Hydrodynamic properties of human cervical-mucus glycoproteins in 6M-guanidinium chloride

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Cervical mucins and fragments thereof were studied by sedimentation-velocity, rotatory viscometry and laser light-scattering performed as photon-correlation spectroscopy as well as low-angle total-intensity measurements. The M_r of the whole mucins is 10×10^6 – 15×10^6 , whereas fragments obtained after reduction of disulphide bonds ('subunits') have M_r 2.1×10^6 – 2.9×10^6 , depending on the method used. Subsequent trypsin digestion of subunits afforded glycopeptides with M_r approx. 0.4×10^6 . The high frictional ratio for the whole mucins is interpreted as a large degree of expansion. The Stokes radius calculated from the diffusion coefficient is approx. 110 nm for the whole mucins, which is in agreement with that estimated from the radius of gyration (130 nm) by using the concept of the equivalent hydrodynamic sphere. The ratio of the concentration-dependence parameter for the reciprocal sedimentation coefficient (K_s) to the intrinsic viscosity ($[\eta]$) for the whole mucins is 1.42, suggesting that the individual macromolecule occupies a spheroidal domain in solution. The relationship between $[\eta]$ and M_r for whole mucins, subunits and T-domains suggests that they are linear flexible macromolecules behaving as somewhat 'stiff' random coils. This conclusion is supported by the relationships between the sedimentation coefficients, the diffusion coefficients and the M_r . The hydrodynamic behaviour of the mucins is thus close to that expected for coiling macromolecules entrapping a lot of solvent, which is consistent with the postulated polymeric structure.

Cervical mucus plays an important role in the physiology of reproduction. During the ovulatory cycle profound changes occur in the rheology of the mucus gel, affecting, for example, spermatozoal migration. The gel is formed by the mucus glycoproteins (the mucins), and knowledge of their characteristic macromolecular features is a prerequisite for an understanding of how these biopolymers determine and regulate the properties of mucus. No structural differences between mucins isolated from different phases of the ovulatory cycle have been revealed.

The particular features of the mucins, apart from their size, contributing to the formation and properties of the mucus gel are not clearly defined. It has been proposed that entanglement alone of the macromolecules is sufficient to explain the properties of cervical mucus (Lee *et al.*, 1977a,b). Silberberg & Meyer (1982) suggest that, in addition

to entanglement, specific 'lectin-like' regions contribute to the formation of the gel. A study on mucins from cystic-fibrotic sputum has provided evidence for a weak isodesmic association between the macromolecules (Harding & Creeth, 1982).

We have developed a protocol for the isolation of mucins under conditions that should minimize degradation by tissue proteinases (Carlstedt *et al.*, 1983a). A tentative model for their macromolecular architecture has also been proposed (Carlstedt *et al.*, 1983b). The mucins were described as linear flexible macromolecules with M_r approx. 10×10^6 containing two characteristic structural regions, namely rod-like segments, which are heavily substituted by oligosaccharides, interspersed with more flexible, non-glycosylated stretches of the protein core. In the present work the hydrodynamic properties of the mucins were studied. The measurements were performed in 6M-guanidinium chloride to avoid a possible tendency for the mucins to self-associate.

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Experimental procedures

Materials

Guanidinium chloride (practical grade) was obtained from Sigma Chemical Co., and stock solutions were treated with charcoal before use. Colloidal silica (Ludox HS-40) was a gift from Dupont Chemical Department. Benzene (AristaR) was bought from BDH Chemicals, and was passed through silica gel before use. Filters (nominal cut-off 1.22 μm and 0.45 μm) were obtained from Millipore.

Mucins and fragments thereof were prepared as described previously (Carlstedt *et al.*, 1983a,b). In summary, human cervical pregnancy mucus was treated with 2mM-di-isopropyl phosphorofluoridate and solubilized in 6M-guanidinium chloride/5mM-N-ethylmaleimide/5mM- Na_2EDTA /10mM-sodium phosphate buffer, pH6.5. After high-speed centrifugation the soluble mucins (approx. 85% of the total) were subjected to isopycnic density-gradient centrifugation in CsCl /4M-guanidinium chloride (initial density 1.39g/ml). This purification step was repeated to ensure complete removal of non-mucin proteins. Nucleic acids were finally removed by isopycnic density-gradient centrifugation in CsCl /0.2M-guanidinium chloride (initial density 1.50g/ml). Purity was assessed by analytical density-gradient centrifugation and by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate.

