Biochem. J. (1991) 279, 251–256 (Printed in Great Britain)

Rat gastric mucin is synthesized and secreted exclusively as filamentous oligomers

Jan DEKKER,*† Arie VAN DER ENDE,* Patrick H. AELMANS,* and Ger J. STROUS*‡
*Department of Cell Biology, University of Utrecht, Heidelberglaan 100, Utrecht, and
†Laboratory of Pediatric Gastroenterology, University of Amsterdam, Academic Medical Center, Meibergdreef 9, Amsterdam, The Netherlands

Oligomeric gastric mucin was isolated from the fundic part of the rat stomach. Previously we have shown by biochemical analysis that this oligomeric mucin consists of disulphide-linked homo-oligomers, which contain no other covalently attached proteins [Dekker, Aelmans & Strous (1991) Biochem. J. 277, 423–427]. Electron-microscopic images of the oligomeric mucin revealed a heterogeneous population of long filamentous molecules of 300–3000 nm length. After reduction and carboxymethylation the monomeric mucins displayed a length distribution with a single peak at about 279 nm. Length-distribution analysis of oligomeric molecules with length up to 1000 nm revealed three subpopulations with one, two or three times the length of the monomeric mucin. The oligomers displayed small globular domains of about 15 nm, which were equally spaced along the molecule's length. As the distance between these globular domains was similar to the monomer length, these domains most likely indicate attachment sites of the monomers. These results show that the mucin monomers attached end-to-end in the oligomer. Biosynthesis of the mucin oligomers was studied by labelling of stomach explants *in vitro* with [^{35}S]methionine, [^{3}H]galactose or [^{35}S]sulphate and subsequent immunoprecipitation of the mucin with a specific antiserum. Analysis by electrophoresis and gel filtration revealed that the oligomerization takes place by formation of disulphide bonds between the 300 kDa mucin precursors. The mucin was exclusively synthesized and secreted as fully glycosylated oligomers, as neither precursor proteins nor monomeric mucin were detected in the culture medium. A model for the biosynthesis of rat gastric mucin is proposed in which the filamentous mucin monomers are linked end-to-end by disulphide bonds.

INTRODUCTION

An adherent mucus layer on the stomach epithelium protects the gastric mucosa against endogenous agents, like HCl and pepsin, as well as against exogenous substances which have been swallowed (Allen *et al.*, 1986; Kerss *et al.*, 1982; McQueen *et al.*, 1983; Neutra & Forstner, 1987). The mucus forms a visco-elastic gel that covers the epithelial cells, separating them from stomach contents. It has unique abilities allowing it to flow and to anneal if severed (Allen *et al.*, 1982). Mucus glycoproteins (mucins) are the most important structural components of the mucus, which are synthesized by highly polarized specialized cells. Reconstituted gels formed *in vitro* of isolated intact gastric mucins at physiological concentrations (about 50 mg/ml) display rheological properties similar to those of the intact mucus gel (Allen *et al.*, 1982; Bell *et al.*, 1984; Sellers *et al.*, 1988). It is therefore generally accepted that the mucins are responsible for the unique visco-elastic properties of the gastric mucus gel.

On the molecular level, gel formation by mucins depends mainly on two features of the molecules. One mucin subunit contains hundreds of oligosaccharides comprising up to 80 % of the weight of the molecule, which are *O*-linked at serine and threonine residues. In a mucus gel the mucin molecules interact by formation of hydrophilic non-covalent bonds between the oligosaccharides (Mian *et al.*, 1982; Verdugo *et al.*, 1983; Bell *et al.*, 1984; Carlstedt & Sheehan, 1984; Sterk *et al.*, 1987; Sellers *et al.*, 1988). Therefore the number and structure of the oligosaccharides partly determine the rheological properties of the mucin gel. The second feature of gastric mucin molecules, and one that is essential for gel formation, is their disulphide-bound oligomeric structure (Allen, 1981; Carlstedt & Sheehan, 1984; Neutra & Forstner, 1987; Roussel *et al.*, 1988; Sellers *et al.*, 1988). Chemical reduction of the disulphide bonds of oligomeric gastric mucins results in complete loss of gel-forming properties (Allen & Snary, 1972; Allen, 1978; Allen *et al.*, 1980; Bell *et al.*, 1984).

