The macromolecular structure of human cervical-mucus glycoproteins

Studies on fragments obtained after reduction of disulphide bridges and after subsequent trypsin digestion

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Human cervical-mucus glycoproteins (mucins) were extracted with 6 M-guanidinium chloride in the presence of proteinase inhibitors and purified by isopycnic density-gradient centrifugations. The whole mucins (M_r approx. 10×10^6) were degraded into 'subunits' (M_r approx. 2×10^6) by reduction of disulphide bonds. Trypsin digestion of the 'subunits' produced glycopeptides with M_r approx. 380 000, which appear to be rod-like with a length of approx. 105 nm. The relationship between the radius of gyration and the M_r value obtained by light-scattering for whole mucins, 'subunits' and 'domains' suggest that cervical-mucus glycoproteins are linear flexible macromolecules composed of, on the average, four or five 'domains'/subunit and four subunits/whole mucin macromolecule. The shape-dependent particle scattering function for the whole mucins and the 'subunits' are in accordance with that of a linear flexible chain. No evidence for a branched or a star-like structure was found. A tentative model for cervical mucins is proposed.

The biological function of cervical mucus varies with its physical properties during the ovulatory cycle. The thin mucus gel found at the periovulatory period is penetrated much more easily by the spermatozoa than is the tenacious mucus plug of the luteal phase (Chantler, 1982). The properties of the mucus gel are critically dependent on the mucus glycoproteins (the mucins). To understand how the macromolecular features of these high- M_r glycoproteins are related to their structure, undegraded mucins representative of those present *in situ* must be investigated.

In a previous paper (Carlstedt *et al.*, 1983) we devised a protocol for the isolation and purification of human cervical mucins with the use of conditions that should minimize degradation of the macromolecules during handling. In the present investigation we have extended the description of these glycoproteins to a physical and biochemical study of fragments obtained after reduction of disulphide bonds and after subsequent trypsin digestion of the reductive fragments.

Experimental

Materials

Trypsin (EC 3.4.21.4; type XI, diphenylcarbamoyl chloride-treated) and guanidinium chloride

(practical grade) were obtained from Sigma Chemical Co. Stock solutions of the latter were treated with charcoal before use. Ultrapure guanidinium chloride was bought from Bethesda Research Laboratories and was used in analytical ultracentrifugation, whereas the practical grade was used for extractions, preparative density-gradient centrifugations and column chromatography. Diisopropyl phosphorofluoridate was obtained from Fluka, and dithiothreitol, *N*-ethylmaleimide as well as HCl (AristaR) were from BDH Chemicals. Sepharose CL-2B was a product of Pharmacia.

Analytical methods

Hexose was determined by the method of Goa (1955), and sialic acid as described by Jourdan *et al.* (1971). An automated version of the latter method (Lohmander *et al.*, 1980) was used to analyse fractions from column chromatography and from preparative density-gradient centrifugation. Amino acids and hexosamines were determined quantitatively with a Durrum automatic amino acid analyser after hydrolysis under O_2 -free Ar at 110°C for 24 h in 6 M-HCl (AristaR) and at 100°C for 10 h in 4 M-HCl (AristaR) respectively. Neutral sugars were determined as the corresponding alditol acetates by g.l.c. (Lindahl, 1970) after hydrolysis in 2 M-trifluoroacetic acid at 100°C for 4 h in sealed

tubes under N_2 . Densities of fractions from preparative density-gradient centrifugation were assessed with a Carlsberg pipette as a pycnometer.

The water content of freeze-dried mucins and fragments thereof was estimated as the loss in weight after drying for 18 h at reduced pressure over P_2O_5 at 110.6°C (refluxing toluene). Drying for longer periods of times gave no further loss in weight.

Preparation of whole mucins, subunits and T-domains

Mucins were prepared from human cervical pregnancy mucus as described previously (Carlstedt *et al.*, 1983). In summary, the mucus gel was treated with di-isopropyl phosphorofluoridate, solubilized by slow stirring in 6M-guanidinium chloride/5 mM- Na_2EDTA /5 mM-*N*-ethylmaleimide/10 mM-sodium phosphate buffer, pH 6.5, and subjected twice to density-gradient centrifugation in CsCl/4 M-guanidinium chloride (initial density 1.39 g/ml) followed by density-gradient centrifugation in CsCl/0.2 M-guanidinium chloride (initial density 1.50 g/ml). The purity was assessed by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate and by analytical density-gradient centrifugation.