Reductive fragments of the mucins ('subunits') were prepared by isopycnic density-gradient centrifugation in CsCl /4M-guanidinium chloride (initial density 1.39g/ml) after reduction in 6M-guanidinium chloride/10mM-dithiothreitol/0.1M-Tris/HCl buffer, pH8.0, for 5h at 37°C followed by alkylation with 2.5-fold molar excess of iodoacetamide over dithiothreitol in the dark overnight.

Glycopeptides (T-domains) were prepared by trypsin digestion of subunits in 0.1M-Tris/HCl buffer, pH8.0, at 37°C overnight, and were isolated by isopycnic density-gradient centrifugation as described for the subunits.

Light-scattering

A Malvern Molecular Analyser System 4300 (Precision Devices and Systems, Malvern, Worcs., U.K.) equipped with a 5mW He/Ne laser (Coherent Radiation, Cambridge, U.K.) was used for both quasi-elastic and total-intensity measurements. The photon-count autocorrelation function of the scattered light was obtained with a 48-channel K7023 correlator, and data were analysed, on-line, with an MPD 70235 minicomputer. The correlation function $[g^2(\tau)]$ is related to the translational diffusion constant (D_T) by $g^2(\tau) = 1 + A \cdot \exp(-2D_T\mu^2)$, where μ is the

scattering vector $(4\pi n_0/\lambda_0) \cdot \sin \theta/2$. D_T is calculated from the slope of the plot of $\ln[g^2(\tau)]$ versus τ . For a polydisperse sample the correlation function is a sum of exponential terms and the slope becomes non-linear. The $\ln[g^2(\tau)]$ -versus- τ function was fitted to a second-order polynomial by least-squares analysis. The diffusion coefficients were calculated from a forced linear fit, as well as from the linear term of the quadratic fit, and are referred to below as $(D_T)_1$ and $(D_T)_2$ respectively. The degree of polydispersity was expressed as $Q = \mu_2/\Gamma_2^2$ (Pusey, 1974), where μ_2 is the quadratic term and Γ_2 is the linear term of the quadratic fit. Readings were taken at several angles and extrapolated to $\theta = 0^\circ$, when a pronounced angular dependence was present. All measurements were performed at 25°C. The relative viscosity of 6M-guanidinium chloride/1mM- Na_2EDTA /1mM-sodium phosphate buffer at 20°C compared with 25°C was determined by capillary viscometry to be 1.085, and was used to correct the diffusion coefficients to 20°C.

Total-intensity measurements were performed with the photomultiplier connected to a photon-count rate-meter. For vertically polarized incident light and unpolarized scattered light $\Delta R(\theta) = [\Delta I(\theta)/I_B(90)]R_B \cdot \sin \theta$, where $\Delta I(\theta)$ is the excess scattering of the solution over solvent at angle θ , $I_B(90)$ is the intensity of light scattered from benzene at 90° and R_B is the Rayleigh ratio for benzene. The optical constant (K) was defined as $K = 4\pi^2 n_B^2 \cdot (\partial n/\partial c)^2 / \lambda_0^4 N$, where n_B is the refractive index for benzene, $\partial n/\partial c$ is the refractive-index increment, λ_0 is the wavelength of the incident light *in vacuo* and N is Avogadro's number. By this definition the correction factor $(n_B/n_0)^2$ that accounts for the difference in refractive index between benzene and the buffer (n_0) is included in the optical constant. $Kc/\Delta R(\theta)$ is related to M_r and to the radius of gyration (R_G) as described in standard text-books (see, e.g., Tanford, 1961a). Measurements were performed at 25°C and data were plotted in accordance with Zimm (1948).

The refractive index (n_B) of benzene at the laser wavelength (632.8nm) was determined by extrapolation from a plot of $(n - 1)$ versus λ^{-2} obtained by using data from Coumou (1960). A value of 1.495 was found. The R_B for benzene at 632.8nm was determined with aqueous suspensions of colloidal silica (Ludox). Ludox solutions were treated and clarified with the precautions outlined by Utiyama (1972). A calibration constant is given by $C = (3/8\pi)(2.303A/l)/\Delta I(90)$, where $\Delta I(90)$ is the excess scattering at 90° and A is the absorbance for the Ludox solution in a cell of path-length l cm (Jolly & Eisenberg, 1976). The procedure was performed at a number of Ludox concentrations and extrapolated to zero. An R_B value for

benzene of $12.5 \times 10^{-6} \text{ cm}^{-1}$ was finally obtained from the relation $R_B = CI_B(90)$, where $I_B(90)$ is the intensity of light scattered by benzene at 90° . This value was used to calibrate the instrument for all further measurements.