The biogenesis of oligomeric mucins is poorly understood. Previously we showed that the oligomeric rat gastric mucin consists exclusively of disulphide-linked mucin-type monomers without other proteins (Dekker *et al.*, 1989*b*, 1991). In the present study we investigated the structure of newly synthesized and secreted oligomeric rat gastric mucin. We analysed oligomeric mucin isolated from rat stomach by electron microscopy. The biosynthesis of the oligomeric mucin was studied by '*in vitro*' metabolic labelling experiments in stomach explants. On the basis of our results we propose a mechanism for oligomerization of rat gastric mucin in which the filamentous mucin subunits are linked end-to-end by disulphide-bond formation before *N*-glycosylation, sulphation and secretion from the cells.

METHODS

Isolation of oligomeric rat fundic mucus glycoprotein

Mucins were isolated from rat fundus as described (Dekker *et al.*, 1989*a*, 1991). Briefly, oligomeric mucins were isolated in the presence of 100 mM-iodoacetamide to inhibit rearrangement of disulphide bonds. Fundic tissue was obtained from 20 male Wistar rats. About 1 g (wet weight) of mucosa was homogenized at 4 °C in a 50 mM-Tris/5 mM-EDTA buffer, pH 7.5, containing 6 M-guanidinium chloride, 1 mM-phenylmethanesulphonyl fluoride and 100 mM-iodoacetamide. The mucin was isolated using three subsequent isopycnic-density-centrifugation steps

‡ To whom correspondence should be sent.

