of the value before heating, but the rotation of the control enzyme decreased further, suggesting that cross-links were favouring the acquisition of native-like structure, whereas the unsubstituted protein was irreversibly denatured at 80°C.

Penicillinase activity was determined from the decrease in either $A_{400}$ (1 mM-benzylpenicillin) or $A_{278}$ (55 mM-cephaloridine) in 1 mM-EDTA (disodium salt) and 39 mM-KH$_2$PO$_4$/61 mM-Na$_2$HPO$_4$, pH 7.0 at 20°C. The benzylpenicillinase activity of the control enzyme was 201 μmol/min per mg. Treatment with dimethyl suberimidate for either 90, 150 or 1140 min resulted in a decrease in benzylpenicillinase specific activity to 78% (s.d. = 2.0 (n = 3)) of that of the control enzyme. No deactivation by benzylpenicillin was observed with either the control or the treated enzyme. Both control and treated enzymes were deactivated by cephaloridine. However, the deactivated rate of cephaloridine hydrolysis with the treated enzyme was 45.5% (s.d. = 1.3 (n = 3)) of the rate with the control enzyme.

In summary, treatment with dimethyl suberimidate at pH 8.4 resulted in a product with almost normal activity with benzylpenicillin but with less than half of the normal deactivated activity with cephaloridine. The small effect on the activity with benzylpenicillin suggests that cross-linking did not directly involve the active site. We suggest that some of the (presumably heterogeneous) cross-links stabilize part of the protein conformation that is barely involved in any isomerization during the catalytic cycle with benzylpenicillin. The additional stability could contribute to lower activity in the deactivated state by an indirect effect on the active site. Alternatively, if the deactivated enzyme consists of an equilibrium mixture of active and inactive molecules, cross-linking could retard a conformational change that is necessary for the reactivation process but that is not necessarily for deactivation. Our results contrast with the correlation between destruction of conformational flexibility and protection against deactivation that has been reported for another penicillinase (Klemes & Citri, 1979).

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Pig gastric and small-intestinal mucus glycoproteins: proposed role in polymeric structure for protein joined by disulphide bridges

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The glycoprotein from pig gastric mucus (mol.wt. $2 \times 10^6$; Scawen & Allen, 1977) and pig small-intestinal mucus (mol.wt. $1.8 \times 10^5$) are cleaved into four subunits by proteolytic enzymes. Exhaustive Pronase digestion of the small-intestinal glycoprotein produces subunits that sediment as an unimodal polydisperse peak on sedimentation-velocity analysis ($s_{20,w} = 10.4S$) and have a mol.wt. of $4.47 \times 10^5$. The intestinal glycoprotein contained 18% by weight and 30% of this was removed after Pronase digestion. The proportions of serine, threonine and proline in the digested glycoprotein were increased, whereas those of all the other amino acids were decreased. Similar results have been reported for pig gastric glycoprotein, with a protein content of 13% by weight, 30-40% of which is lost on proteolytic digestion (Scawen & Allen, 1977).

Reduction of pig small-intestinal mucus glycoprotein with 0.2 M-mercaptoethanol produced subunits that sedimented as an unimodal polydisperse peak on sedimentation analysis ($s_{20,w} = 8.2S$) with a mol.wt. of $2.36 \times 10^5$, about half the size of the Pronase-digested subunits. Reduction also cleaved the Pronase-digested subunits into two smaller components of the same size as those obtained by direct reduction of the undigested intestinal glycoprotein. However, reduction of gastric glycoprotein with 0.2 M-mercaptoethanol only cleaved the glycoprotein into four subunits (mol.wt. $5 \times 10^5$; Snary et al., 1970), the same size as the Pronase-digested intestinal glycoprotein, and therefore these two glycoproteins differ in their polymeric structure formed by disulphide bridges. A further distinction was that the intestinal glycoprotein was considerably more resistant to reduction than the gastric glycoprotein: for example, after treatment with 10 mM-mercaptoethanol for 24 h, the former was only 40% dissociated, whereas the latter was all in the subunit form. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the intestinal glycoprotein showed that it was free of non-covalently-bound protein, but, after reduction in 0.2 M-mercaptoethanol, a major protein band of mol.wt. 90000 and minor bands of 60000 and 50000 mol.wt. were observed. This protein released after reduction was isolated from the reduced glycoprotein subunits by equilibrium centrifugation in a CsCl density gradient and in sufficient amounts for one 90000-mol.wt. protein per undegraded intestinal glycoprotein molecule. This protein was not released on mercaptoethanol reduction of the Pronase-digested intestinal glycoprotein and therefore it has been proteolytically digested.