Solutions for light-scattering (photon-correlation as well as total-intensity measurements) were clarified by centrifugation (20000*g* for 1–2 h at 20°C ; $10 \times 10 \text{ ml}$ angle rotor, MSE 50 centrifuge). Solutions of mucin fragments were, in addition, filtered ($1.22 \mu\text{m}$ for subunits; $0.45 \mu\text{m}$ for T-domains). Glass cells (sample volumes 200–500 μl) provided by Malvern were used for measurements down to 15° .

The refractive-index increment ($\partial n/\partial c$) at constant chemical potential of diffusible components (Casassa & Eisenberg, 1964) was determined with a Waters R401 differential refractometer as described previously (Carlstedt *et al.*, 1983b).

Ultracentrifugation

Sedimentation-velocity experiments were performed with an MSE 75 analytical ultracentrifuge (Centriscan) with the schlieren optics. Samples were dialysed against 6M-guanidinium chloride/1 mM- Na_2EDTA /1 mM-sodium phosphate buffer, pH 6.5. The apparent sedimentation coefficients were determined from the peak positions and $1/s_{\text{app}}$ was extrapolated to zero concentration.

Viscosity

Viscosity was measured with a Couette-type viscometer similar to that described by Ogston & Stanier (1953) but modified to the cone-and-plate principle (Mooney & Ewart, 1934). The conical bob made of stainless-steel was suspended on a CuPd wire, and the deflexion (ϕ) of light reflected from a mirror on the top of the bob was measured with a torsion head to return the beam of light to the zero position. The relative viscosity ($\eta_{\text{rel.}}$) defined as the ratio of the viscosity of sample over solvent is obtained as ϕ_s/ϕ_0 , where ϕ_s and ϕ_0 are the deflexions of light in arbitrary units for sample and solvent respectively. The pot was filled with 1.2 ml of sample and was maintained at 17°C . The reduced viscosity $[(\eta_{\text{rel.}} - 1)/c]$ was extrapolated to zero shear rate and the values obtained were extrapolated to zero concentration to determine the intrinsic viscosity.

Determination of concentrations

Concentrations were assessed after the physical measurements by sialic acid determination (Jourdian *et al.*, 1971), with the cognate mucin stock solution as a standard. The concentration of the stock solution was determined gravimetrically and corrected for moisture as described previously (Carlstedt *et al.*, 1983a).

Results and discussion

Total-intensity light-scattering

A Zimm plot of data for the whole mucins is shown in Fig. 1(a). The M_r was 11.1×10^6 and the R_G was 200 nm, in agreement with values reported previously (Carlstedt *et al.*, 1983b). The Malvern instrument permitted measurements at much lower angles than the instrument used previously (Carlstedt *et al.*, 1983a,b), and with the higher wavelength of the incident light used (632.8 nm as compared with 436 nm) a limiting value for $h^2 R_G^2$ (h is the scattering vector as defined above) of 0.55 was reached as compared with 4.55. This should ensure that the limiting slope of the particle

Fig. 1. Zimm plot of light-scattering data (a) and c/R_θ versus concentration (b) for whole mucins

Samples were dialysed into 6M-guanidinium chloride/1 mM- Na_2EDTA /1 mM-sodium phosphate buffer, pH 6.5. Readings were taken at $\theta = 15^\circ, 20^\circ, 25^\circ, 30^\circ, 35^\circ, 40^\circ$ and 60° , and extrapolation to $\theta = 0^\circ$ was performed from 15° to 35° . (a) The following concentrations were used: 0.06, 0.135, 0.30 and 0.715 mg/ml. The optical constant was $9.32 \times 10^{-8} \text{ cm}^2 \cdot \text{g}^{-2}$. (b) Data for c/R_θ were extrapolated to $\theta = 0^\circ$ as described above and plotted against the concentration. Points $\circ$ are obtained from (a) whereas points $\bullet$ are from a separate experiment. Extrapolation to $c = 0$ at $c > 0.5 \text{ mg/ml}$ would give M_r approx. 23×10^6 .

scattering function is reached and thus that correct estimates of M_r and R_G are obtained. An upward curvature is apparent above approx. 0.5 mg/ml for the line c/R_θ (at $\theta = 0^\circ$) versus concentration (Fig. 1b). Extrapolation to zero concentration for data at $c > 0.5$ mg/ml would give M_r approx. 23×10^6 , suggesting a tendency to self-associate.