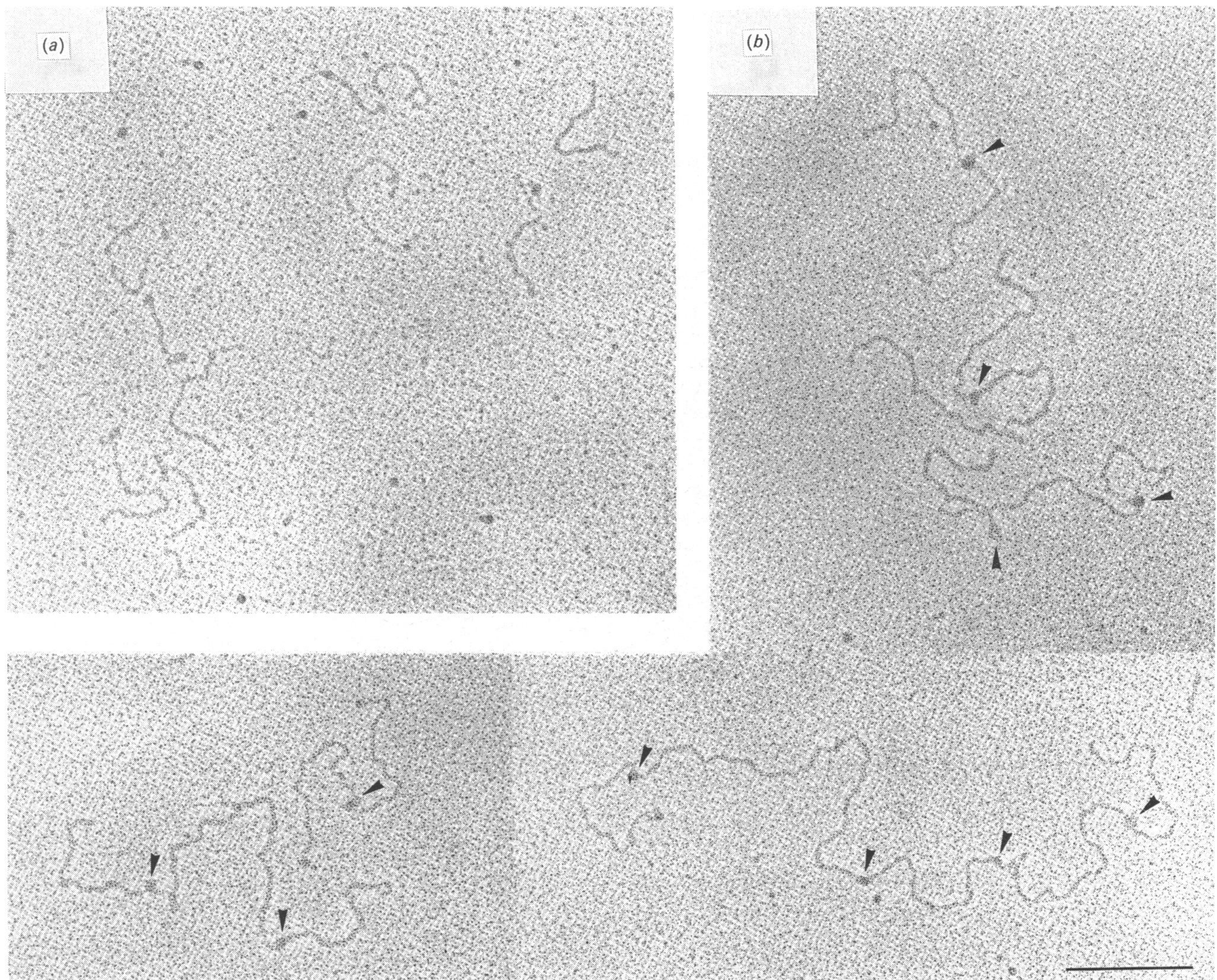

Fig. 1. Rat gastric mucin revealed by electron microscopy

(*a*) Mucin after reduction and carboxymethylation; (*b*) mucin before reduction. Globular structures on the oligomers, which probably indicate the attachment site of two adjacent monomers, are indicated by arrowheads. The bar represents 200 nm.

[two 2.4 M-CsCl/4 M-guanidinium chloride gradients (density 1.4 g/ml) and one 2.9 M-CsCl/1 M-guanidinium chloride gradient (density 1.5 g/ml)]. The mucin in the density gradients was detected after dialysis of small aliquots of the gradient fractions against water and subsequent SDS/PAGE. Mucin-containing fractions were pooled. After the third centrifugation step the isolated mucin was dialysed against water and stored at −20 °C.

Analytical methods

Gel filtration on Sepharose CL-2B (Pharmacia) in the presence of guanidinium chloride was performed as described by Carlstedt *et al.* (1983). The gel-filtration column was 100 cm long with 1.6 cm internal diameter. Reduction and carboxymethylation of oligomeric mucins by dithiothreitol and iodoacetamide respectively were performed as described by Fontana & Gross (1986). SDS/PAGE was performed by the method of Laemmli (1970). Samples were applied to the 4 % (w/v) polyacrylamide gels after boiling for 3 min in sample buffer containing 1 % SDS, with or without 5 % (v/v) 2-mercaptoethanol. Reduced and non-reduced thyroglobulin were used as molecular-mass standards. Fluorograms of gels were recorded as described by Bonner & Laskey (1974). The radiolabelled glycoproteins were detected by exposure to pre-exposed Kodak SB-5 X-ray films.

Electron microscopy of isolated gastric mucin

Sample preparation of intact, reduced and carboxymethylated mucin was essentially the same as described for nucleic acids by Labhart & Koller (1981). Alcian Blue-treated carbon-coated grids were allowed to float on 10 μl of mucin in water (0.5 μg/ml) for 1–2 min. After adsorption, the grids were washed by floating on distilled water for 15 min. Staining and dehydration was performed as described by Vollenweider *et al.* (1976). The grids were rotary-shadowed with platinum at an angle of 7°. Micrographs were taken in a Jeol 1200 EX microscope, operating at 80 kV at a magnification of 20000 or 40000 ×.

Metabolic labelling of gastric mucin

The metabolic labelling of rat gastric tissue was performed as described previously (Dekker *et al.*, 1989*b*). Briefly, the stomach of inbred male Wistar rats was obtained after killing the rats by cervical dislocation. Small sections (2 mm × 2 mm) were cut from the fundic part of the stomach. The segments were placed in

prewarmed Eagle's minimal essential medium (Gibco) and incubated for 30 min at 37 °C under O_2/CO_2 (19:1). The tissue was metabolically labelled for 15 min with either [^{35}S]methionine (sp. radioactivity 900 Ci/mmol), [^{3}H]galactose (sp. radioactivity 22 Ci/mmol) or [^{35}S]sulphate (sp. radioactivity 25 Ci/mg) (all from Amersham International) and homogenized. The radiolabelled mucin was isolated by immunoprecipitation, a specific antiserum (Dekker *et al.*, 1989*b*) being used. The radiolabelled mucin was analysed either by SDS/PAGE or by Sepharose CL-2B gel filtration. The immunoprecipitated samples were boiled in sample buffer containing SDS or 6 M-guanidinium chloride before electrophoresis and gel filtration respectively.

