The mechanism of thermal degradation of a high-molecular-weight glycoprotein complex from bovine cervical mucus

Frank A. MEYER and Gaio PARADOSSI*

Polymer Research Department, The Weizmann Institute of Science, Rehovot, Israel 76100

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Gel-like oestrus bovine cervical mucus can be brought to the point of dissolution by thermal treatment. The glycoprotein complex so produced was isolated on CsCl density gradients, and found to be of a size comparable with that of a complex purified from mucus that had been brought to the point of dissolution by mild mechanical stirring. The latter material (GP-S) had a mol.wt. of 15.9×10^6 and was used to study further the effect of thermal treatment. Time and temperature lead to a gradual breakdown of GP-S, which is characterized by a single activation energy of 93.3 kJ/mol (22.3 kcal/mol) over the temperature range of 21–99°C. The process responsible is thermal hydrolysis of peptide bonds, particularly next to aspartic acid residues. This conclusion is consistent with the appearance of aspartic acid as a new *N*-terminal amino acid and the activation energy of the process. After thermal degradation there is an increase in the buoyant density of GP-S and a change in the amino acid composition. These findings were found to be consistent with the loss of the naked peptide region and the preponderance of aspartic acid residues in this region. Thermal degradation therefore does not involve dispersion of non-covalent bonds, and indeed GP-S is quite unaffected by media commonly used to disperse such bonds.

The functions of mucus secretions are dependent, in large measure, on their gel character (King *et al.*, 1974; Wolf *et al.*, 1978; Gelman & Meyer, 1979). The cross-links that give rise to the gel structure depend, at least in part, on disulphide bonds that are believed to occur between the network-producing biopolymer. The latter is a glycoprotein whose protein backbone involves two types of domains, a naked peptide region and a glycosylated region (Donald, 1973; Gibbons & Sellwood, 1973; Gelman & Vered, 1976; Roberts, 1976; Bhushana Rao & Masson, 1977; Scawen & Allen, 1977; Roberts, 1978; Marshall & Allen, 1978; Mantle *et al.*, 1981), which differ in amino acid composition and in susceptibility to proteinases. The glycosylated region is protected against enzymic attack which affects the naked peptide regions, resulting in their removal. Although the naked peptide regions account for only a few per cent of the glycoprotein, they seem to be the site of the cross-links. The naked peptide contains most, if not all, of the cystine residues and

hence of the disulphide cross-links; cysteine residues are not present in the glycoprotein. Reduction of disulphide bonds or proteolysis thus both lead to mucus solubilization. The end product of reduction and/or proteolysis is commonly found to be a single chain of mol.wt. approx. 500 000 (Degand *et al.*, 1973; Hatcher *et al.*, 1977; Meyer, 1977; Scawen & Allen, 1977; Marshall & Allen, 1978; Sachdev *et al.*, 1978; Pearson *et al.*, 1980; Mantle *et al.*, 1981).

Non-covalent bonds between molecular units have also been proposed in maintaining the integrity of the mucus gel. This would help explain, for example, how mucus anneals when sectioned (Silberberg, 1979; Allen *et al.*, 1982) or how mucus droplets exteriorized from individual membrane-bound intracellular storage granules form bulk mucus (Silberberg, 1979). However, the nature of these bonds and of the molecular unit obtained after such bonds have been selectively broken has not been established.

The finding that mucus is readily solubilized merely by thermal treatment could be considered as evidence in support of non-covalent bonds (Meyer & Silberberg, 1978). Studies (Meyer, 1976; Gelman & Meyer, 1979) of the dissolution of oestrus bovine cervical mucus indicate that it occurs spontaneously with time and is very rapid at 100°C. At 4°C,

Abbreviation used: PBS, phosphate-buffered saline (0.127 M-NaCl, 0.017 M-Na₂HPO₄, 12H₂O, 0.0085 M-HCl, 0.02% NaN₃, adjusted to pH 7.0).

* Present address: Istituto di Chimico Fisica, Università di Roma, 00185-Roma, Italy.

however, this mucus is stable for relatively long periods. Preliminary findings, moreover, indicate that the glycoprotein complex present when mucus first comes into solution is also thermally unstable (Meyer, 1976; Meyer & Silberberg, 1978).

The purpose of the present study is to investigate the mechanism of thermal degradation. This has been done with the glycoprotein complex, obtained at the point of mucus dissolution, which has a mol.wt. of approx. 16×10^6 .

