New and Notable

19 Å Solution Structure of the Filarial Nematode Immunomodulatory Protein, ES-62

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In the January issue of the *Biophysical Journal* Claire Ackerman, Margaret Harnett, William Harnett, Sharon Kelly, Dmitri Svergun, and Olwyn Byron published the solution structure of a parasite protein to 19 Å resolution. ES-62 is a glycoprotein, post-translationally modified by the addition of phosphorylcholine to glycan groups. It is secreted by filarial nematode worms (ES stands for excreted-secreted) and is believed to contribute to the survival of the parasite by modulating the immune system of the vertebrate host. In fact, phosphorylcholine has well-documented hapten activity. A hapten is a small molecular group that is incapable of eliciting antibody response but which can be immunologically active when associated to a protein. There was no structural information on ES-62 when the authors undertook the study. They combined analytical ultracentrifugation, circular dichroism, and small-angle x-ray scattering, with sequence analysis to establish that the protein is a tetramer and to propose a low-resolution structural model.

Hydrodynamics provided the methods that laid the foundation of biophysics early in the twentieth century. Svedberg, in the 1920s, built the first analytical centrifuge and proved that proteins were macromolecules of well-defined dimensions and molar mass. The fundamental advances in structural molecular biology achieved with sedimentation analysis include the discovery of ribosomes (ribosomes and ribosomal subunits are named according to their sedimentation coefficients, 70S, 30S, 50S, etc., where *S* is the Svedberg unit), of changes in the dimensions of hemoglobin upon oxygenation, and the proof for the semiconservative mechanism of DNA replication (by isotope labeling of strands).

Protein crystals had already been obtained in the 1930s, but it was not until 1957 that the structure of hemoglobin to 5.5 Å resolution was published. At the time, difficulties encountered with protein crystallization and the labor-intensive nature of the crystallographic study itself (this was before powerful computers…) appeared to doom crystallography to providing rare, unique information on the three-dimensional structure of only a few proteins. Biophysicists continued to develop the methods to study structures and interactions in solution at the boundary between thermodynamics and structure, which had already played a crucial role in the pre-double-helix days. Several years before the crystal structure of transfer RNA was solved, for example, J. Ninio deduced its L-shape from small-angle scattering for his 1970 doctorate thesis (Ninio et al., 1972). Then the advent of synchrotron radiation sources coupled to high-speed x-ray detectors and powerful crystallographic computing led to a scientific revolution following the sharp increase in the number of protein structures solved by x-ray crystallography. In parallel, the number of known protein sequences grew at an enormous rate. The first complete sequence of a bacterial genome (of *Mycoplasma genitalium*) was published in 1995. Now, structural genomics is meeting the challenge of matching gene sequences with three-dimensional structures from crystallography and NMR. In the wake of these events, there is a tendency to consider solution methods as old-fashioned—it would appear that it is much more rewarding to invest the effort into crystallizing proteins and even large complexes such as the ribosome to obtain the chemical information inherent in their high resolution structures. But a protein crystal structure provides a starting point to the understanding of structure-function relationships and is not an end in itself. X-ray, neutron scattering, and analytical centrifugation solution methods were again recruited to address questions arising after a high-resolution structure was solved, questions such as: does it undergo oligomerization or conformational changes under different environmental conditions or when it interacts with its functional partners? Zaccai and Xian (1988), for example, have shown, by comparing small-angle neutron scattering data with crystal structure calculations, that the L-shaped structure of transfer RNA could ''open up'' in certain environments, establishing its conformational flexibility. More recently, the density of the hydration shell around three proteins was measured experimentally to be significantly higher than that of bulk water, by combining x-ray and neutron small-angle scattering with calculations based on the crystal structures (Svergun et al., 1998). Characterizing of the physical properties of protein surface hydration is essential for understanding protein folding, and the experimental work of Svergun et al. provided the basis for a molecular dynamics simulation to explain the origin of the increased density (Merzel and Smith, 2002). In the last decade, new approaches have been proposed to meet the challenge of relating hydrodynamics parameters and macromolecular structure, which was first tackled in the 1930s in terms of simple geometrical shapes such as ellipsoids of revolution. The program HYDROPRO (Garcia de la Torre et al., 2000) was initially intended for the calculation of hydrodynamics parameters

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from atomic resolution structures of macromolecules. It was used to calculate the sedimentation coefficient of the low resolution structure of ES-62 proposed from the scattering experiments, to compare with the experimental value. The result not only confirmed the structure of the protein but also provided an accurate estimate of its degree of hydration.

The article of Claire Ackerman et al. reminds us that there are very important families of proteins that do not form crystals easily and are too large for solution state NMR study; these include glycoproteins and membrane proteins, which together represent a large fraction in a genome. Scattering and hydrodynamics methods to study structures in solution, therefore, are regaining great topical interest. The article provides a beautiful description of the complementarity between different state-of-the-art solution methods (circular dichroism to study secondary structure; analytical centrifugation; and x-ray small-angle scattering for tertiary and quaternary structure analysis), the highly sophisticated modeling approaches that have been developed to make best use of the different data (such as the HYDRPRO program, for example) and bioinformatics-based sequence analysis (to analyze domain homology with other proteins showing similar enzymatic activity). Nematode-associated diseases affect hundreds of millions in the world, and at present there is no hope for a vaccine. As the authors have written, the low-resolution structure of ES-62 is a first step in the structural study of this protein. The structure can be seen as providing a scaffold around which further studies will build. Biochemical methods such as cross-linking will be combined with biophysical approaches in the quest to understand the multifunctional properties of this protein and its interactions with the host immune system.

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