Reduction of disulphide bridges was performed in 6 M-guanidinium chloride/5 mM- Na_2EDTA /10 mM-dithiothreitol/0.10 M-Tris/HCl buffer, pH 8.0, for 5 h at 37°C. Free thiols were subsequently alkylated by the addition of 2.5 molar excess of iodoacetamide over dithiothreitol for approx. 15 h at room temperature in the dark. After dialysis against 6 M-guanidinium chloride/5 mM- Na_2EDTA /10 mM-sodium phosphate buffer, pH 6.5, the fragments were subjected to density-gradient centrifugation in CsCl/4 M-guanidinium chloride (initial density 1.42 g/ml). The major glycosylated fragments obtained by this procedure are referred to below as 'subunits'. The purity was assessed by analytical density-gradient centrifugation.

Trypsin digestion of subunits was performed in 0.10 M-Tris/HCl buffer, pH 8.0, for 5 h at 37°C with approx. 50 µg of trypsin/mg of subunit. After digestion the fragments were dialysed against 6 M-guanidinium chloride/5 mM- Na_2EDTA /10 mM-sodium phosphate buffer, pH 6.5, and subjected to density-gradient centrifugation as described for the subunits. The major glycosylated tryptic fragments are referred to below as 'T-domains'.

Light-scattering

The intensity of light scattered at angles between 30° and 150° was determined with a Sofica model 42000 photo-goniometer as described previously (Carlstedt *et al.*, 1983). Samples were dialysed against 6 M-guanidinium chloride/1 mM- Na_2EDTA /1 mM-sodium phosphate buffer, pH 6.5, at room temperature. Results were evaluated as

described by Tomimatsu *et al.* (1968) and plotted in accordance with Zimm (1948). The particle scattering function $[P(\theta)]$ was calculated (see, e.g. Tanford, 1961), and $P(\theta)^{-1}$ was plotted against $h^2R_G^2$, where h is the scattering vector $(4\pi\lambda_0/\lambda_0) \cdot \sin \theta/2$ and R_G is the radius of gyration. $P(\theta)$ for a random coil with a Gaussian distribution of the mass segments was calculated from the expression $P(\theta) = (2/w^2)(e^{-w} + w - 1)$, where $w = h^2R_G^2$ (see, e.g., Tanford, 1961).

The apparent refractive-index increment $(\partial n/\partial c)$ at constant chemical potential of diffusible components (Casassa & Eisenberg, 1964) was determined at room temperature with a Waters R401 differential refractometer. The sample solutions were pumped directly from the dialysis bags into the low-volume flow-cell used with the instrument. The signal (Δn) was read in millivolts against the solvent-solvent base-line on the chart-recorder, and the system was calibrated with sucrose solutions of known concentrations and refractive indices. Whole mucins, subunits and T-domains were studied at 2 mg/ml, 7.1 mg/ml and 10.7 mg/ml respectively.

The concentrations of the mucin solutions were always assessed after the physical measurements by sialic acid determination, with the cognate mucin stock solution as a standard. The concentration of this standard was determined gravimetrically by freeze-drying after extensive dialysis of a sample of the preparation and correcting for moisture as described above.

Analytical ultracentrifugation

Sedimentation equilibrium was performed with an MSE 75 Centriscan analytical ultracentrifuge, by the meniscus-depletion method (Yphantis, 1964). Samples were dialysed against 6 M-guanidinium chloride/1 mM- Na_2EDTA /1 mM-sodium phosphate buffer, pH 6.5, and 150 µl of sample was used together with 50 µl of Fluorinert FC-70 (Beckman). The apparent M_r values were obtained from the slope of the $\ln c$ -versus- r^2 line with the absorbance optics at 280 nm, and $1/M_{app}$ was plotted against the initial concentration and extrapolated to zero. For subunits the concentrations were 0.06, 0.12 and 0.17 mg/ml, and centrifugation was continued for approx. 50 h at 7200 rev./min after an overspeed period of 6 h at 11000 rev./min. T-domains were studied at 11000 rev./min and at 13000 rev./min, at the concentrations 0.19 and 0.76 g/ml and 0.08, 0.13 and 0.23 g/ml respectively. In both cases centrifugation was continued for approx. 50 h after an overspeed period for 6 h at 15000 rev./min. Samples were monitored several times during the last 12 h of the experiments to ensure that equilibrium had been achieved.