Photon-correlation spectroscopy

The translational diffusion coefficient (D_T) for the whole mucins showed a pronounced angular dependence (Fig. 2a). This behaviour is characteristic for large molecules and has been described, for example, for cervical mucins (Lee *et al.*, 1977a,b) and for DNA (see, e.g., Schurr, 1977). The apparent diffusion coefficients were extrapolated to zero angle and plotted against the concentration (Fig. 2b). The values of $(D_T)_1$ were always somewhat lower than those of $(D_T)_2$, which is probably explained by a larger contribution from the largest particles to the forced linear fit than to the linear term of the quadratic fit. The difference between $(D_T)_1$ and $(D_T)_2$ was more pronounced at high concentrations, suggesting the presence of a larger fraction of big particles formed by self-association and/or an increased restriction of random motions. The increase of the polydispersity parameter (Q) with concentration is also consistent with a broader range of correlation times, which could be interpreted as a formation of larger macromolecular units at higher concentrations. The angular dependence of D_T was not very pronounced for the subunits and the T-domains (Fig. 3a), and further measurements were consequently only performed at 20° . The concentration-dependence of $(D_T)_2$ together with the cognate values of Q is shown in Figs. 3(b) and 3(c). The apparent diffusion coefficients were extrapolated to zero concentration by linear regression with the use of least-squares analysis and corrected to 20°C (6M-guanidinium chloride) as well as to standard conditions (20°C , water) (Table 1). The z -average of D_T is obtained for a polydisperse sample by photon-correlation spectroscopy.

Ultracentrifugation

The sedimentation coefficients obtained by linear regression with the use of least-squares analysis are shown in Table 1. The Svedberg equation [$M = s^0 RT/D^0(1 - \bar{v}_2\rho)$] was used to calculate M_r (Table 1), with the partial specific volumes obtained in 6M-guanidinium chloride (Carlstedt *et al.*, 1983b). With the assumption that the sedimentation coefficient is the weight-average, combination with the z -average D_T will yield the weight-average relative molecular mass (Kinell, 1959).

A higher M_r for the whole mucins was obtained by sedimentation-diffusion than by light-scatter-

Fig. 2. Angular dependence (a) and concentration-dependence (b) of the translational diffusion coefficient for whole mucins

All measurements were performed in 6M-guanidinium chloride/1 mM- Na_2EDTA /1 mM-sodium phosphate buffer, pH 6.5. (a) The data were analysed with a forced linear fit of the correlogram (see the text). Mucin concentration was 0.30 mg/ml. (b) The apparent diffusion coefficients (D_T) were obtained after extrapolation to zero angle ($\theta = 15^\circ, 20^\circ, 25^\circ, 30^\circ$ and 35°). $(D_T)_1$ was calculated from a forced linear fit (▲ and ○), and $(D_T)_2$ from the linear term of the quadratic fit (●) of the log correlogram. Points, ▲, ● and ○ are data from two separate experiments. Points ● and ○ (same experiment) show the different ways to analyse the data. The cognate polydispersity term (Q ; ■) tends to increase with concentration.

ing. Part of the difference could be due to errors in the refractive-index increment and the partial specific volume, but the discrepancy is too large, in our opinion, to be explained solely by this. Two other possible explanations should be considered. Firstly, the total-intensity light-scattering data (Fig. 1b) suggest that a partial aggregation might take place at concentrations above 0.5 mg/ml. As sedimentation-velocity experiments could only be performed at concentrations of approx. 0.5 mg/ml and above, the s values might be too high to be combined with the cognate D_T that could be measured down to approx. $50 \mu\text{g/ml}$. A second possibility is that the frictional coefficients for the macromolecules during sedimentation and during diffusion are not identical, which is an implicit assumption in the derivation of the Svedberg equation.