Experimental

Oestrus bovine cervical mucus

This was collected from healthy cows before artificial insemination. Samples were pooled and immediately frozen. Mucus samples that were coloured or contaminated by more than traces of insoluble material were discarded. Mucus was allowed to thaw out at 4°C before use.

Thermal and mechanical degradation of mucus to the point of dissolution

Two mild methods, mechanical stirring and thermal treatment, are available to bring mucus to the point of dissolution.

(i) *Mechanical stirring.* To 1 vol. of mucus, 3 vol. of PBS was added. The two-phase system (insoluble mucus gel and solution phase), of volume 210 ml, was stirred on a magnetic stirrer (6 cm bar stirring at 80 rev./min) at 4°C until the presence of a single phase just indicated dissolution.

(ii) *Thermal treatment.* Mucus that had been dialysed against PBS for 2 days at 4°C was heated at 80°C. Dissolution is obtained when the mucus can just be poured as a homogeneous liquid. This generally occurred after some 20 min of heating.

Isolation of glycoproteins solubilized by thermal and mechanical dissolution of mucus

The glycoprotein complexes brought into solution were isolated on CsCl density gradients as previously described (Meyer *et al.*, 1973; Meyer, 1977). Centrifugation was performed at 100 000 *g* at 4°C for 48 h on a Beckman model L preparative ultracentrifuge with the no. 50 rotor. The first centrifuge run was performed at an initial density of 1.42 g/ml to sediment the glycoprotein (buoyant density approx. 1.52 g/ml) into the bottom half of the tube; proteins (buoyant density approx. 1.33 g/ml) stay at the top of the tube. The glycoprotein fraction was then re-centrifuged in CsCl (initial density 1.50 g/ml) so as to remove trace amounts of protein contaminants and nucleic acids (buoyant density about 1.70 g/ml). The fraction of density 1.43–1.64 g/ml from this centrifugation run was collected and exhaustively dialysed against PBS at 4°C. The purified glycoproteins so prepared from

thermal and from mechanical dissolution of mucus are referred to as GP-T and GP-S respectively. GP-S was used further to prepare reduced GP-S and Pronase-digested GP-S. It also provided the starting material for investigation of the effect of thermal degradation.

Reduced GP-S

This was prepared from GP-S (1 mg/ml) by using 2 mM-dithiothreitol in 0.135 M-Tris/HCl buffer, pH 8.0. Reduction was performed at 37°C for 1 h under an atmosphere of N₂. Iodoacetamide was then added to give a final concentration of 3 mM and the solution was incubated at room temperature for 30 min and then dialysed exhaustively against PBS buffer.

Determination of thiol groups liberated

The thiol groups liberated by the above reduction of GP-S and by thermal degradation were determined by alkylation with iodo[1-¹⁴C]acetamide. To 1 vol. of GP-S, 0.01 vol. of a solution prepared by dissolving 50 µCi of iodo[1-¹⁴C]acetamide (49 Ci/mol; The Radiochemical Centre, Amersham, Bucks., U.K.) in 1.0 ml of 0.30 M-iodoacetamide in 0.135 M-Tris/HCl buffer, pH 8.0, was added. Uptake of label was determined, after extensive dialysis against PBS, in a Packard Tri-Carb 3255 liquid-scintillation spectrometer with scintillation fluid (Bray, 1960). GP-S initially has 0.2 nmol of free thiol groups/mg of glycoprotein.

Pronase-digested GP-S

This was prepared by incubation of 2.5 mg of GP-S/ml in PBS buffer, pH 7.0, with 0.25 mg of Pronase (B grade; Calbiochem, San Diego, CA, U.S.A.)/ml at 37°C for 10 h, followed by a further addition of the same amount of Pronase. Fig. 1 shows that the viscosity rapidly decreases initially and that by 9 h little further change occurs. An incubation time of 16 h was therefore considered adequate for digestion. After proteolysis, the digest was centrifuged in a CsCl density gradient at an initial density of 1.42 g/ml, as described above, and the Pronase-digested GP-S was collected from the bottom half of the centrifuge tube. CsCl was removed by exhaustive dialysis against PBS at 4°C.

Thermal treatment of glycoproteins

Solutions in closed bottles containing about 0.5–2 mg of glycoprotein/ml were heated in a thermostatically controlled bath in the dark. Unless otherwise stated thermal treatment was performed in PBS, pH 7.0.