The apparent specific volume ($\bar{v}$) at constant chemical potential of diffusible components

(Casassa & Eisenberg, 1964) was calculated from the density increment ($\Delta\rho/\Delta c$) measured with a Kratky densitometer (Anton Paar, Graz, Austria) at 20°C. Solutions were dialysed against 6 M-guanidinium chloride/1 mM- Na_2EDTA /1 mM-sodium phosphate buffer, pH 6.5, for at least 72 h under stirring. Whole mucins were studied at approx. 3 mg/ml, subunits at approx. 5.2 mg/ml and 11.1 mg/ml, and T-domains at approx. 6.3 mg/ml and 12.1 mg/ml. The concentrations of the samples were assessed after the measurements as described above.

Results

Isolation of mucin fragments

The effect of dithiothreitol on the cervical mucins was studied by monitoring their size as a function of time with light-scattering (Fig. 1). The apparent M_r decreased rapidly after the addition of the reagent (3.3 mM) and reached a plateau after approx. 10 min, and no further decrease was noted for 72 h. A 5-fold increase in the concentration of dithiothreitol or a

Fig. 1. Effect of dithiothreitol on the M_r of cervical mucins

Dithiothreitol (final concn. 3.3 mM) was added to cervical mucins (0.19 mg/ml) in 6 M-guanidinium chloride/0.10 M-Tris/HCl buffer, pH 8.0, and the intensity of light scattered at 30° (I_{30}) was followed as a function of time. The decrease in I_{30} corresponds to a change in apparent M_r from approx. 10.6×10^6 to 1.8×10^6 . No further decrease was observed from 30 min to 72 h.

gradual rise in the temperature to about 70°C was not accompanied by any further decrease in the apparent M_r .

In a preparative experiment subunits were isolated after reduction and alkylation by isopycnic density-gradient centrifugation (Fig. 2a). Mucins subjected to this procedure were recovered as subunits (84%) and proteins from the top of the gradient (11%). The subunits banded between the densities 1.38 g/ml and 1.48 g/ml, with the peak position close to 1.43 g/ml. The protein fraction gave rise to four to six bands on polyacrylamide-gel electrophoresis in sodium dodecyl sulphate (results not shown). No protein bands were seen when the whole mucins were subjected to electrophoresis without prior reduction.

Subunits subjected to trypsin digestion were recovered as T-domains (80%) and peptide material (4%) by density-gradient centrifugation (Fig. 2b). The peak position of the T-domains was at 1.46 g/ml, with a distribution between the densities 1.39 g/ml and 1.50 g/ml.

Chemical characterization of mucin fragments

The chemical composition of subunits and T-domains are shown in Table 1. The relative proportions of the monosaccharides are similar for the two fragments and also close to those of the whole mucins (Carlstedt *et al.*, 1983). A small decrease in the protein content was observed for the subunits, whereas a much larger one was noted for the T-domains compared with the whole mucins. This was also reflected in the buoyant densities of the respective macromolecules. Whole mucins banded at a density of 1.42 g/ml in CsCl/4 M-guanidinium chloride, close to that of the subunits, whereas the T-domains appeared at a significantly higher buoyant density (1.46 g/ml). The amino acid composition is shown in Table 2. An enrichment of serine, threonine and proline is observed in the T-domains, accounting, together with glycine and alanine, for approx. 75% of the total protein.

Physical characterization of mucin fragments

Subunits and T-domains appeared as single unimodal peaks with no evident heterogeneity on gel chromatography (Sephacrose CL-2B) in 6 M-guanidinium chloride (Fig. 3). Whole mucins chromatographed with the void volume, whereas the peak positions of subunits and T-domains were at K_{av} 0.19 and 0.60 respectively. The gel chromatograms of subunits from four different mucin preparations were very similar. No further decrease in molecular size was observed when mucins were treated by 6 M-guanidinium chloride/5 mM- Na_2EDTA /10 mM-dithiothreitol/0.5 M-2-mercaptoethanol/0.10 M-Tris/HCl buffer, pH 8.0, for 72 h at room temperature followed by gel chromatography.

