SPEED–DEPENDENT SEDIMENTATION VELOCITY
OF HUMAN CERVICAL MUCINS IN THE ANALYTICAL ULTRACENTRIFUGE

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INTRODUCTION

Highly purified human cervical mucins were prepared by isopycnic density-gradient centrifugation (Carlstedt et al., 1981). The macromolecules elute in the void volume of a Sepharose CL 2B column and five separate preparations gave molecular weights between 4.8 and 6.5 x 10^6 by light-scattering in 6M guanidine hydrochloride (GuHCl). To study the size heterogeneity and polydispersity, sedimentation-velocity experiments were carried out in the analytical ultracentrifuge. In the course of this work new observations were made on the sedimentation behaviour of large mucin molecules.

EXPERIMENTAL

Sedimentation-velocity experiments were carried out in an MSE Centriscan 75 analytical ultracentrifuge. Approximately 350 ul of sample were used in cells (1 cm path length) fitted with quartz windows. Samples (0.8–1.1 mg/ml) were extensively dialysed to the appropriate starting conditions. The sedimenting boundary was monitored by schlieren optics with a knife edge angle of 75°. Experiments were performed at 20°C in 0.2M NaCl, 4M, 6M and 8M GuHCl.

RESULTS

A typical sedimentation experiment in 4M GuHCl carried out at a rotor speed of 30k rpm is shown in Fig.1a. The material sedimented as a single peak with an apparent sedimentation rate of 13.3 S. Three features of the experimental result arouse suspicion (i) boundary broadening occurred to such a degree that the boundary could not be monitored for more than 80–90 minutes, (ii) a perturbed baseline
Fig. 1. Sedimentation-velocity patterns obtained with human cervical mucins at (a) 30k rpm (scans every 8 minutes) and (b) at 10k rpm (scans every 90 minutes). Sedimentation is from left to right. Samples (approximately 1 mg/ml) were extensively dialysed against 4M GuHCl. Schlieren optics at 550 nm was used.