Analytical methods

Sialic acid was determined by the method of Warren (1959), after hydrolysis in 0.05 M-H₂SO₄ at

Fig. 1. Effect of Pronase on the reduced viscosity of GP-S as a function of time at 37°C in PBS, pH 7.0 GP-S (2.5 mg/ml) was digested with Pronase (0.25 mg/ml). A second addition of an equal amount of fresh Pronase solution was made after 10 h. Viscosity measurements were performed at 20°C in a semi-micro Cannon–Ubbelohde viscometer.

80°C for 1 h, with *N*-acetylneuraminic acid as standard, and protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Amino acid composition (Spackman *et al.*, 1958) was determined on a Dionex model D-500 amino acid analyser (Dionex, Sunnyvale, CA, U.S.A.) on samples that had been dialysed against distilled water, freeze-dried and then hydrolysed in 6M-HCl at 100°C for 20 h *in vacuo*. *N*-Terminal amino acids were identified by chromatography of their dansyl (5-dimethylaminonaphthalene-1-sulphonyl) derivatives (Gray, 1972) on polyamide thin layers (Woods & Wang, 1967).

Glycoprotein concentrations were estimated from dry-weight determinations. For GP-S and reduced GP-S, concentrations were also determined by analysis for sialic acid, which represents 9.0% (w/w) of the dry weight. Glycoprotein concentrations in the various experiments were adjusted by dilution, or by concentration with a Minicon Macrosolute Concentrator, type B15 (Amicon, Oosterhout, Holland), at 4°C.

Sedimentation-velocity runs

These were performed at 20°C in a Beckman model E analytical ultracentrifuge equipped with schlieren optics by using solutions that had been extensively dialysed against PBS, pH 7.0.

Experiments were performed over the concentration range 0.5–5.0 mg/ml. Linear plots of $1/s$ against concentration were obtained and used to derive sedimentation coefficients ($s_{25,w}^0$) by using a relative viscosity and a density for the PBS buffer at 25°C of 1.013 and 1.006 g/ml respectively.

Light scattering

Total-intensity light-scattering experiments were performed with a modified Malvern photon correlator [Malvern Molecular Analyser 4300; Precision Devices and Systems (U.K.) Ltd., Malvern, Worcs., U.K.] with polarized laser light of wavelength 623.8 nm (Jolly & Eisenberg, 1976; Voordouw *et al.*, 1978). The total intensities of the scattered light measured at 23°C between 20° and 150° were treated by the method of Zimm (1948). A specific refractive-index increment for glycoprotein of 0.147 was used (Meyer, 1977).

Dust was removed by the techniques developed by Dubin (1972). Solutions that had been dialysed against PBS were clarified by centrifugation at 15000g for 1 h and transferred, in a clean-air cabinet, to dust-free cuvettes. The solvent PBS (diffusate) was cleaned by filtration through a 0.22 µm Millipore filter.

Viscosity measurements

These were performed on solutions that had been well dialysed and clarified by a prior centrifugation at 12000g for 10 min. Measurements were performed at 20°C in a thermostatically controlled bath by using a semi-micro no. 50 Cannon–Ubbelohde viscometer with an approximate flow time for water of 210 s. Dilutions were made with diffusate *in situ*.

Plots of reduced viscosity against concentration were made to obtain the reduced viscosity at zero concentration, here referred to as the intrinsic viscosity. Absolute values for the latter, which for non-Newtonian systems require extrapolation both to zero concentration and zero shear rate, were not determined.

Determination of the activation energy for the thermal degradation process

From samples incubated at various temperatures, portions were periodically withdrawn for viscosity determinations at 20°C. For each temperature a plot of viscosity against time was made and a smooth curve could be fitted. The initial rate of viscosity change with time, i.e. the time required for a defined initial viscosity change at each temperature, was obtained and used to make an Arrhenius plot. This was linear, and the activation energy of the process could thus be obtained. It was found that the various decay curves when plotted against the logarithm of time could be superimposed by a suitable shift factor (Ferry, 1970). An Arrhenius plot of these again gave a linear curve over the entire temperature range. From this plot, too, an activation energy could be calculated.

Results

The purified preparations obtained from mucus that had been brought to the point of dissolution by

Fig. 2. Effect of temperature and time on the intrinsic viscosity of GP-S in PBS buffer, pH 7.0

A solution of GP-S (1.60 mg/ml) in a closed vial was heated in the dark in a thermostatically controlled bath. Samples were withdrawn at various times and immediately cooled in an ice bath. Viscosity measurements were subsequently performed at 20°C.