The M_r values of the subunits and of the

Fig. 2. Isopycnic density-gradient centrifugation in CsCl/4 M-guanidinium chloride of subunits (a) and T-domains (b) (a) Mucins were reduced and alkylated as described in the text, and CsCl was added to an initial density of 1.42 g/ml. After centrifugation (approx. 65 h; 36000 rev./min; 15°C; 8 × 25 ml rotor; MSE 65 ultracentrifuge), fractions were collected from the bottom of the tubes and analysed for sialic acid (●), hexose (Δ), absorbance at 280 nm (○) and density (■). Fractions were pooled as shown by the horizontal bars. (b) Subunits were digested with trypsin as described in the text and isopycnic density-gradient centrifugation, and analyses were performed as described above. Fractions were pooled as shown by the horizontal bars.

Table 1. Chemical composition of fragments derived from cervical mucins

All numbers are the mean of two independent measurements. Amino sugars were determined with the amino acid analyser and neutral sugars by g.l.c. Sialic acid is expressed as *N*-acetylneuraminic acid, and protein is the sum of the individual values for the amino acids. All numbers are corrected for moisture (subunits, 11%; T-domains, 14%).

	Subunits		T-domains	
	(g/100 g of subunit)	(molar proportions of monosaccharides)	(g/100 g of T-domains)	(molar proportions of monosaccharides)
<i>N</i> -Acetylgalactosamine	12.0	1.00	14.5	1.00
<i>N</i> -Acetylglucosamine	15.1	1.26	16.6	1.14
Galactose	20.3	2.11	23.9	2.49
Fucose	9.2	1.06	9.7	1.12
Sialic acid	15.5	0.90	19.7	1.14
Protein	18.1		11.9	

T-domains were determined by light-scattering (Figs. 4a and 4b) and by sedimentation equilibrium (Table 3). The somewhat lower values obtained with the ultracentrifuge could be explained if the largest species of a polydisperse distribution were too close to the bottom of the cell to be included in the

average. The differences are, however, well within the possible errors, which are critically dependent on accurate measurements of $\partial n/\partial c$ and of $\bar{v}$. The sialic acid determinations used to assess concentrations were performed with a 1–2% variation between replicate analyses. An error of approx. 1–2% in the

concentrations of the solutions used to calibrate the refractometer would thus provide $\partial n/\partial c$ values that are correct to within 5%. Similar considerations suggest that the values for the apparent specific volumes also are accurate to within 5%. Determinations of $\partial n/\partial c$ were performed with white light, as the instrument cannot be used with filters. As the $\partial n/\partial c$ is likely to vary slightly with wavelength, T-domains were studied at 436 nm, 578 nm and 621 nm in a Shimadzu model DR-4 differential refractometer, as described previously (Carlstedt *et al.*, 1983). The differences in the readings were within the random errors for this instrument.

Table 2. Amino acid composition of fragments derived from cervical mucins

Methionine could not be evaluated owing to interference by glucosamine. Abbreviation: N.D., not detected.

Amino acids	Composition (mol/1000 mol of total amino acids)	
	Subunits	T-domains
Asx	58.0	27.3
Thr	237.5	312.3
Ser	131.1	164.7
Glx	56.7	43.4
Pro	116.5	124.4
Gly	72.2	69.7
Ala	95.6	100.8
CyS	N.D.*	N.D.
Val	38.7	35.1
Met	—	—
Ile	26.3	24.1
Leu	59.1	51.0
Tyr	21.7	N.D.*
Phe	25.6	N.D.*
His	19.4	20.1
Lys	12.7	9.4
Arg	29.1	17.7

* When the samples were re-run at a much higher column load, material was detected in positions that could correspond to these amino acids.

However, a trend to lower values of $\partial n/\partial c$ with increasing wavelength was discerned (approx. 1–2% lower at 621 nm than at 436 nm). The values obtained for white light were thus used without further corrections.

