

Cinderella will go to the ball

Analytical ultracentrifugation has revived as a tool for protein characterisation

Analytical ultracentrifugation has undergone an extraordinary evolution since its inception by T. Svedberg and co-workers in the 1920s.

After many years of decline, it is now rapidly re-emerging as a powerful tool for the characterisation of the size, solution conformation (in dilute solution), and, particularly, interaction properties of protein systems and other classes of biological macromolecule.

The renaissance has had two clear effects. The first was the growth of the National Centre for Macromolecular Hydrodynamics (with laboratories at the Universities of Nottingham and Leicester),

supported by the Science & Engineering Research Council and industry in response to increasing demands for collaborative work (a similar facility has been set up in the United States with the National Ultracentrifuge Facility at Storrs). The second was the launch last year of the new Optima XLA Analytical Ultracentrifuge from Beckman Instruments, with full on-line computer control and analysis facilities making the technique available for the first time to the lay bimolecular and polymer scientist without the need for extensive specialist training.

For conformational analysis (using sedimentation velocity in the ultracentrifuge), this

revival is probably the result of a realisation amongst biological scientists that:

- not all biological macromolecules can be crystallised — many are available in too small a quantity (especially newly-engineered proteins) or are simply not amenable to full structural analysis (for example, intact, immunologically-active antibody molecules);
- the requirement of very high concentrations for NMR analysis of biological macromolecules — particularly those with a molecular weight greater than 10,000 — can lead to serious difficulties in data interpretation.

Solution techniques, such as sedimentation analysis or X-ray scattering, although of low resolution, may represent for many systems the only realistic "handle" on macromolecular conformation in solution. It is possible to address:

- gross conformation types (whether the macromolecule is first of all behaving as a randomly-coiled structure, a rigid-rod structure or a globular structure); and,
- by combining sedimentation data with data from other solution techniques, such as X-ray scattering or viscometry, more detailed information, such as the degree of flexibility (if it is a coiled or rod shape) or a representation using "bead models"

if it is more globular.

For molecular weight analysis (using sedimentation equilibrium), the revival in the technique has derived largely from an increasing — sometimes painful — awareness of the limitations of gel electrophoresis and gel chromatography methods. Both of these methods, although relatively straightforward, have limitations for many systems. Problems may arise from non-uniform binding of SDS in the SDS-PAGE analysis of some proteins (for example histone, glycoproteins) or more than just the polypeptide chain molecular weight may be desired.

With gel chromatography (including HPLC), difficulties may occur in obtaining suitable calibration standards and also in terms of interaction phenomena with the material of the gel column. Sedimentation equilibrium is an absolute molecular weight technique, not requiring calibration standards or assumptions concerning conformation. It is superior to other absolute techniques in that, unlike mass spectrometry, it gives the molecular weight of systems containing more than one subunit and also the molecular weights of very large single polypeptides (found in, for example, some muscle proteins, such as "titin" (molecular weight greater than 10⁶) and it does not suffer from problems of dust or any other supramolecular contaminations which plague light-scattering measurements.

For interaction studies, sedimentation velocity can be used as a quick, easy-to-use assay for interaction in systems containing different types of molecule (for example an enzyme with its cofactor (Figure 1)) or for the evaluation using sedimentation equilibrium of the association constants in a self-associating system.

For purity assays, the sedimentation velocity technique and the technique of isopycnic density gradient equilibrium are ideal for looking at the

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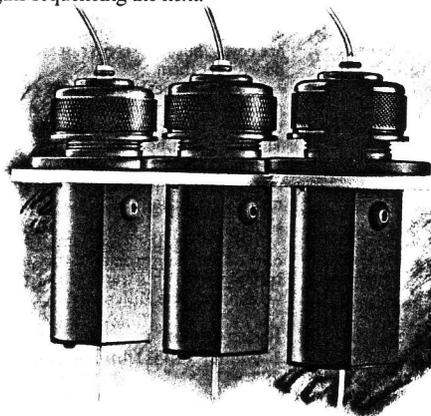
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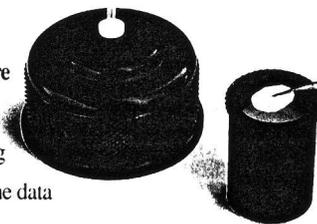
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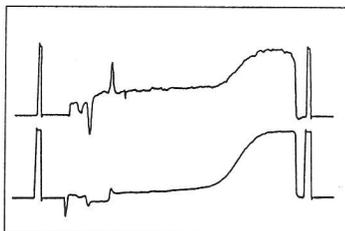
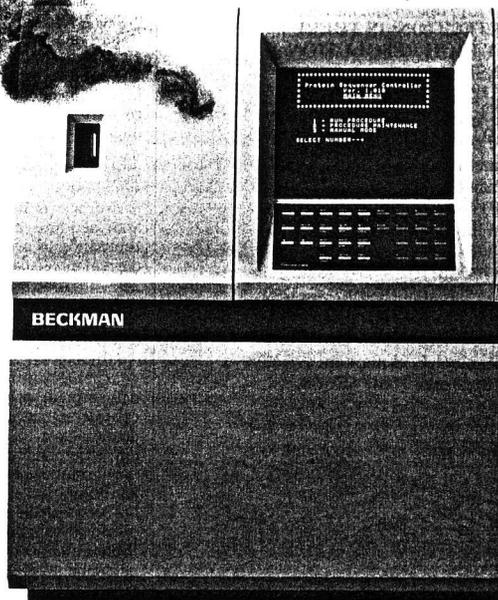


Figure 1: Co-sedimentation diagram for the enzyme mutase (bottom scan across the ultracentrifuge cell at a wavelength of 295nm) and its B12 cofactor (top scan, 608nm and taken 2 minutes after the bottom scan). The centre of the sedimentation boundaries for both are identical, with no residual absorbance left behind, indicating complete binding of the cofactor. Rotor speed was 44,000rpm.

purity of bimolecular preparations (for example for glycoprotein systems, checking for any contamination through free protein or nucleic acid).

Apart from work on newly-engineered protein molecules — especially those of immunological importance — we find that a large part of interest in the technique is coming from academic and industrial groups interested in pharmaceutical applications, such as the behaviour of biopolymer drug-delivery systems.

For most work, we feel that biochemists and molecular biologists should be able to provide the answers themselves using the new Optima XLA Ultracentrifuge, which doubles up with a preparative capability; for more specialist problems (such as those involving heterogeneous systems), the facility offered by the National Centre for Macromolecular Hydrodynamics should be able to help. The current capability of the technique, including a comprehensive description of the new XLA, has been described².

References

1. Morgan, P., Byron, I. and Harding, S. (1992) "The solution conformation of novel antibody fragments studied using the Optima XLA Analytical Ultracentrifuge", *Discovery (Beckman Instruments)* 1, 2-4
2. Harding, S., Rowe, A. and Horton, J. (eds) (1992) "Analytical Ultracentrifugation in Biochemistry and Polymer Science" (630 pages) Royal Society of Chemistry, Cambridge (ISBN 085186-345-0)

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NEW PRODUCTS START ON PAGE 23