A protein of 70000 mol.wt. is joined to the native gastric-mucus glycoprotein by disulphide bridges on a 1:1 molar basis.

Fig. 1. Suggested structure for pig gastric-mucus glycoprotein

The spatial arrangements of the subunits with respect to the 70000-mol.wt. protein is not known.
Dissociation of the native gastric glycoprotein into subunits by 0.2 M-mercaptoethanol or by proteolysis is associated with the release or digestion of this 70000-mol.wt. protein (Pearson & Allen, 1980). From these data a model can be constructed for gastric-mucus glycoprotein where, on average, four subunits are each joined to this protein by disulphide bridges (Fig. 1). An alternative model, where the subunits are joined to each other by disulphide bridges with the protein bound separately to one or more subunits, is less likely, but cannot be excluded. Intestinal mucus glycoprotein appears to possess a similar overall structure, since cleavage into an average of four subunits by Pronase results in the loss of the 90000-mol.wt. protein, but, in contrast with gastric glycoprotein, each subunit would have to consist of two further components joined by disulphide bridges.

The proteins released from these glycoproteins by reduction of the disulphide bridges are a covalent part of the molecule and quite distinct from the non-covalently-bound link proteins of the proteoglycans (Muir & Hardingham, 1975). The 70000-mol.wt. protein from the gastric glycoprotein does not cross-react with human serum albumin and has a different amino acid analysis.


(i.e. high frequencies) where there is insufficient time for the chains to entangle, but at lower frequencies the network can rearrange, thus allowing the material to flow, and G" predominates.

Gastric mucus does, however, show substantial qualitative differences from more rigid gels such as those of agar or gelatin, particularly in the ratio of G' to G" which is only ~3, rather than 10–100, suggesting that an appreciable proportion of the glycoprotein is not incorporated in the network, but is present as a sol fraction that enhances the viscosity of the interstitial fluid rather than conferring solid-like character. The mucus-gel structure is also capable of re-forming after mechanical damage (e.g. it will eventually anneal if sectioned) and, on a sufficiently long time scale, shows flow behaviour. This indicates that the lifetime of the intermolecular junctions, although long in comparison with polymer entanglements, is finite.

The concentration of glycoprotein in native pig gastric mucus is 55 mg ml⁻¹ (18 determinations). Purified glycoprotein (Sephrose 4B) at the same concentration gives a gel with closely comparable mechanical properties (six replications). We therefore conclude that the gel structure arises predominantly from intermolecular associations of glycoprotein molecules, which can ‘make and break’, thus conferring on the mucus its characteristic flow properties.

Prolonged incubation of the native gel for 24h, 37°C, (with azide added as bacteriostat) resulted in very much lower values of G' and G", with G' equal to, or even lower than, G", thus indicating complete collapse of the gel structure. A shorter incubation for 4h under comparable conditions produced only a limited change in G' (<10%). Incubation in the mild acid conditions of pH 2 resulted in a much greater decrease in G' (~75%) over the same period. This was shown, however, not primarily to be due to acid hydrolysis, since incubation at 4°C for 4h in 1 M-HCl produced less than 20% decrease in G'. The mucolytic effect on incubation at low pH appears therefore to be enzymic in nature, presumably due to endogenous peptidase associated with the mucus gel. This has been confirmed by gel-filtration studies on Sepharose 2B, which showed that 83, 37.7 and 14.8% of the total glycoprotein in the mucus was present as peptidase-degraded subunits after the 24h incubation, the 4h incubation at 37°C, pH 2, and the 4h incubation at 4°C, 1 M-HCl respectively.