Fig. 3. Gel chromatography on Sepharose CL-2B of cervical mucins and fragments thereof
Chromatography was performed on a column (13.5 mm × 1585 mm) equilibrated with 6 M-guanidinium chloride / 1 mM-Na₂EDTA / 1 mM-sodium phosphate buffer, pH 6.5, at a flow rate of approx. 7 ml/h. Fractions (approx. 3 ml) were analysed for sialic acid. —, Whole mucin; ·····, subunits; ----, T-domains. V₀, Void volume; V_i, total volume.

Table 3. Summary of physical data for cervical mucins and fragments derived therefrom
All measurements were performed in 6 M-guanidinium chloride/1 mM-Na₂EDTA/1 mM-sodium phosphate buffer, pH 6.5. R_G is the radius of gyration, $\partial n/\partial c$ the refractive-index increment and $\bar{v}$ the partial specific volume.

	$10^{-6} \times M_w$ (sedimentation equilibrium)	$10^{-6} \times M_w$ (light-scattering)	R _G (nm)	$\partial n/\partial c$ (ml/g)	$\bar{v}$ (ml/g)
Whole mucin*	—	10.6	197	0.101	0.67
Subunits	1.6	2.1	74	0.098	0.66
T-domains	0.29	0.38	31	0.096	0.65

* Data for the M_w and the radius of gyration are from Carlstedt *et al.* (1983). The value for the M_w is recalculated with the value for $\partial n/\partial c$ given here.

Fig. 4. Zimm plots of light-scattering data from solutions of (a) subunits and (b) T-domains

All measurements were performed in 6M-guanidinium chloride/1mM- Na_2EDTA /1mM-sodium phosphate buffer, pH 6.5. The following concentrations were used: (a) 0.326, 0.245, 0.163 and 0.082 mg/ml; (b) 3.39, 2.54, 1.70 and 0.85 mg/ml. The optical constants were (a) 1.784×10^{-7} and (b) $1.714 \times 10^{-7} \text{ cm}^2 \text{ g}^{-2}$.

The value of $\partial n/\partial c$ with the Waters instrument for whole mucins is significantly lower than that reported previously (Carlstedt *et al.*, 1983). The latter value (0.135 ml/g) appear to be 'high' compared with those obtained for subunits and T-domains. Re-measurement of $\partial n/\partial c$ for the whole mucins with the Shimadzu instrument gave values of approx. 0.1, but with much less precision compared with the Waters instrument. The values for the T-domains obtained with the Shimadzu instrument

Fig. 5. Particle scattering function $P(\theta)$ as a function of the scattering vector for (a) whole mucins and (b) subunits

Light-scattering was performed in 6M-guanidinium chloride/1mM- Na_2EDTA /1mM-sodium phosphate buffer, pH 6.5. $P(\theta)$ was calculated and $P(\theta)^{-1}$ plotted against $h^2 R_G^2$ (●), where h is the scattering vector and R_G the radius of gyration. The continuous line shows the expected $P(\theta)^{-1}$ values for a Gaussian coil calculated as described in the text. Note that the shape-dependent $P(\theta)$ function is very similar for whole mucins and for subunits and always falls below this line. This suggests that whole mucins and subunits have rather similar macromolecular properties, both behaving as linear flexible macromolecules.

during the study of the wavelength dispersion were also close to those obtained with the Waters instrument (but with less precision), suggesting that no systematic deviation exists between measurements performed with the two instruments.

Light-scattering data were also used to determine the radius of gyration (Table 3) and the shape-dependent particle scattering function $[P(\theta)]$. The curve of $P(\theta)^{-1}$ versus $h^2R_G^2$ for the whole mucins (Fig. 5a) is very similar to that for the subunits (Fig. 5b), suggesting principally similar macromolecular designs. The particle scattering functions for both macromolecules are compatible neither with a rod-like nor with a compact symmetrical structure, but would fit that expected for a 'stiff' random coil. The $P(\theta)$ function was not calculated for the T-domain, as these fragments are too small to extend the function over a sufficient range of $h^2R_G^2$ to yield any useful information.