Fig. 3. Angular dependence (a) and concentration-dependence (b and c) of the translational diffusion coefficient for subunits (b) and T-domains (c)

Measurements were performed in 6M-guanidinium chloride/1 mM- Na_2EDTA /1 mM-sodium phosphate buffer, pH 6.5, and the diffusion coefficients (D_T)₂ were obtained from the linear term of the quadratic fit of the log correlogram. (a) The angular dependence of subunits (●) and T-domains (■). (b) The concentration-dependence of subunits (●). The polydispersity term (Q , ○) shows a slight increase with concentration. (c) The concentration-dependence of T-domains (■). There is no discernible change in the polydispersity term (Q ; □).

Viscometry

A pronounced shear-dependence was observed for the whole mucins (Fig. 4a), whereas subunits and T-domains were little affected (results not shown). The reduced viscosity (η_{sp}/c) plotted against the concentration (c) for whole mucin showed a distinct upward curvature with a sharp rise at approx. 1.5–2 mg/ml (Fig. 4b). A much smaller concentration-dependence was noted for the subunits, whereas the T-domains were virtually not affected at all. The intrinsic viscosities $[\eta]$ were

Fig. 4. Shear-dependence of the reduced viscosity for whole mucins (a), reduced viscosity versus concentration (b) and $\log \eta_{sp}$ versus $\log c \cdot [\eta]$ (c) for whole mucins, subunits and T-domains

All measurements were performed in 6M-guanidinium chloride/1 mM- Na_2EDTA /1 mM-sodium phosphate buffer, pH 6.5. (a) Whole mucins were studied at the following concentrations: ▲, 0.4 mg/ml; □, 0.8 mg/ml; ■, 1.2 mg/ml; ○, 1.6 mg/ml; ●, 2.0 mg/ml. Extrapolation to zero shear rate was done manually. (b) The viscosity at zero shear rate was plotted against the concentration and extrapolated to zero concentration. ●, Whole mucins; ■, subunits; ▲, T-domains. (c) $\log \eta_{sp}$ was plotted against $\log c \cdot [\eta]$. A value of 1.3 was obtained for the slope by linear regression with the use of least-squares analysis. Symbols are as in (b).

obtained by manual extrapolation (Table 1). When $\log \eta_{sp}$ was plotted versus the logarithm of the coil-overlap parameter ($c[\eta]$) (Morris *et al.*, 1981), it was noted that the whole mucins as well as the fragments could be described by the same line with a slope of approx. 1.3, close to the behaviour of many linear coiling macromolecules in dilute solution (Fig. 4c). The cervical mucins thus behave as a dilute solution of linear coiling macromolecules under these conditions.

The intrinsic viscosities and the sedimentation coefficients were used to estimate M_r with the Scheraga-Mandelkern equation $\beta = (Ns^0[\eta]^3\eta_0)/M^3(1 - \bar{v}_2\rho)$, where η_0 is the viscosity of the

Fig. 5. Plots of $\log[\eta]$, $\log(1/D^0)$ and $\log s^0$ versus $\log M_r$ for whole mucins (● and ○), subunits (■ and □) and T-domains (▲).

All measurements were performed in 6M-guanidinium chloride/1 mM- Na_2EDTA /1 mM-sodium phosphate buffer, pH 6.5. (a) Plot of $\log[\eta]$ versus $\log M_r$. A value of the slope $\alpha = 0.71$ was obtained by linear regression with the use of least-squares analysis (—) with $M_r \times 10^6$ for whole mucins (●) and $M_r \times 10^6$ for the subunits (■). With the relative molecular masses obtained by the Svedberg equation (whole mucins, 15.5×10^6 ; subunits, 2.88×10^6) the slope $\alpha = 0.62$ (○, □, ----). Expected α -values are: rod, 1.8; random coil, 0.5–0.8; sphere, 0.33. (b) Plot of $\log(1/D^0)$ versus $\log M_r$ (symbols and calculation are as in a). A value of the slope $\alpha = 0.64$ was obtained with $M_r \times 10^6$ for the whole mucins and $M_r \times 10^6$ for the subunits. With the relative molecular masses obtained by the Svedberg equation the slope $\alpha = 0.56$. Expected values are: rod, 0.8; solid sphere, 0.33; random coil, 0.5–0.6. (c) Plot of $\log s^0$ versus $\log M_r$ (symbols and calculations are as in a). A value of the slope $\alpha = 0.46$ was obtained, with 10×10^6 as the M_r for the whole mucin and 2.1×10^6 for the subunits. With the relative molecular masses obtained by the Svedberg equation the slope $\alpha = 0.41$. Expected α -values are: rod, 0.2; solid sphere, 0.66; random coil, 0.4–0.5.

solvent, and all other symbols have their usual meaning (see, e.g., Tanford, 1961b). For a flexible chain β is typically 2.6×10^6 when $[\eta]$ is expressed in dl/g. The results (Table 1) agree with those obtained by light-scattering and by sedimentation-diffusion. The average for a polydisperse sample is, however, not well defined, and the relative molecular masses calculated are also dependent on the value assigned to β . This parameter does not usually vary to such an extent as to invalidate an estimation of the relative molecular mass.

