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PREFACE

Olwyn Byron · Stephen E. Harding

New developments in analytical ultracentrifugation and related macromolecular modelling techniques

On March 25th and 26th 1996, the fourth United Kingdom Analytical Ultracentrifuge Users' Group Meeting was held at the University of Leicester and hosted by the National Centre for Macromolecular Hydrodynamics. The focus areas of the meeting were twofold. One comprised new developments in terms of instrumentation (the launch of the new XL-I analytical ultracentrifuge) and analysis procedures (molecular mass algorithms and dilute solution conformation algorithms). The other comprised specific applications to biological macromolecules of both biomedical and commercial importance. From this meeting a number of articles were invited and the following collection of refereed papers is the result. These contributions testify to the expansion of popularity and potential of the analytical ultracentrifuge in modern biomolecular science.

Introduction

Seven years on from an article by H. K. Schachman (1989) in which he announced the renaissance of the analytical ultracentrifuge, the technique has now been firmly re-established as a powerful tool for the analysis of biomolecular systems in terms of molecular mass, molecular mass distribution (polydispersity), mass action phenomena (interactions) and solution conformation. Since this time, a plethora of scientific papers has been published, together with two books (Harding et al. 1992; Schuster and Laue 1994).

To keep pace with these developments, in Europe, an annual Users' Group meeting is held alternately in the United Kingdom and the Federal Republic of Germany. The main focus of the United Kingdom symposia has been in developments and applications to biomolecular systems, whereas the German symposia have considered also colloidal and synthetic polymer systems. For several years special editions of *Progress in Colloid and Polymer Science* have been published based on the German symposia, and now for the first time a collection of invited papers based on the meeting hosted by the United Kingdom is being published in the *European Biophysics Journal*. The papers are arranged in the order: instrumentation; analysis; and specific applications to biological macromolecules.

O. Byron (✉)
National Centre for Macromolecular Hydrodynamics,
Department of Biochemistry, University of Leicester,
University Road, Leicester LE1 7RH, UK

S. E. Harding
National Centre for Macromolecular Hydrodynamics,
Department of Applied Biochemistry and Food Science,
University of Nottingham, Sutton Bonington LE12 5RD, UK

Developments in instrumentation

It was particularly pleasing to be able to use the 4th UK Meeting to host the European launch of the most recent modern analytical ultracentrifuge – the Beckman Optima XL-I, which has integrated Rayleigh interference and absorption optics. This is described in the paper by Furst. The addition of the Rayleigh interference system permits more sensitive optical detection of concentration distributions in the ultracentrifuge cell, and without the requirement of a chromophore: the ability to make absorption and interference optical records should be particularly advantageous for the analysis of interactions in mixed solute systems. For very concentrated systems – necessary, for example for the study of interacting systems of molecules where the affinity is very low, the schlieren optical system, still available only on older analytical ultracentrifuges, is the only solution. A modern data acquisition system for such an instrument is the subject of the paper by Clewlow et al.

Developments in mass and conformation analysis

A series of papers describes developments in the analysis and interpretation of ultracentrifuge data. A paper by

Behlke provides a general overview followed by a paper by Behlke and Ristau which considers specifically the analysis of interacting biopolymer systems. The analysis of molecular mass is then considered, and Colfen and Harding describe the use of two fundamental programmes, MSTAR-A and MSTAR-I, which handle absorption and interference sedimentation equilibrium ASCII data that are produced, for example, from the XL-A and XL-I ultracentrifuge. These programmes are used to obtain weight, z-average and point average molecular weight information. The output data can then be easily transported to more specific analysis programmes (self-association, polydisperse distribution etc.) where appropriate. Three further software papers then follow. Harding et al. describe the ELLIPS suite of PC algorithms for both simple ellipsoid of revolution (ELLIPS1) and more advanced triaxial ellipsoid (ELLIPS2–4) representations of biomolecular conformation in solution using both hydration dependent and independent "Universal" shape functions. For molecules and biomolecular assemblies with more complicated shapes, modelling based on the hydrodynamic bead approximation is more appropriate, and Garcia de la Torre et al. describe a PC routine SOLPRO which also uses hydration dependent and independent Universal shape functions. It is now possible to model molecular shape without the necessity also to consider size and potential ambiguities therein. Another PC bead modelling algorithm BEAMS is also described by Spotorno et al. which uses the ancillary programmes PROMOLP and GRUMB for helping the user with both the choice of number and radius of beads and in the interactive construction of spatially pre-defined models. ELLIPS, SOLPRO and BEAMS now provide a complementary set of conformation algorithms for fairly rigid biological macromolecules in solution. In the final analysis paper, Pavlov considers more general macromolecular shapes as represented by polysaccharides, a class of biomolecule with a huge range of possible structural architectures, in terms of the concentration dependence of the sedimentation coefficient, and describes the importance of using the concentration dependence term k_s (ml/g) in combination with the sedimentation coefficient for the analysis of the equilibrium rigidity of extra-rigid, rigid, semi-flexible and flexible chain polysaccharides.

Applications to biomolecular systems

The largest section in this issue, happily, is devoted to reports on the successful use of the analytical ultracentrifuge and associated techniques in the study of biomolecular systems, and we have attempted to order these from studies on small interacting peptides, through to proteins, large protein assemblies, proteins and glycoproteins involved in the immune response and finally the structure of large mucin glycoproteins.

Two papers on peptides are included. One, described by Liu et al. concerns an HIV-enhancer-binding peptide which dimerises in the presence of a leucine zipper; the other, described by Thomas et al concerns a trimerising alpha-helical coiled-coil phenylalanine zipper-type peptide. Colfen et al. then describe the heterodimeric properties of the electron transferring flavoprotein (ETF), and the paper by Dean et al. describes how a point mutation (Asp165His), which is distant from the subunit interface, changes the homohexameric wild-type glutamate dehydrogenase into a dimeric state. The theme of association and oligomeric structure is continued with papers by Byron et al. on DT-diaphorase, confirming the predominance of dimer and identifying the presence of tetramer; Flood et al. on the self-association of α-actinin fragments; Aerts et al. on the effect of increasing concentrations of amphiphilic and neutral detergent on the quaternary structure of the lens protein α-crystallin; and Varley et al. on large molecular weight association products of the macrophage inflammatory protein-1α (MIP-1α) and a biologically active mutant thereof.

The proteins and glycoproteins involved in the immune response are the subject of papers by Silkowski et al., who consider weak interactions between the cell adhesion molecules CD2 and CD48 and obtain results which complement those from surface plasmon resonance, and Beavil and Beavil, who consider the conformation of a complex between a human IgE fragment and the IgE high affinity receptor Fcε RI-α. Keown et al. then consider the factors underpinning the stoichiometry of this interaction and exclude the possibility that steric hindrance by Cε2 accounts for the unexpected 1:1 stoichiometry. The theme of glycoproteins is considered by Jumel et al., who derive a linear random coil model for the large colonic mucin glycoproteins, based on sedimentation analysis in conjunction with size-exclusion chromatography coupled to laser light scattering for molecular weight distribution analysis. Emanating from the famous Uppsala laboratories of Svedberg and Tiselius, the techniques of ultracentrifugation and electrophoresis have been inextricably linked, particularly as complementary tools for the study of macro-ion diffusion phenomena, and the final article by Shepard et al. considers very recent developments in this area.

Finally the editors of this special issue of the European Biophysical Journal are very grateful to the Managing Editor, Dr. Peter Bayley, for his enthusiasm, help and patience in bringing this collection of papers to press.

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