

Analytical centrifuge facility

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Introduction

The Centre has two Beckman XL-I analytical ultracentrifuges installed in the Wellcome Trust JIF Centre for Biomolecular Interactions. Both instruments are equipped with absorbance and interference optics, 4-place, and 8-place rotors, and velocity and equilibrium cells with a choice of quartz or sapphire windows. We employ a range of data analysis methods, enabling the determination of properties of macromolecules in free solution including species distribution, mass, degree of asymmetry and association constants of interacting species.

Work carried out in 2004

The facility was used by a number of researchers groups, some making extensive AUC studies while others did simple experiments to determine the properties in free solution of their material. The results from one of these latter experiments is shown as an example.

Sedimentation velocity analysis of a GFP fusion protein

This work was part of a postgraduate research programme into directed evolution of aldolases being carried out by Chris Plummer in the laboratory of Dr Alan Berry. In order to select soluble mutants of GatY, an insoluble tagatose bisphosphate aldolase, fusions with green fluorescent protein (GFP) were used. The GFP serves as a visual screen for soluble mutants. Insoluble mutants would be cleared into inclusion bodies where GFP would not have time to fold and so be non-fluorescent. The soundness of this strategy was tested by constructing a GFP fusion with a soluble aldolase of the same class, fructose bisphosphate aldolase (FBP aldolase). Sedimentation velocity analysis was performed on this construct to check that the oligomeric state of the aldolase-GFP fusion was the same as the aldolase alone - in this case a dimer.

Three samples of the protein at 0.1, 0.25 and 0.5 mg/ml were centrifuged at 35000 rpm for 5 hours. The changing distribution of protein was recorded by Rayleigh interference optics and radial absorbance scans at 390nm - the absorbance peak of the GFP used. The whole boundary profiles were used to calculate the distribution of sedimentation coefficients [$c(s)$] by fitting to the Lamm equation using the program Sedfit v 8.9 (P. Schuck, NIH).

In all 6 datasets there was one major component having a sedimentation coefficient lying in the range 6.9 to 7.4 Svedbergs, the value being inversely correlated with concentration, as expected. The area underneath these peaks is proportional to the concentration of the component (Fig 1.)

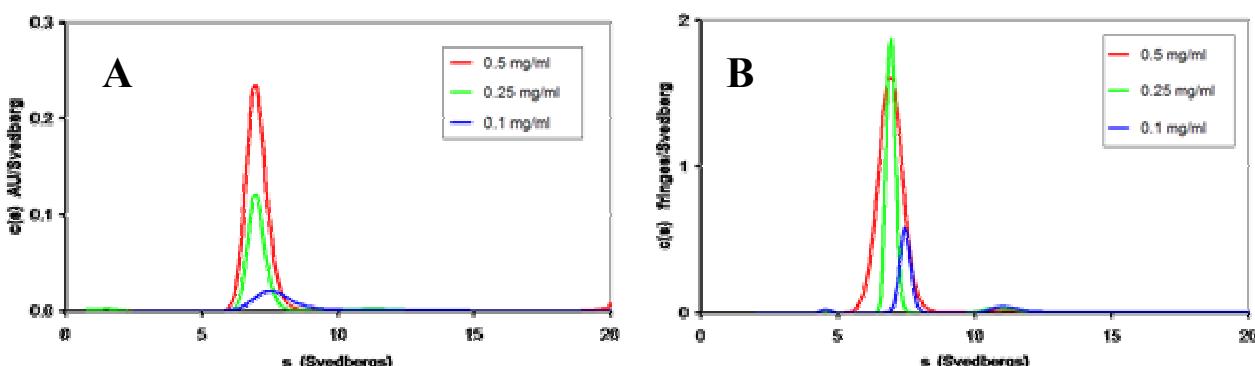


Fig 1. $c(s)$ analyses

A: Absorbance data, B: Interference data

Comparison of the sedimentation coefficient measured with that of a perfect sphere having the same mass as the protein indicated that a 7.1 S solute must be at least as large as the dimer (135 kDa), with a frictional ratio of 1.3. There were small amounts of material sedimenting faster than 7 S, but none smaller. In c(s) analyses of the interference data there is a peak at around 11 S which is absent in analyses of the A₃₉₀ data. This is probably a non-GFP contaminant, or possibly unfolded fusion protein; the interference system measures concentration changes of total solute, whereas the absorbance system is selective.

This experiment showed that the natural oligomeric state of the control protein was maintained when fused with GFP. Therefore it is highly likely that the GFP fusions with GatY soluble mutants will also exist in the same oligomeric state as the unfused protein - in this case tetramer.

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