Calculation of hydrodynamic parameters

The radius of the equivalent hydrated sphere (R_e) was calculated from the diffusion coefficient by using the Stokes equation and the expression $f = kT/D^0$. The value of R_e was also estimated from the intrinsic viscosity by using the expression $[\eta] = vV_h N/M$, where v is the Simha shape factor and V_h is the volume of the hydrated macromolecule. The results are shown in Table 2. Random coils can be treated as spherical particles with an equivalent hydrodynamic radius, $R_e = R_G/1.51$, where R_G is the radius of gyration (Flory, 1953). R_e values of 132 nm and 50 nm were obtained for the whole mucins and the subunits respectively, showing that the dimensions observed by light-scattering are in agreement with those calculated from the diffusion coefficients and the intrinsic viscosities.

The frictional ratio ($f/f_{\min.}$) is defined as the ratio between the actual frictional coefficient and that calculated for the corresponding unsolvated sphere ($f_{\min.}$). The frictional ratio was analysed to estimate limits on shape (axial ratio, a/b) and 'hydration' ($\rho_{\max.}$) as outlined by, for example, Tanford (1961b) and Cantor & Schimmel (1980). The high frictional ratios obtained can be interpreted as either a very high axial ratio (i.e. the molecules are extremely asymmetric) or as a large degree of 'hydration' (i.e. the molecules are highly expanded). As data from light-scattering (Carlstedt *et al.*, 1983b) are not compatible with a rod-like structure but would rather fit a flexible chain, we conclude that the high frictional coefficient must be due to expansion. Although the numbers for the axial ratios and the maximum hydration calculated from the intrinsic viscosities are somewhat different from those derived from the diffusion coefficients, they point towards the same conclusions.

The radii of gyration predicted from the intrinsic viscosities with the Flory-Fox treatment of a random coil (Flory, 1953) are 127 nm, 53 nm and 20 nm for whole mucins, subunits and T-domains respectively, substantially lower than those observed by light-scattering (Carlstedt *et al.*, 1983b). The radius of gyration was also calculated from the intrinsic viscosities by assuming rod-like struc-

Table 1. *Summary of physical data for cervical mucins and fragments thereof*

All measurements were performed in 6M-guanidinium chloride/1 mM- Na_2EDTA /1 mM-sodium phosphate buffer, pH 6.5. The values within parentheses are corrected to standard conditions (water, 20°C). The partial specific volume used were: whole mucins, 0.67 ml/g; subunits, 0.66 ml/g; T-domains 0.65 ml/g. The same values were used for water as for 6M-guanidinium chloride. The z-average diffusion coefficients were obtained from the linear term of the quadratic fit of the correlogram and were corrected to 20°C. The $M(s^0[\eta])$ was determined with the Scheraga-Mandelkern equation with a value of $\beta = 2.6 \times 10^6$ ($[\eta]$ expressed in dl/g). The $M(s^0/D^0)$ is a weight average (Kinell, 1959).

	Whole mucin	Subunits	T-domains
s^0 (S)	17.4 (40.4)	8.5 (19.2)	3.9 (8.7)
$D^0 \times 10^8$ (cm ² /s)	1.2 (1.9)	2.9 (4.7)	9.3 (15.1)
R_G (nm)	197*	74*	31*
	201†	—	—
$[\eta]$ (ml/g)	635	224	65
$M_w \times 10^{-6}$ (light-scattering)	10.6*	2.10*	0.38*
	11.1†	—	—
$M_w \times 10^{-6}$ (sedimentation-equilibrium)	—	1.61‡	0.29‡
$M(s^0/D^0) \times 10^{-6}$	15.5	2.88	0.40
$M(s^0[\eta]) \times 10^{-6}$	11.9	2.22	0.35

* Values obtained with conventional light-scattering (Carlstedt *et al.*, 1983b).

† Value obtained with laser light-scattering.