Fig. 3. Arrhenius plot of the shift factors, a_T , against temperature, T , for the degradation of GP-S in PBS buffer, pH 7.0

The shift factors were obtained from the data of Fig. 2 and are relative to the 98.5°C curve. The slope of the line of best fit gives an activation energy of 94.1 kJ/mol (22.5 kcal/mol).

thermal treatment (GP-T) or by mild mechanical stirring (GP-S) sedimented as single peaks in the analytical ultracentrifuge and were of similar size. The latter is demonstrated by the physicochemical data of the materials. Thus the sedimentation coefficients obtained for GP-T and GP-S are 67S and 72S respectively and their intrinsic viscosities

are 720 ml/g and 760 ml/g respectively. For GP-S, light-scattering measurements give a mol.wt. of 15.9×10^6 and a radius of gyration of 210 nm. Since essentially similar-sized complexes are indicated, GP-S was used as the starting material for further studies of thermal degradation.

Stability of GP-S

Fig. 2 presents the results of a typical experiment showing the effect of temperature on the intrinsic viscosity of GP-S as a function of time. At each temperature there is a decrease in viscosity with time which is irreversible. The rate of the process increases as the temperature is raised. Fig. 2 shows that the viscosity plotted against the logarithm of time at each temperature gives a series of parallel curves which can be superimposed by a shift by the amount of $\log a_T$ along the abscissa. The parameter a_T is the shift factor, and an Arrhenius plot of these against temperature is shown in Fig. 3. A straight line is obtained, indicating that the same monomolecular degradation process, characterized by a single activation energy, is occurring over the entire temperature range. The activation energy calculated from several experiments, whether by the temperature-dependence of the shift factors or of the initial rates of degradation, was 93.3 ± 7.1 kJ/mol (22.3 ± 1.7 kcal/mol). [This value is somewhat lower than that previously reported (Meyer, 1976). In that preliminary study only a limited number of experimental points over a smaller temperature range were determined.]

The effect of various solvent environments on the stability and rate of thermal degradation of GP-S is shown in Table 1. Measurements performed in the presence of 3M-NaCl, 8M-urea, 6M-guanidine hydrochloride, 0.2M-KCNS or 1% sodium dodecyl sulphate all gave similar viscosities to that in PBS. The solvent environment, moreover, had little effect on the rate of the thermal degradation of the glycoprotein. Conditions known to influence the oxidative-reductive depolymerization reaction (Pigman & Rizvi, 1959; Pigman *et al.*, 1961), such as the presence of 20% (v/v) ethanol or bubbling N_2 through the solution, were also without effect. Thermal degradation, performed in 0.15M-NaCl adjusted to pH 2.6 with HCl, proceeded with an activation energy of 93.3 ± 13.0 kJ/mol (22.3 ± 3.1 kcal/mol), which is very similar to that found at neutral pH. On the other hand, when performed in 0.15M-NaCl adjusted to pH 12.8 with NaOH, a lower activation energy for breakdown was found, 57.3 ± 2.1 kJ/mol (13.7 ± 0.5 kcal/mol).

Sedimentation-velocity runs on GP-S during thermal degradation in PBS, pH 7.0, were performed. The sedimentation patterns after GP-S had been heated at 96°C for 6 min, 18 min, 27 min, 2 h

Table 1. *Effect of solvent conditions on the stability and thermal degradation rate of GP-S at pH 7.0*

Solutions of GP-S (approx. 0.5 mg/ml) in 2 ml of PBS were dialysed at 4°C for 2 days against 100 ml of the solvent to be tested. The latter were made up in PBS and adjusted to pH 7.0. After dialysis the solutions were diluted to 2.5 ml and viscosity measurements were performed at 20°C. The effect of dissolving out gases was determined after nitrogen had been bubbled (2–5 bubbles/s) through a solution for 20 min at room temperature.

	Reduced viscosity (% of control)	Reduced viscosity after incubation for 45 min at 80°C (% decrease)
PBS (control)	100	50
3 M-NaCl	89	51
8 M-Urea	105	43
6 M-Guanidine hydrochloride	80	43
0.2 M-KCNS	110	46
1% Sodium dodecyl sulphate	94	49
20% Ethanol	98	53
Nitrogen	100	51

Fig. 4. *Molecular weight of GP-S as a function of time at 96°C in PBS buffer, pH 7.0*

A solution of GP-S was used from which samples were withdrawn at various times for molecular-weight determination by light scattering at 23°C.

and 4 h indicated in each case a single unimodal peak.