RESULTS AND DISCUSSION

Electron-microscopic image of rat gastric mucin

Previously we showed that the oligomeric mucin isolated from the rat stomach was composed exclusively of mucin-type subunits, which were defined as mucin monomers (Dekker *et al.*, 1989*b*, 1991). To reveal the oligomeric mucin structure and its subunits, we recorded electron micrographs of rat gastric mucin before and after reduction and carboxymethylation. After reduction molecules were observed with a linear filamentous appearance (Fig. 1*a*). The length distribution of these molecules displayed in Fig. 2(*c*) showed a single peak with a mean length of 279 ± 94 nm (S.D., $n = 171$). The structure of the oligomeric mucin, observed without reduction, appeared very similar to that of the monomers. The oligomeric mucin appeared as filamentous molecules without any branching points, with lengths varying between 300 and 3000 nm (Fig. 1*b*). The oligomers tend to cluster on the grids; therefore it was impossible to record the length distribution, as the chance of recording a separated, stretched, oligomer was inversely proportional to its length. Recorded mucin molecules scored up to 1000 nm consisted mainly of three subpopulations, which showed mean lengths similar to one, two or three times the length of the monomeric subunit (Fig. 2*a*). These recordings indicate that the monomer is the smallest unit in the mucin oligomer, as the length of the oligomer is a multiple of the monomer length.

In addition, the oligomers showed small globular structures (diameter about 15 nm) at regular intervals that were absent on the monomeric subunit (Fig. 1). The distance between the globular domains on the oligomers [350 ± 128 nm mean ± S.D.); $n = 286$) was virtually identical with the mean length of the reduced monomer (Figs. 2*b* and 3*c*). This suggests that the globular structures indicate attachment sites of two subunits in the oligomers. From biochemical analysis it was clear that the oligomers were free from other proteins (Dekker *et al.*, 1989*b*, 1991). Therefore the globular domain on the oligomers most likely represents a structure within the mucin molecule that is abolished upon reduction.

Our conclusion is that the filamentous monomeric subunits in rat oligomeric mucin are linked together end-to-end in the oligomers. The occurrence of oligomers longer than dimers indicates that monomers are able to form intermolecular disulphide bonds at both ends of the molecule (i.e. on both the *N*- and *C*-terminus of the polypeptide chain). Similar electron-microscopic studies on human cervical and tracheo-bronchial mucin showed that these mucins consist of monomeric subunits of 390 and 490 nm length respectively, linked end-to-end by disulphide bonds into oligomers with lengths up to 3 μm (Sheehan & Carlstedt, 1990; Thornton *et al.*, 1990). The main difference between these mucins and the rat gastric mucin is the sensitivity towards proteolytic enzymes. In a previous study we have shown that proteinase treatment of monomeric mucin results in a length decrease of 28 % (Dekker *et al.*, 1989*a*). Taken together with the present data we conclude that the rat gastric mucin monomer consists of one large proteinase-resistant glycopeptide of about 300 nm. This is clearly different from the subunit structure of human cervical and tracheo-bronchial mucins, which contain several proteinase-resistant glycopeptides of about 100 nm (Sheehan & Carlstedt, 1990; Thornton *et al.*, 1990). Apparently in these mucins the glycosylated part of the protein backbone is interrupted by non-glycosylated amino acid sequences that are sensitive to proteolytic enzymes.

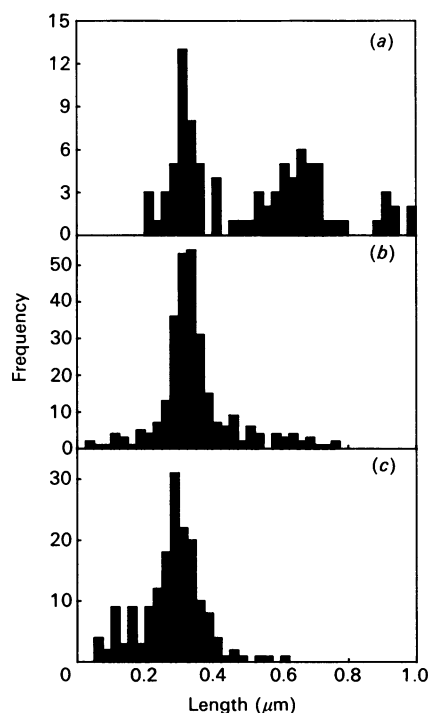

Fig. 2. The length of rat gastric mucin

The length of mucin molecules was determined from electron micrographs as shown in Fig. 1. Only clearly separated molecules of 0–1000 nm, free from kinks, were measured. The number of molecules in a class was plotted against their length in classes of 25 nm. (*a*) Length distribution of oligomeric mucin; (*b*) distribution of the distance between the globular domains in the oligomeric mucin, indicated by arrowheads in Fig. 1; (*c*) length distribution of reduced monomeric mucin.