‡ From Carlstedt *et al.* (1983b).

Table 2. *Summary of hydrodynamic parameters for cervical mucin and fragments thereof*

The radius of the equivalent hydrated sphere (R_e) was calculated from D_T and from $[\eta]$ as described in the text. M_r 10×10^6 and 2.1×10^6 were used for the whole mucins and the subunits respectively. The frictional ratio ($f/f_{\min.}$) is the ratio between the actual frictional coefficient and that of the cognate unsolvated sphere, and was used to obtain limits on shape (a/b) and 'hydration' ($\rho_{\max.}$) of the macromolecules. The axial ratio (a/b) for a prolate ellipsoid and the maximum 'hydration' ($\rho_{\max.}$) expressed as g of water/g of macromolecule was estimated with the assumption that the molecules are unsolvated and spherical respectively. The axial ratio and the maximum 'hydration' was also estimated from the Einstein-Simha equation $[\eta] = v(\bar{v}_2 + \rho\bar{v}_1) = v(V_h N/M)$, where v is the shape-dependent Simha factor and V_h is the volume of the hydrated macromolecule. The ρ -ratio is defined as R_G/R_e (Burchard *et al.*, 1980) and K_s as $s^0 \cdot d(1/s)/dc$ (see, e.g., Creeth & Knight, 1965).

	Whole mucins	Subunits	T-domain
D^0 $\left\{ \begin{array}{l} R_e \text{ (nm)} \\ f/f_{\min.} \\ a/b \\ \rho_{\max.} \end{array} \right.$	$\left\{ \begin{array}{l} 112 \\ 8.1 \\ > 395 \\ 353 \end{array} \right.$	$\left\{ \begin{array}{l} 46 \\ 5.6 \\ 190 \\ 113 \end{array} \right.$	$\left\{ \begin{array}{l} 14 \\ 3.0 \\ 52 \\ 18 \end{array} \right.$
$[\eta]$ $\left\{ \begin{array}{l} R_e \text{ (nm)} \\ v \\ a/b \\ \rho_{\max.} \end{array} \right.$	$\left\{ \begin{array}{l} 100 \\ 948 \\ 130 \\ 253 \end{array} \right.$	$\left\{ \begin{array}{l} 42 \\ 339 \\ 75 \\ 89 \end{array} \right.$	$\left\{ \begin{array}{l} 16 \\ 100 \\ 35 \\ 25 \end{array} \right.$
ρ -ratio	1.76	1.61	2.21
$K_s/[\eta]$	1.42	1.42	1.28

and T-domains. The estimate for the T-domains is in agreement with that observed by light-scattering, whereas the values for the whole mucins and the subunits are too high. The whole mucins and the subunits thus behave as somewhat 'stiff' coils in a good solvent, whereas the T-domains appear to be rod-like.

The ratio of $K_s/[\eta]$, where K_s is the concentration-dependence parameter of the reciprocal sedimentation coefficient $K_s = s^0 \cdot d(1/s)/dc$, has been used to evaluate the contribution of asymmetry and expansion to the intrinsic viscosity. The ratio is predicted to be close to 1.6 for random-coil polymers (Wales & Van Holde, 1954). It has been shown, however, that a ratio of approx. 1.6 is characteristic for all macromolecules with a spherical conformation whether compact or expanded, and that a lower value suggests an increasing contribution by asymmetry (Creeth & Knight, 1965). A value of 1.42 was obtained for whole mucins and subunits, whereas that of the T-domains was 1.28 (Table 2). The whole mucin and the subunits thus appear to occupy spheroidal domains in solution, and the high intrinsic viscosities are interpreted as expansion and solvent entrainment rather than as a high degree of asymmetry. The value for the T-domains, although somewhat lower, is also in keeping with an expanded structure.

The relationship between the intrinsic viscosity and the relative molecular mass for a series of similar polymers can be expressed by the Mark-Houwink equation $[\eta] = kM_r^\alpha$, where α is a shape-dependent parameter. A plot of $\log[\eta]$ versus $\log M_r$ for whole mucins, subunits and T-domains is

tures, and the values obtained were 226 nm, 91 nm and 32 nm respectively for whole mucins, subunits

shown in Fig. 5(a). The slope ($\alpha = 0.71$) obtained is close to that expected for a somewhat 'stiff' linear flexible macromolecule. The diffusion coefficients and sedimentation coefficients can be related to M_r in a similar way (Fig. 5b and 5c), and the corresponding α -values also fit those expected for linear flexible macromolecules in a good solvent. The slopes were determined with the relative molecular masses obtained by light-scattering (continuous lines in Fig. 5), as we suspect that those obtained by the Svedberg equation might be somewhat high, owing to, for example, the formation of aggregates. With the latter relative molecular masses the slopes will be somewhat lower, but the hydrodynamic description of the macromolecules is not altered.

