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SELF ASSOCIATION, POLYDISPERSITY AND THERMODYNAMIC NON-IDEALITY IN A CYSTIC FIBROTIC GLYCOPROTEIN

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Rayleigh sedimentation equilibrium patterns of mucus glycoproteins are rather difficult to interpret quantitatively: the substances are polydisperse in both molecular weight and partial specific volume ($\bar{v}$) owing to a quasi-continuous distribution of oligosaccharide chain length. Many are also polydisperse with respect to disulphide cross-linked aggregation. In addition, many give Rayleigh equilibrium patterns with very high positive slopes at the cell base – this is suggestive of self association despite the fact that they are extremely non-ideal thermodynamically (arising from a very high water affinity). We report here a study of a glycoprotein CFPHI from the bronchial secretion of a cystic fibrotic.

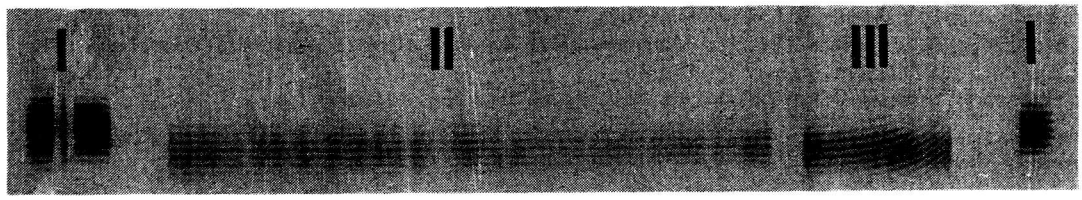

Figure 1 : Rayleigh equilibrium pattern for the glycoprotein CFPHI from the bronchial secretion of a cystic fibrotic. c_0 ~ 0.2 mg/ml, equilibrium speed 2231 rpm. I: reference fringes, II: air fringes, III: solution fringes.

Materials and methods: Freeze dried glycoprotein from cystic fibrosis patient CFPHI (code no., see ref. 1) was dissolved in a 1M NaCl solvent buffered to pH = 6.8 with phosphate. Point average molecular weights were determined from 3 mm solution columns using a low speed sedimentation equilibrium method analogous to that of Teller *et al.* (2). Equilibrium patterns were recorded using Rayleigh optics. Apparent molecular weights (for determination of the second virial coefficient B) were determined using the technique of ultrashort columns (see for example, ref. 3) and interpreted following method I of van Holde and Baldwin (3).

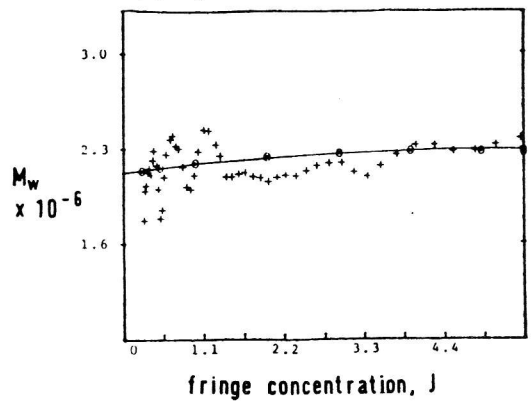

Figure 2 : Plot of observed (+) and simulated (⊙—⊙) point average molecular weight values against concentration. The data refer to figure 1; simulation is based on an isodesmic association characterised by B = 1.5 × 10^4 ml . mole/g^2, k = 0.26 dm^3/g, M_1 (monomer molecular weight) = 2.15 × 10^6.

Results and discussion: From figure 1 it appears that the association is relatively weak since even at low loading concentrations (c_0 ~ 0.2 mg/ml) the effect of non-ideality virtually completely counteracts it so that a pseudo-ideal, monodisperse single solute Rayleigh pattern is produced. The number, weight, and z point-average molecular weights (M_n, M_w, M_z) remain remarkably constant (~ 2.1 × 10^6) throughout the whole 3 mm solution column in a low speed experiment. We have shown that it is not a real ideal single solute by: (i) measuring B using ultrashort (~ 0.7 mm) columns (4) (the value obtained, 1.5 ± 0.3 × 10^{-4} ml . mol/g^2, despite being a minimum value because of association is ~ 10 times that for globular proteins); (ii) demonstrating that a 1mm column Rayleigh pattern at c_0 = 2.0 mg/ml in a 12 mm cell shows parallel straight fringes, whereas c_0 = 0.8 mg/ml in a 1mm column of a 30 mm cell (and hence the same fringe concentration as the 12 mm) gives fringes of normal curvature.

To a first approximation, if we assume monomers associate indefinitely with constant free energy increment (i.e., isodesmically (5)), neglect effects of polydispersity and take $B = 1.5 \times 10^{-4}$ ml . mol/g^2, a value for the intrinsic equilibrium constant k (ref. 2) of 0.26 dm^3/g is found to give a good fit to the observed fringe and point average molecular weight data (figure 2). For the static case (i.e., no solute redistribution due to an ultracentrifugal field) this corresponds to ~90 % monomer, 8.5 % dimer and 0.6 % trimer. We have also however demonstrated that polydispersity is significant since M_w (c) *vs* c plots for different c_o do not overlap (6) and we have determined the extent of polydispersity in $\bar{v}$ and are currently investigating by simulation the contributions of this and other polydispersity to the observed Rayleigh patterns. Finally, the acquisition of a more accurate value for B may now be possible by combining M_{yl} (6) with M_w data. A new function M* for determining M_n (and hence M_{yl}) data more accurately will be used.

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