Biosynthesis of oligomeric rat gastric mucin

Previously we showed by metabolic labelling of gastric tissue that rat gastric mucin is synthesized as a 300 kDa *N*-glycosylated precursor protein that oligomerizes in the rough endoplasmic reticulum by formation of intermolecular disulphide bonds (Dekker *et al.*, 1989*b*; Dekker & Strous, 1990). Here, we address the question how rat gastric mucous cells synthesize and secrete mature oligomeric mucin. To this end, we pulse-labelled the mucin protein backbone in gastric tissue explants *in vitro* with [^{35}S]methionine and chase-incubated the tissue to record the synthesis and secretion of mature oligomeric mucin. Similarly, we labelled gastric mucous tissue with [^{3}H]galactose or [^{35}S]sulphate, which are incorporated into oligosaccharides of the mucin in the *trans* cisternae of the Golgi complex (Kramer *et al.*, 1978). The metabolically labelled mucin was extracted from the tissue homogenates and the incubation media by immunoprecipitation and was analysed by both SDS/4 %-PAGE and gel filtration on Sepharose CL-2B.

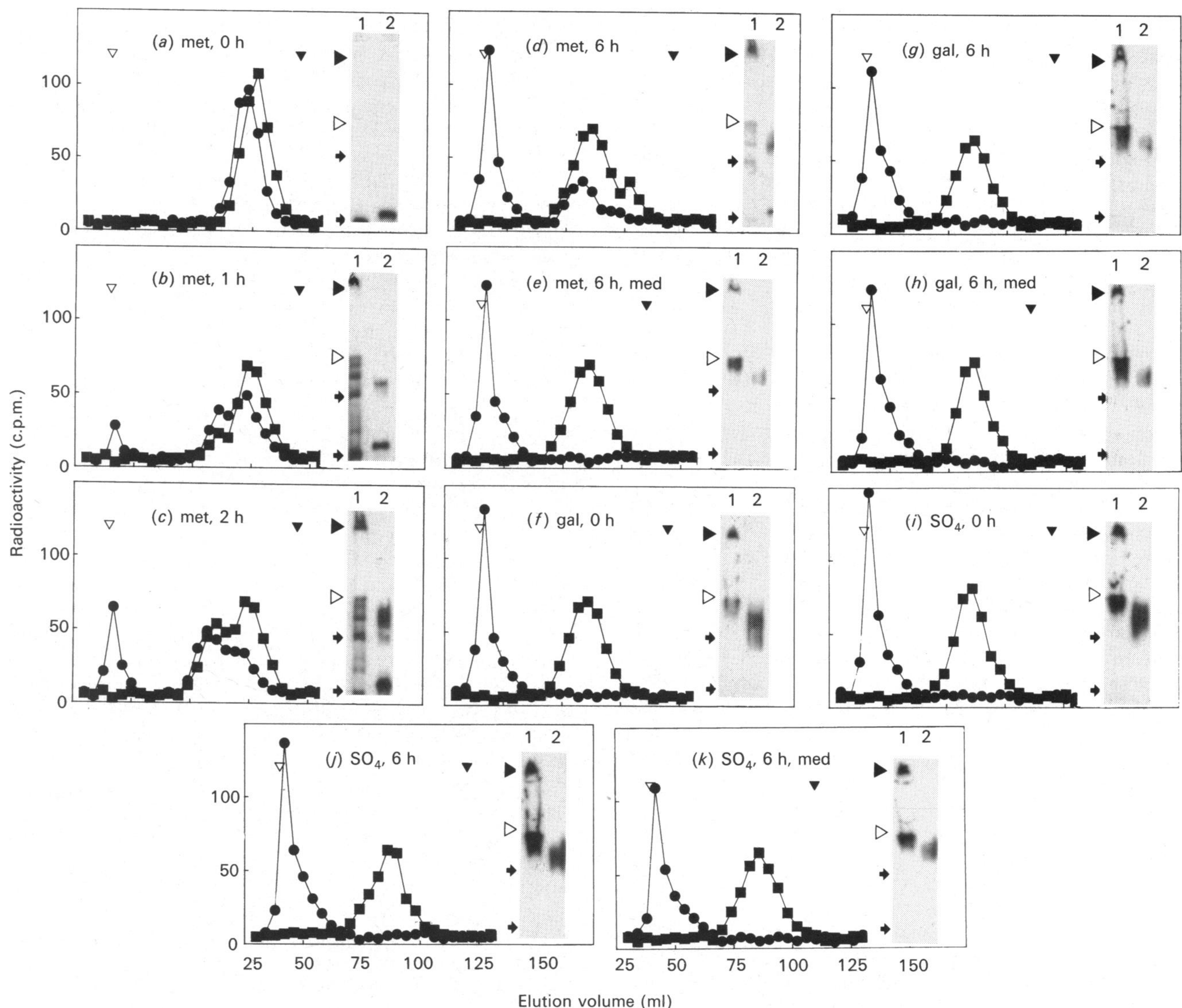

Fig. 3. Biosynthesis of oligomeric rat gastric mucin

Gastric tissue from rat was labelled for 15 min with either [^{35}S]methionine (met), [^{3}H]galactose (gal) or [^{35}S]sulphate (SO_4) and chase-incubated for the time indicated. The tissue was homogenized and mucin was immunoprecipitated from the homogenate or culture medium (med) using a specific antiserum. The immunoprecipitated mucin was divided into four aliquots, two of which were reduced and carboxymethylated. The mucin was analysed by gel filtration on Sepharose CL-2B, before (●) and after (■) reduction. The open and closed arrowheads in the gel-filtration analyses indicate the void and total volume of the gel filtration column respectively. In each case the radiolabelled immunoprecipitated mucin was also analysed by reducing and non-reducing SDS/4%-PAGE (3% stacking gel) (insets). Lanes 1, non-reduced mucin; lanes 2, reduced mucin. Molecular-mass standards are indicated by arrows at 300 and 660 kDa. The open and closed arrowheads in the SDS/PAGE analyses indicate the border of the running and stacking gel and the top of the stacking gel respectively.