The relationship between the radius of gyration (R_G) and the relative molecular mass (M_r) tells us

Fig. 6. Plot of $\log R_G$ versus $\log M_r$ for whole mucins (●), subunits (■) and T-domains (▲).

The relationship between the radius of gyration (R_G) and the relative molecular mass (M_r) can be expressed as $R_G = KM_r^\alpha$, where α depends on the molecular shape. Expected α values are: solid spheres, 0.33; random coils, 0.5–0.6; long rods, 1.0. For a macromolecule growing via extensive 'branching', α decreases as M_r increases from the value for a flexible random coil to that for a solid sphere. Stiff coils are expected to yield α values between 0.5 and 1.0, as a rod could be regarded as the extreme version of such a coil. The value obtained by linear regression with the use of least-squares analyses is 0.55, compatible with a somewhat 'stiff' random coil.

how size and shape of a polymer are affected by an increase in total mass. This is usually expressed as $R_G = KM_r^\alpha$, where α is a parameter sensitive to the shape of the macromolecules. A log-log plot of the data for whole mucins, subunits and T-domains (Fig. 6) provided an α value of 0.55, close to that expected for a random coil in a good solvent. The results are not compatible with the fragments being fitted together to form a rod-like (α close to 1.0) or a compact symmetrical structure (α approx. 0.33).

Discussion

The size of the cervical mucins described in the present paper is close to that suggested by Lee *et al.* (1977a,b) for mucins from bovine oestrus and human mid-cycle mucus, and conforms also with data on bovine oestrus mucins from Silberberg & Meyer (1982). Cervical mucins studied by Wolf *et al.* (1977) are eluted with the void volume of a Sepharose 2B gel, in agreement with our results. Cervical mucins from the bonnet monkey (Hatcher *et al.*, 1977) appear to be much smaller (M_r approx. 10^6), as are those from pig gastric and pig small-intestinal mucus (M_r approx. 2×10^6). Pig colonic mucins have M_r approx. 15×10^6 . For a summary of data on gastrointestinal mucins see, for example, Allen (1981). M_r values reported for respiratory mucins are: 3×10^6 – 7×10^6 (Creeth *et al.*, 1977), 2×10^6 (Woodward *et al.*, 1982) and 2×10^6 (Feldhoff *et al.*, 1979).

Fragments obtained after reduction of disulphide bonds in mucins are generally referred to as subunits. Subunits studied in the present work are similar in size to those prepared by Yurewicz & Moghissi (1981) from human cervical mucus, whereas such fragments from the bonnet monkey have M_r approx. 0.5×10^6 , i.e. similar in size to those obtained from pig gastric, pig small-intestinal and pig colonic mucins (see Allen, 1981). Respiratory mucins give rise to subunits varying in size from M_r 600 000 to M_r 2.3×10^6 (Creeth *et al.*, 1977). Houdret *et al.* (1981) showed that subunits isolated after solubilization of bronchial mucus by reduction in 6M-guanidinium chloride were larger than those obtained after reduction in 0.2M-NaCl. However, reduction of cervical mucins in 0.1M-Tris/HCl buffer alone gave rise to somewhat larger fragments (gel chromatography) than those observed after reduction in 6M-guanidinium chloride (results not shown).

High- M_r glycopeptides (M_r approx. 300 000–600 000) are characteristic products after proteolytic digestion of mucins from mucus-producing epithelia. There is ample evidence that the major part of the carbohydrate in mucins is present in these glycopeptides as clusters of oligosaccharides (domains) protecting the protein core from further

proteinase action. Some mucins (e.g. pig gastric mucin) give rise to reductive fragments and proteolytic fragments that are of similar size, and the reductive fragments are not degraded to any large extent by proteolysis. Mucins from different tissues and species thus show a broad distribution in molecular sizes and also distinctly different fragmentation patterns (see, e.g., Clamp *et al.*, 1978).