The dependence of the molecular weight (from light scattering) of GP-S in PBS, pH 7.0, as a function of time at 96°C is shown in Fig. 4. A continuous decrease in molecular weight is seen with time. Initially there is a rapid decrease, followed by a phase where the molecular weight continues to fall,

but at a much slower rate. Viscosity measurements of the temperature-dependence of the rate of breakdown of a sample withdrawn during this phase (after 2½ h of heating) indicate an activation energy of 95.4 ± 2.1 kJ/mol (22.8 ± 0.5 kcal/mol), showing that the same degradation process as that observed initially is occurring during this phase.

Pronase-digested GP-S without the naked peptide region (see below) also exhibits the process of slow thermal degradation, with a similar activation energy of 99.6 ± 2.1 kJ/mol (23.8 ± 0.5 kcal/mol).

Compositional changes resulting from thermal degradation

Preliminary experiments indicated that thermal degradation of GP-S leads to a progressive increase in the buoyant density of the glycoprotein on CsCl-density-gradient centrifugation. In Fig. 5 the effect of thermal degradation on the banding behaviour of GP-S is compared with that of reduced GP-S and Pronase-treated GP-S. Thermal degradation here involved heating GP-S (approx. 1 mg/ml) in PBS, pH 7.0, for 210 min at 96°C; this produced an entity with an intrinsic viscosity of 108 ml/g, i.e. comparable in size with Pronase-digested GP-S (93 ml/g). Fig. 5 shows that thermal degradation is accompanied by the release of protein and a shift of the glycoprotein to a higher density of 1.550 g/ml, similar to that of Pronase-digested GP-S. This suggests that the naked peptide region has been cleaved by thermal degradation, which is further demonstrated by the fact that treatment of the thermally degraded GP-S with Pronase produces little subsequent change in the position of the peak. On the other hand, degradation of GP-S involving disulphide-bond cleavage does not result in a density change. Indeed, studies of disulphide-bond cleavage as a result of the thermal treatment indicate the liberation of only some 2.5 nmol of free thiol groups/mg of glycoprotein, as compared with the 16.7 nmol/mg that is liberated on reduction with dithiothreitol.

The sialic acid-containing glycoprotein fractions representing the banded materials, GP-S, thermally degraded GP-S and Pronase-digested GP-S, were each pooled and subjected to amino acid analyses. Fig. 6 shows that the amino acid composition of the thermally degraded GP-S is very similar to that of Pronase-digested GP-S. Both treatments cause an increase in the threonine, serine, proline, valine and alanine contents and a decrease in isoleucine, phenylalanine, arginine, glycine, leucine, glutamic acid and aspartic acid. These changes, moreover, follow the pattern previously reported (Gibbons & Sellwood, 1973; Bhushana Rao & Masson, 1977; Roberts, 1978) upon proteolytic cleavage of the naked peptide region from bovine cervical glycoproteins.

Fig. 5. Effect of thermal degradation on the banding pattern of GP-S in a preparative CsCl density gradient (a) ●, ○, GP-S; ▲, reduced GP-S. (b) ●, ○, GP-S after thermal degradation (approx. 1 mg/ml in PBS, pH 7.0, at 96°C for 210 min); ■, Pronase-digested GP-S; ▲, Pronase digestion of thermally degraded GP-S. The data points for sialic acid (closed symbols) and protein (open symbols) for each preparation have been normalized to a peak sialic concentration of 300 μg/ml. Data points for sialic acid in (a) and (b) lie on common curves (shading) and those for protein on the broken lines. Gradient centrifugation was performed at 100 000 *g* for 50 h at 4°C; the density at the top of the tube on completion of the run is indicated by the arrow on the abscissa. Pronase digestion is as described in the Experimental section.

N-Terminal amino acid analysis performed on GP-S that had been thermally degraded for 90 min at 96°C in PBS, pH 7.0, results in the appearance of aspartic acid as a new *N*-terminal amino acid in addition to alanine, the *N*-terminal amino acid characteristic of the intact glycoprotein (Bhushana Rao & Masson, 1977).