As Fig. 3(*a*) shows, the mucin is initially synthesized as a 300 kDa protein, a fact which is demonstrated by SDS/PAGE after labelling with [^{34}S]methionine. This species can be labelled with neither [^{3}H]galactose nor [^{35}S]sulphate (Figs. 3*f* and 3*g*). The molecule is eluted on gel filtration at about 100 ml (Fig. 3*a*). The [^{35}S]methionine-labelled 300 kDa protein oligomerized into di-, tri- and tetra-mers by disulphide-bond formation, as shown by SDS/PAGE (Figs. 3*a*–3*e*). These di-, tri- and tetra-meric precursors, which were resolved by SDS/PAGE, could not be resolved on gel-filtration analysis and were eluted as one peak at around 85 ml (Figs. 3*b*–3*d*). Reduction of the oligomeric 300 kDa precursors resulted in quantitative recovery of the monomeric precursors in the 100 ml peak on gel filtration (Figs. 3*a*–3*e*).

The mature rat gastric mucin is heavily *O*-glycosylated and sulphated (Dekker *et al.*, 1989*a*,*b*; Dekker & Strous, 1990). On gel filtration this glycosylation and sulphation results in the accumulation of [^{35}S]methionine-labelled high-molecular-mass molecules in the void volume of the column (Figs. 3*d* and 3*e*). On reduction and carboxymethylation this mucin was quantitatively recovered in a peak at about 85 ml. We have shown previously that this reduced, high-molecular-mass mucin behaved similarly to unlabelled 'mature' mucin isolated from rat stomach on both gel filtration and SDS/PAGE (Dekker *et al.*, 1991). The metabolically labelled oligomeric mucin found in the void volume of the gel-filtration column is therefore most likely similar to the oligomeric mature mucin isolated from rat stomach.

To establish the moment of maturation in the biosynthesis of the oligomeric rat gastric mucin we incubated the tissue with radiolabelled galactose and sulphate. The elution patterns of

immunoprecipitated [^{35}S]sulphate-labelled mucin from rat tissue and culture medium were similar to the patterns obtained after labelling with [^{3}H]galactose (Figs. 3*f*–3*k*). Both [^{3}H]galactose and [^{35}S]sulphate are exclusively incorporated into oligomers, no radiolabelled subunits being detected on gel filtration. Therefore we conclude that only oligomerized precursors reach the *trans* cisternae of the Golgi complex. Neither precursor protein nor monomeric mature mucin was detected in the medium after labelling in any of the metabolic labelling experiments (Figs. 3*e*, 3*h* and 3*k*). Thus only mature oligomers were secreted.