The number of amino acid residues per T-domain was estimated to be approx. 400, on the basis of an average M_r of 110 for the amino acids and 380 000 for the entire T-domains. The rise along the axis/amino acid is dependent on the secondary structure and is 0.15 nm, 0.31 nm and 0.35 nm for an α -helix, a polyproline helix and a β -pleated sheet respectively. The high content of proline and the extensive oligosaccharide substitution would certainly not allow a continuous α -helix in the T-domains, and the high proline content is not compatible with a fully extended β -structure. With an estimated average translation along the axis of 0.25–0.30 nm/amino acid, the length of the T-domain would be about 100–120 nm, consistent with data on bronchial-mucus glycoproteins of similar size (Lamblin *et al.*, 1979). The predicted radius of gyration for a rod with this length is 30–35 nm, from the equation $R_G^2 = L^2/12 + r^2/4$, where L is the length and r is the radius of the rod. A radius of 5 nm was used, as it is compatible with the estimated lateral projection of a decasaccharide from the protein core. The calculated radius of gyration is close to the experimentally found one (31 nm), suggesting that a major part of the protein core must be extended to

account for this value, i.e. the T-domains appear to be rod-like structures. This conclusion has also been reached by Silberberg & Meyer (1982).

The relationship between size parameters and the M_r for polymers often provides important clues to their macromolecular architecture, in particular when the intact macromolecules are studied together with well-defined fragments thereof. The $\log R_G$ -versus- $\log M_r$ values for the whole mucins, the subunits and the T-domains fall on a straight line, with a slope close to that expected for a macromolecule growing as a flexible but somewhat stiff linear chain. The particle scattering functions for the whole mucins and for the subunits are also in accordance with this design. Polydispersity and/or excluded-volume effects could, however, displace the particle scattering function for a branched structure to resemble that of a random coil. Comb-like structures, in particular, are difficult to distinguish from linear flexible molecules (see, e.g., Casassa & Berry, 1966). Monodisperse stars with four equal arms give $P(\theta)^{-1}$ values that are higher than those obtained for a random coil with the same radius of gyration (Benoit, 1953). The only way, in our opinion, to arrange the T-domains into a star-like subunit structure would be by disulphide bonds between the protein cores. As subunits were derived from the whole mucins by reduction of disulphide bonds, a linear arrangement of T-domains interspersed at regular intervals with non-glycosylated, trypsin-sensitive, regions of the protein core is the most likely model. The relative M_r values of subunits and T-domains, together with their yields, suggest

Fig. 7. *Proposed macromolecular architecture of cervical mucins*

The whole mucins (a) are linear flexible macromolecules composed of, on the average, four subunits (b) obtained after reduction of disulphide bonds. On the average four or five T-domains (c) are produced by trypsin digestion. An enlarged version of the T-domains (d) shows how these are composed of oligosaccharides tightly spaced on a central protein core.

that four or five T-domains account for one subunit. Their similar particle scattering functions, together with the slope in the log-log plot (Fig. 5), suggest that subunits grow into whole mucins without any drastic change in the overall design of the macromolecules. Thus, on the average, four subunits added end to end would account for the whole mucin as a linear macromolecule.

In our opinion, the data presented here are best accommodated into the model depicted in Fig. 7. The whole mucins (Fig. 7a) as well as the subunits (Fig. 7b) are linear flexible macromolecules. Part of the protein detaches on reduction. These polypeptides are believed to be part of the covalent structure of the whole mucins. Their localizations are tentatively suggested in Fig. 7 (small arrows), as there are no overlapping fragments available to provide evidence for how they should be fitted into the model. The subunits (Fig. 7b) contain proteinase-sensitive sites in the 'naked' regions between the oligosaccharide domains. The T-domains (Fig. 7c) are rod-like (Fig. 7d) and are probably not very flexible. To account for the overall flexibility, the mucins must rely on the carbohydrate-free stretches between the T-domains and the subunits. The mucins could thus be regarded as a train of rather stiff rods (length approx. 100–110 nm) interspersed with regions of 'naked' protein that in some places can be severed by reduction. Our description of the mucins is close to that of Gibbons (see, e.g., Gibbons & Sellwood, 1973), who described bovine cervical mucins as 'filiform' with a general behaviour similar to that of a somewhat stiffened random coil.

All measurements were performed in 6 M-guanidinium chloride to circumvent the possible complication by self-association in 0.2 M-NaCl suggested by earlier data (Carlstedt *et al.*, 1983). Hence the model might not express the macromolecular properties of the mucins under physiological conditions, but is used to suggest how the fragments can be fitted into the whole mucin structure. The particle scattering function in 0.2 M-NaCl suggests, however, that the whole mucins behave as linear, although less flexible, macromolecules in this solvent as well (results not shown).

