45 Monomeric behaviour of *Mytilus edulis* (mussel) glue protein in dilute solution

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The mussel adhesive protein Mefp-1 [1] has been isolated and studied with regards its molecular weight and state of oligomerisation. The molecular weight of the monomer species from sequence analysis is 102000 Da which is in good agreement with the result reported from MALDI mass spectrometry of 110000 Da. In this study, sedimentation equilibrium in the analytical ultracentrifuge in dilute solution of pH 4.5 and I=0.10M, at a loading protein concentration of 0.4 mg/ml yielded an apparent molecular weight (whole distribution weight average, $M_{w,app}$) of (120000±10000) Da via the "M*" procedure [2]. This, together with plots of point weight average apparent molar weight [3] versus concentration demonstrate that this protein is essentially monomeric in dilute solution.

The protein Mefp-1 is one of the major adhesive proteins used by marine mussels to bind strongly to underwater surfaces. This has been related to its strong surface active and adsorptive behaviour [4-6]. This and related mussel adhesive proteins are characterised by having high lysine contents and hydroxylated amino acids: Mefp-1 for example consists of tandemly repeated decapeptides each containing two residues of lysine, 1-2 residues of Dopa [7,8], 1-2 residues of *trans*-4-hydroxyproline and 1 residue of *trans*-2,3,*cis*-3,4-dihydroxyproline [9]. These strong adhesive properties have recently inspired a proposed use for these proteins as mucoadhesives for drug delivery [10]. Little is known however of the properties of these molecules in solution. The purpose of the present study is thus to help address this by performing on dilute solutions the technique of low speed sedimentation equilibrium experiments in the analytical ultracentrifuge. All solution measurements were performed in a acetate buffer, pH 4.6 and I=0.10M. An Optima XL-A ultracentrifuge (Beckman Instruments, Palo Alto, USA) was employed.

Sedimentation equilibrium was employed at a rotor speed of 14000 rev/min, temperature of 20.0°C, and 12mm optical path length cells. A low loading concentrations of 0.4mg/ml was employed to minimise the effects of thermodynamic non-ideality. A partial specific volume of 0.7435 ml/g was calculated from the amino acid sequence.

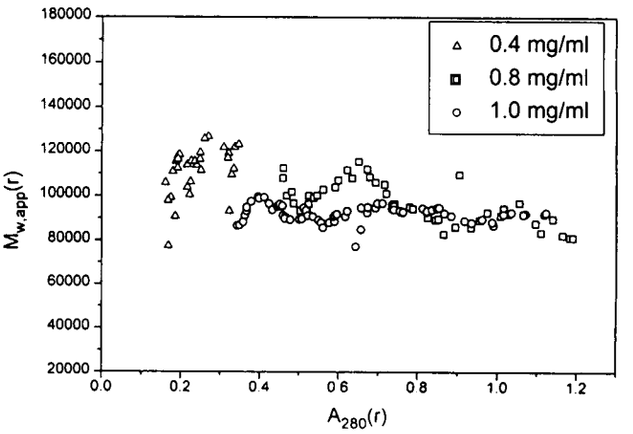
Equilibrium solute distributions were captured as an ASCII data set of concentration (expressed as ultra-violet absorbance at a wavelength of 278nm) versus radial displacement from the rotor centre, r (cm) and then analysed using the molar mass routine MSTARA recently adapted for PC [11].

M* analysis: monomeric behaviour: The weight average molecular weight, $M_{w,app}$ was determined from extrapolation of the "M*" function to the cell base. Since the loading concentration is low, thermodynamic non-ideality effects can be reasonably neglected and hence $M_{w,app} \approx$ the "ideal" weight average molecular weight M_w. Using this procedure, M_w = (120000±10000) Da confirming the view of essentially monomers.

Point average molecular weight analysis: monomeric behaviour. This view is strengthened when we consider plots of point apparent weight average molecular weight, $M_{w,app}$ (r) as a function of local concentration (expressed as absorbance units A(r) at radial positions r from the rotor centre). Fig. 1 shows clearly that for a loading concentration of 0.4 mg/ml there is no evidence of associative behaviour. Fig. 1 also shows corresponding plots obtained at loading concentrations of 0.8mg/ml and 1.0mg/ml, again showing no evidence for an association.

The mussel protein Mefp-1 clearly remains as monomers in these solution conditions (pH 4.6, I=0.10M). In further work, knowledge of this feature will be important in the assay of stoichiometries and strengths of interactions with mucus glycoproteins for understanding the mucoadhesive potential of these molecules [10] and for further hydrodynamic study on conformation in solution.

Fig. 1. Mefp-1: Point average molecular weight plot. Plot of point weight average molecular weight versus local concentration (expressed as absorbance units at 280nm) at various radial positions r in the ultracentrifuge cell for different loading concentrations (0.4, 0.8, 1.0 mg/ml).



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