The ρ -ratio was defined by Burchard *et al.* (1980) and fitted to a number of theoretical models for polymers, including linear, flexible chains and regular stars. We note that the ρ -ratios (Table 2) for whole mucins and subunits are not significantly different, suggesting that no drastic change in the macromolecular architecture occurs after reduction of disulphide bonds in the mucins. Furthermore, the ρ -ratios for whole mucins and subunits are close to those predicted for polydisperse linear flexible chain and significantly higher than that predicted for, for example, a regular star with four or five arms. The ρ -ratio for the T-domains is in keeping with a rod-like structure.

The hydrodynamic data for the whole mucins and the subunits are thus in keeping with the behaviour expected for a random coil, which is in agreement with the proposed polymeric structure for the mucins (Carlstedt *et al.*, 1983b). However, for the T-domains there is a discrepancy between the R_G , and thus the ρ -ratio, favouring a rod-like structure, and the parameter $K_s/[\eta]$, which is consistent with expansion rather than with asymmetry. The latter interpretation is corroborated by the slope of the plot of $\log[\eta]$ versus $\log M_r$. An over-estimation of the R_G (which is a z -average) is a likely explanation, but the possibility of a conformational instability due to the presence of a flow-field should in our opinion, also be considered. The model proposed for the macromolecular structure of the cervical mucins (Carlstedt *et al.*, 1983b) should be modified to account for flexibility within the highly glycosylated regions (the T-domains).

General discussion

Mucus is a hydrated gel containing approx. 95–99.5% of water, depending on its physical properties and physiological function. The gel is formed

by the mucins, which account for about half of the non-diffusible solids in mucus. Disulphide bonds have been implicated in this 'cross-linking', and many investigators have, in addition, postulated the presence of 'cross-linking' factors (e.g. proteins) to explain the physical properties of the gel (see, e.g., Clamp *et al.*, 1978). A 70000- M_r protein has been suggested to 'organize' the 'subunits' in the covalent polymeric structure of gastric mucins (Pearson *et al.*, 1981). In our opinion the basic gel-forming unit of mucus is the largest mucin that can be isolated after the breakage of all non-covalent bonds in the gel. Consequently disulphide bonds neither 'cross-link' the mucins (with or without the aid of 'cross-linking' proteins) nor participate in the polymerization of the mucus gel, although such bonds appear to be vitally important for the integrity of the individual mucin as a macromolecule.

Physical entanglement must be an important factor in the formation of cervical mucus, as the solvent domains of the mucins will overlap at a concentration much lower than that found in mucus. Assuming that the whole mucins occupy a spherical domain with the same radius as the R_G , it is estimated that the macromolecules would start to interpenetrate at approx. 0.5 mg/ml. The degree of interpenetration, and thus entanglement, is dependent on the size and shape of the macromolecules. The M_r of the cervical mucins is in the order of 10×10^6 – 15×10^6 , and we suggest that the true value is close to the lower limit. The hydrodynamic 'model' of the mucins emerging from all hydrodynamic techniques used here is that of a somewhat expanded coil within a spheroidal solvent domain. This is close to the classical description of a random coil in a good solvent, and also fits the model suggested for the polymeric structure of the mucins (Carlstedt *et al.*, 1983b). The large solvent entrapment is in keeping with the high water content of the mucus gel.

The size and the volume occupancy of the mucins will pose a problem for the packaging of the macromolecules in the storage granules of the secretory cell. It is noted that the length of a fully extended cervical mucin would be in the order of 4–6 μm , thus exceeding the diameter of the secretory granule. Before secretion the mucins must be stored in a form where the conformational freedom is severely restricted. If secretion is followed by removal of this constraint, the macromolecules would expand and imbibe water, resulting in a pronounced interpenetration and physical entanglement. The content of the individual secretory granulum will swell and form the mucus gel. This perception of the formation of the mucus gel does not, however, preclude the presence of specific 'site'-mediated interactions.

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