Biochemical analysis of mucin preparations isolated from stomach showed small amounts of monomer-sized mucin fragments (Dekker *et al.*, 1991). The electron-microscopic analysis in our present study also showed these mucin fragments (Fig. 2*a*). However, monomeric-sized species were never found in the metabolically labelled mucin from stomach tissue nor in culture medium (Fig. 3). Thus the presence of these species in the oligomer preparations of the rat most likely indicates that they originate from peptic degradation in the stomach lumen.

Two models have been proposed for the oligomeric structure of mucins. The first model was proposed for porcine gastric mucin in which four identical mucin monomers were individually linked by disulphide bonds to a central link protein (Allen, 1981). Since then, a controversy has existed as to whether mucin oligomers contain covalently linked non-mucin proteins. A 118 kDa 'link' protein was found in salivary, gastric, small-intestinal and colonic mucins from four different species (Robertson *et al.*, 1990). However, in our studies of rat and human gastric mucin we have never found evidence for the presence of covalently bound non-mucin proteins in the mucin oligomers (Dekker *et al.*, 1989*b*; Dekker & Strous, 1990; Dekker *et al.*, 1991). Thus our conclusion is that rat gastric mucin consists of homo-oligomers of mucin-type glycoproteins.

In 1984 a second model was proposed by Carlstedt & Sheehan for human cervical mucin. According to their model the mucin monomers are linked directly together by disulphide bonds to form linear oligomers without a 'link' protein (Carlstedt & Sheehan, 1984). This 'flexible-thread' model has been verified since then by physical and electron-microscopic data for a number of other mucins (Harding *et al.*, 1983; Slayter *et al.*, 1984; Rose *et al.*, 1984; Forstner & Forstner, 1986; Sheehan *et al.*, 1986; Sheehan & Carlstedt, 1990). Values of measured contour lengths must be taken with caution, as accumulated data show that there may be very substantial folding in linear filamentous mucin molecules (Hutton *et al.*, 1988). Our data on the structure and biosynthesis of oligomeric rat gastric mucin suggest that the model of Carlstedt & Sheehan (1984) is also applicable to rat gastric mucin. On the basis of our studies on the structure and maturation we propose the following features for the biogenesis of mucin.

(1) The length of oligomers is determined in the rough endoplasmic reticulum by assembling a limited number of monomers end-to-end via disulphide bonds (Dekker & Strous, 1990). This process is regulated by the presence of *N*-linked oligosaccharides at strategic positions on the polypeptide (Dekker & Strous, 1990).

(2) The rat gastric mucin consists of homo-oligomers, as analysis of the isolated oligomers revealed that no other proteins were covalently attached to the oligomers (Dekker *et al.*, 1989*b*, 1991).

(3) The mature monomeric subunits are long filamentous molecules that are linked end-to-end to form filamentous linear oligomers.

(4) Both ends of the monomeric subunits are capable of disulphide-bond formation, as oligomers larger than dimers are commonly observed in electron microscopy. Thus both *N*- and *C*-terminal ends of the mucin polypeptide are capable of forming intermolecular disulphide bonds.

(5) The mucins exclusively acquire *O*-linked oligosaccharides and sulphate in the Golgi complex in oligomeric state, as no (mature) monomers were observed after metabolic labelling of the mucin with either [^{3}H]galactose or [^{35}S]sulphate. Moreover, previous experiments showed that only 5% of the 300 kDa precursor was retained on a *N*-acetylgalactosamine-specific lectin column, indicating that the initial *O*-glycosylation takes place primarily after precursors have left the rough endoplasmic reticulum (Dekker & Strous, 1990).

The accumulated data on the structure of mucin molecules from various species and organs and their oligomers show considerable similarities within this group of glycoproteins. Therefore the features described above for the biogenesis of rat gastric mucin probably apply to most mucins.

We thank Maurits Niekerk, René Scriwanek and Tom van Rijn for preparation of the Figures. This work was partially financed by the Foundation for Medical Research (NWO; grant 900-522-065) and by NATO (grant 0316/87).

