los Angeles, appears to support that hypothesis.

Riek, Eisenberg, and colleagues studied purified, hydrated inclusion bodies formed separately by three different proteins overexpressed in *E. coli*: early secreted antigen 6-kilodalton protein, secretory human bone morphogenic protein-2, and a portion of the extracellular domain of the human membrane protein myelin oligodendrocyte glycoprotein (*PLoS Biol.* **2008**, *6*, e195). The group studied the aggregates by using several different methods. In each case, they found evidence for intermolecular β-sheet interactions in protein segments that were seven to 10 residues long. When hydrophobic residues in those segments were mutated to arginine, a hydrophilic amino acid, the proteins no longer aggregated into inclusion bodies.

Noting that intermolecular β-sheet interactions are also found in amyloid fibrils formed in

human diseases such as Alzheimer's disease, Eisenberg says that such interactions may be a common motif in protein aggregation. Formation of inclusion bodies could perhaps be prevented, he suggests, by somehow inhibiting β-sheet interactions or by engineering bacteria to make additional protein-folding chaperones to help proteins fold properly and avoid β-sheet interactions.

IN A SEPARATE PAPER, Weliky and coworkers used solid-state nuclear magnetic resonance spectroscopy to detect ^{13}CO groups bonded to ^{15}N in four residues of the protein backbone of flu virus hemagglutinin overexpressed in *E. coli* and aggregated into inclusion bodies (*J. Am. Chem. Soc.* **2008**, *130*, 12568). The group worked with whole cells to eliminate possible artifacts caused by purification. The chemical shifts of the ^{13}CO reveal local protein structure, enabling the researchers to distinguish between α-helices and β-sheets. The team found that the protein appears to retain much of the α-helical structure it adopts in its native form.

A protein such as the flu virus hemagglutinin studied by Weliky's group could adopt nativelike α-helix conformations over much of its sequence yet still have a short segment of residues involved in the intermolecular β-sheet interactions that Riek, Eisenberg, and colleagues postulate are critical for inclusion body formation. Therefore, the results of the two papers could be complementary, Wetzel says.

Robert Tycko, chief of solid-state NMR and biomolecular physics in the Laboratory of Chemical Physics at the National Institute of Diabetes & Digestive & Kidney Diseases, adds, "It may turn out that inclusion bodies have a diversity of structure, possibly depending on the stability and secondary structure content of the protein in its nonaggregated state."

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