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References

- Allen, A. (1981) in *Physiology of the Gastrointestinal Tract* (Johnson, L. R., ed.), pp. 617–639, Raven Press, New York
- Benoit, H. (1953) *J. Polym. Sci.* **11**, 507–510
- Carlstedt, I., Lindgren, H., Sheehan, J. K., Ulmsten, U. & Wingerup, L. (1983) *Biochem. J.* **211**, 13–22
- Casassa, E. F. & Berry, G. C. (1966) *J. Polym. Sci.* **4**, 881–887
- Casassa, E. F. & Eisenberg, H. (1964) *Adv. Protein Chem.* **19**, 287–395
- Chantler, E. (1982) in *Mucus in Health and Disease-II* (Chantler, E., Elder, J. & Elstein, M., eds.), pp. 251–263, Plenum Press, New York
- Clamp, J. R., Allen, A., Gibbons, R. A. & Roberts, G. P. (1978) *Br. Med. Bull.* **34**, 25–41
- Creeth, J. M., Bhaskar, K. R., Horton, J. R., Das, I., Lopez-Vidriero, M.-T. & Reid, L. (1977) *Biochem. J.* **167**, 557–569
- Feldhoff, P. A., Bhavanandan, V. P. & Davidson, E. A. (1979) *Biochemistry* **18**, 2430–2436
- Gibbons, R. A. & Sellwood, R. (1973) in *The Biology of the Cervix* (Blandau, R. J. & Moghissi, K., eds.), pp. 251–265, University of Chicago Press, Chicago
- Goa, J. (1955) *Scand. J. Clin. Lab. Invest. Suppl.* **22**, 19–25
- Hatcher, V. B., Schwarzmann, G. O. H., Jeanloz, R. W. & McArthur, J. W. (1977) *Biochemistry* **16**, 1518–1524
- Houdret, N., Le Treut, A., Lhermitte, M., Lamblin, G., Degand, P. & Roussel, P. (1981) *Biochim. Biophys. Acta* **668**, 413–419
- Jourdian, G. W., Dean, L. & Roseman, S. (1971) *J. Biol. Chem.* **246**, 430–435
- Lamblin, G., Lhermitte, M., Degand, P., Roussel, P. & Slayter, H. S. (1979) *Biochimie* **61**, 23–43
- Lee, W. I., Blandau, R. J. & Verdugo, P. (1977a) in *The Uterine Cervix in Reproduction* (Insler, V. & Bettendorf, G., eds.), pp. 68–77, Georg Thieme, Stuttgart
- Lee, W. I., Verdugo, P., Blandau, R. J. & Gaddum-Rosse, P. (1977b) *Gynecol. Invest.* **8**, 254–266
- Lindahl, U. (1970) *Biochem. J.* **116**, 27–34
- Lohmänder, L. S., De Luca, S., Nilsson, B., Hascall, V. C., Caputo, C. B., Kimura, J. H. & Heinegård, D. (1980) *J. Biol. Chem.* **255**, 6084–6091
- Silberberg, A. & Meyer, F. A. (1982) in *Mucus in Health and Disease-II* (Chantler, E., Elder, J. & Elstein, M., eds.), pp. 53–74, Plenum Press, New York
- Tanford, C. (1961) *Physical Chemistry of Macromolecules*, pp. 275–316, John Wiley and Sons, New York
- Tomimatsu, Y., Vitello, L. & Fong, K. (1968) *J. Colloid Interface Sci.* **27**, 573–580
- Wolf, D. P., Sokoloski, J., Khan, M. A. & Litt, M. (1977) *Fertil. Steril.* **28**, 53–58
- Woodward, H., Horsey, B., Bhavanandan, V. P. & Davidson, E. A. (1982) *Biochemistry* **21**, 694–701
- Yphantis, D. A. (1964) *Biochemistry* **3**, 297–317
- Yurewicz, E. C. & Moghissi, K. S. (1981) *J. Biol. Chem.* **256**, 11895–11904
- Zimm, B. H. (1948) *J. Chem. Phys.* **16**, 1099–1116