REFERENCES

Allen, A. (1978) Br. Med. Bul. **34**, 28–33

Allen, A. (1981) in Physiology of the Gastrointestinal Tract (Johnson L. R., ed.), pp. 617–639, Raven Press, New York

Allen, A. & Snary, D. (1972) Gut **13**, 666–672

Allen, A., Pearson, J. P., Venables, C. W. & Younan, F. (1980) J. Physiol. (London) **306**, 40*P*

Allen, A., Bell, A., Mantle, M. & Pearson, J. P. (1982) Adv. Exp. Med. Biol. **144**, 115–133

Allen, A., Hutton, D. A., Leonard, A. J., Pearson, J. P. & Sellers, L. A. (1986) Scand. J. Gastroenterol. **21** (suppl. 125), 71–77

Bell, A., Allen, A., Morris, E. R. & Ross-Murphy, S. B. (1984) Int. J. Biol. Macromol. **6**, 309–315

Bonner, W. M. & Laskey, R. A. (1974) Eur. J. Biochem. **46**, 83–88

Carlstedt, I. & Sheehan, J. K. (1984) CIBA Found. Symp. **109**, 157–166

Carlstedt, I., Lindgren, H., Sheehan, J. K., Ulmsten, U. & Wingerup, L. (1983) Biochem. J. **211**, 13–22

Dekker, J. & Strous, G. J. (1990) J. Biol. Chem. **265**, 18116–18122

Dekker, J., Van Beurden-Lamers, W. M. O., Oprins, A. & Strous, G. J. (1989*a*) Biochem. J. **260**, 717–723

Dekker, J., Van Beurden-Lamers, W. M. O. & Strous, G. J. (1989*b*) J. Biol. Chem. **264**, 10431–10437

Dekker, J., Aelmans, P. H. & Strous, G. J. (1991) Biochem. J., in the press

Fontana, A. & Gross, E. (1986) in Practical Protein Chemistry (Darbre A., ed.), pp. 67–120, Wiley, New York

Forstner, G. G. & Forstner, J. F. (1986) in Molecular and Cellular Basis of Digestion (Desnuelle, P., Sjöström, H. & Norén, O., eds.), pp. 125–143, Elsevier, Amsterdam

Harding, S. E., Rowe, A. J. & Creeth, J. M. (1983) Biochem. J. **209**, 893–896

Hutton, D. A., Allen, A. & Slayter, H. S. (1988) Biochem. Soc. Trans **16**, 584–585

Kerss, S., Allen, A. & Garner, A. (1982) Clin. Sci. **63**, 187–195

Kramer, M. F., Geuze, J. J. & Strous, G. J. (1978) Ciba Found. Symp. **54**, 25–51

Labhart, P. & Koller, T. (1981) Eur. J. Cell Biol. **24**, 309–316

Laemmli, U. K. (1970) Nature (London) **227**, 680–685

McQueen, S., Hutton, D. A., Allen, A. & Garner, A. (1983) Am. J. Physiol. **8**, G388–G394

Mian, N., Pope, A. J., Anderson, C. E. & Kent, P. W. (1982) Biochim. Biophys. Acta **717**, 41–48

Neutra, M. R. & Forstner, J. R. (1987) in Physiology of the Gastrointestinal Tract, 2nd edn. (Johnson L. R., ed.), pp. 975–1009, Raven Press, New York

Robertson, A. M., Mantle, M., Fahim, R. E. F., Specian, R. D., Benneck, A., Kawagishi, S., Sherman, P. & Forstner, J. F. (1990) Biochem. J. **261**, 637–647

Rose, M. C., Voter, W. A., Sage, H., Brown, C. F. & Kaufman, B. (1984) J. Biol. Chem. **259**, 3167–3172
Roussel, P., Lamblin, G., Lhermitte, M., Houdret, N., Lafitte, J.-J., Perini, J.-M., Klein, A. & Scharfman, A. (1988) Biochimie **70**, 1471–1482
Sellers, L. A., Allen, A., Morris, E. R. & Ross-Murthy, S. B. (1988) Carbohydr. Res. **178**, 93–110
Sheehan, J. K. & Carlstedt, I. (1990) Biochem. J. **265**, 169–178
Sheehan, J. K., Oates, K. & Carlstedt, I. (1986) Biochem. J. **239**, 147–153
Slayter, H. S., Lamblin, G., Le Treut, A., Galabert, C., Moudret, N., Degrad, P., Roussel, P. (1984) Eur. J. Biochem. **142**, 209–218
Sterk, H., Fabian, W. & Hayne, E. (1987) Int. J. Biol. Macromol. **9**, 48–62
Thornton, D. J., Davies, J. R., Kraayenbrink, M., Richardson, P. S., Sheehan, J. K. & Carlstedt, I. (1990) Biochem. J. **265**, 179–186
Verdugo, P., Tam, P. Y. & Butler, J. (1983) Biorheology **20**, 223–230
Vollenweider, H. J., Koller, T., Weber, H., Weissmann, C. J. (1976) J. Mol. Biol. **101**, 367–377

Received 18 February 1991/18 April 1991; accepted 8 May 1991