Discussion

The thermal degradation process found in the present study is very similar to that described for proteins. For the latter, peptide bonds next to aspartic acid residues have been shown to be

particularly sensitive to hydrolytic cleavage at acidic or neutral pH (Courts, 1954; Light, 1967; Schultz, 1967). Such cleavage is associated with an activation energy of 95.0 kJ/mol (22.7 kcal/mol). It occurs at the amino end of aspartic acid and, if degradation is more extensive, at the carboxyl end as well, leading to the liberation of free aspartic acid. The activation energy for the thermal degradation of the 15.9×10^6 -mol.wt. GP-S is very similar, and the process is also accompanied by the appearance of aspartic acid as a new *N*-terminal amino acid. In view of the relatively large amount of aspartic acid residues in the naked peptide region (approx. 80% of the total; Bhushana Rao & Masson, 1977), it is expected that this part of the peptide chain in particular should be singled out for rapid thermal hydrolysis. This is so, since the amino acid composition of GP-S that has been thermally degraded to a size comparable with that of Pronase-digested GP-S clearly matches that of GP-S where the naked peptide has been removed by proteolysis. As expected, loss of the naked peptide region on thermal hydrolysis produces the glycosylated region, which now forms a band at a higher buoyant density similar to that seen for Pronase-digested GP-S. Moreover, Pronase treatment of the thermally degraded GP-S does not cause a further shift in buoyant density.

Hydrolysis of peptide bonds next to aspartic acid residues may occur to some extent also in the glycosylated regions, since a further breakdown with essentially the same activation energy is obtained when Pronase-digested GP-S is subjected to thermal degradation.

Thermal degradation of GP-S at pH 12.8 is accompanied by a much smaller activation of 57.3 kJ/mol (13.7 kcal/mol). This value too is consistent with the preferential hydrolysis of peptide bonds next to serine and threonine residues that is seen for proteins at alkaline pH (Courts, 1954).

That degradation involves an enzymic process can be ruled out. Firstly, the glycoprotein preparation used in the studies has been purified on CsCl density gradients to remove extraneous proteins. Also the rate of thermal degradation increases monotonically up to the highest accessible temperature (Fig. 4), in contrast with the effect of enzymes, which have maximal activity around physiological temperatures. In addition, degradation was unaffected by solvents that would be expected to denature enzymes (Table 1).

The finding that thermal breakdown of the 15.9×10^6 -mol.wt. GP-S involves scission of covalent bonds, rather than the 'melting' of non-covalent bonds or the dissociation of physical entanglements, and the stability of GP-S in media that disperse ionic, hydrogen or hydrophobic bonds, indicate that GP-S is most probably a covalently

Fig. 6. Effect of thermal degradation on the amino acid composition of GP-S

The amino acid compositions of GP-S, Pronase-digested GP-S and thermally degraded GP-S are given by the middle, left and right bars for each amino acid respectively. The respective preparations are the combined sialic acid-containing fractions in Fig. 5 banding in the ranges 1.45–1.62 g/ml, 1.47–1.64 g/ml and 1.47–1.64 g/ml. The amino acids are grouped according to whether there is an increase or decrease in a particular amino acid after treatment of GP-S.

linked unit or the breakdown product of a still larger covalent entity. A secondary bond system, on the other hand, that remains stable under the conditions to which GP-S was subjected to would have to be of a most unusual nature. On this basis, therefore, it is likely that the lower-molecular-weight glycoprotein fraction (4×10^6), isolated by Gibbons (1959) and Gibbons & Glover (1959) after dispersion of mucus, is a breakdown product of a larger covalent entity.

The details of the process of thermal degradation were investigated on GP-S and not on the mucus gel. It is thus still possible that only secondary-bond cross-links or entanglements exist between units of the size of GP-S or larger. Whether or not GP-S itself is a clearly delineated molecular unit of the gel is uncertain. In this regard it is noteworthy that the first units solubilized either by mild mechanical stirring (GP-S) or by thermal degradation (GP-T) are of similar size. It is possible, however, that the size of these entities is an artifact of the breakdown process, which in both cases is stopped at the same end-point, i.e. at the point of mucus dissolution. On the other hand, the dimensions of the storage granules (200–1800 nm diameter; Jones & Reid,

1978) in which mucus is packaged before secretion are comparable with the coil dimensions of GP-S (approx. 500 nm diameter, as estimated from its radius of gyration), suggesting that the GP-S particle may be more than just an artifact of preparation. This would also be consistent with the laser-light-scattering studies of Lee *et al.* (1977), who find independently moving entities of not too dissimilar size (mol.wt. 10×10^6) in bovine cervical mucus.

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