The Glycoproteins of Tendon and their Interaction with Collagen

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Bowes *et al.* (1957) first showed that non-collagenous proteins were present in connective tissue, and that these were removed by procedures used to purify collagen. Some workers (Robert & Comte, 1968; Barnes & Partridge, 1968) have prepared glycoproteins from soft connective tissues, but fractionation utilized high temperatures and extremes of pH. However, Dunstone & Franek (1967) and Hascall & Sajdera (1969) isolated glycoproteins by milder procedures while fractionating proteoglycans. Similar methods were used to isolate glycoproteins from tendon (Anderson & Jackson, 1972).

Glycoproteins A and B were prepared from homogenized bovine achilles tendon. The tendon was extracted exhaustively with 0.2M-NaCl, and then exhaustively with 3M-MgCl₂. Chromatography of the combined 0.2M-NaCl extracts on sulphoethyl-Sephadex removed neutral proteins. The acidic fraction was freed from proteoglycans by CsCl-density-gradient ultracentrifugation, and the purified protein fraction was designated glycoprotein A.

The neutral protein fraction from sulphoethyl-Sephadex contained serum proteins, and had a definite lysozyme-like activity.

Dialysis of the 3M-MgCl₂ extract gave a collagenous precipitate with adsorbed glycoprotein. Exhaustive extraction with 0.2M-NaCl removed the latter, which was fractionated as described above for glycoprotein A. The purified protein fraction was designated glycoprotein B.

The amino acid profiles of glycoproteins A and B were typical of previously isolated connective-tissue glycoproteins (Hascall & Sajdera, 1969; Dunstone & Franek, 1967; Robert & Comte, 1968), aspartic acid, glutamic acid and leucine predominating. Glycoprotein B, unlike glycoprotein A, appeared to contain a significant amount (8%) of a collagenous component. Each glycoprotein had 4% hexosamine (glucosamine predominating over galactosamine) and 3-4% hexose (galactose and mannose being the major sugars, fucose and glucose also being present). They also contained 2% sulphate.

On isoelectric focusing, each glycoprotein showed several bands centred around pH4. However, on interaction with calf skin tropocollagen, only one band was observed in each case. The interaction of collagen with glycoproteins observed *in vitro* may parallel an interaction *in vivo* that could be important in the organization of collagen fibrils. This finding

supports the suggestion (Jackson & Bentley, 1968) that glycoproteins maintain the structural stability of collagen fibrils above a certain diameter.

Anderson, J. C. & Jackson, D. S. (1972) *Biochem. J.* **127**, 179

Barnes, M. J. & Partridge, S. M. (1968) *Biochem. J.* **109**, 883

Bowes, J. H., Elliott, R. G. & Moss, J. A. (1957) in *Connective Tissue, A Symposium* (Tunbridge, R. E., ed.), p. 264, Blackwell, Oxford

Dunstone, J. R. & Franek, M. D. (1967) *Biochem. Biophys. Res. Commun.* **27**, 39

Hascall, V. C. & Sajdera, S. W. (1969) *J. Biol. Chem.* **244**, 2384

Jackson, D. S. & Bentley, J. P. (1968) in *Treatise on Collagen* (Gould, B. S., ed.), vol. 2, p. 189, Academic Press, London

Robert, L. & Comte, P. (1968) *Life Sci.* **7**, 493

Age-Related Changes in the Stability of Human Skin Polymeric Collagen

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It is generally accepted that the number of cross-links between collagen molecules increases with the age of the animals from which the collagen is obtained (Jackson, 1965; Bailey, 1969). However, few studies have been carried out on human collagens (Bornstein, 1970). In particular, distinctions between the changes that may occur during growth and thereafter in adult life remain obscure (Steven, 1966a,b; Hamlin & Kohn, 1971). We now report age-related changes in the stabilities of a polymeric collagen fraction obtained from skin samples of 86 humans aged 0-93 years.

Polymeric collagen was extracted from the skin over the greater trochanter; 28 skin samples were taken *post mortem* and 58 by biopsy. Of these samples 29 were from normal individuals with no evidence of connective-tissue disorder. The stabilities of the polymeric collagen fractions were measured by their resistance to thermal denaturation in water for 1 h at 100°C after treatment with either 2M-NaOH for 6 days at 4°C or with Pronase for 18 h at 20-22°C (Francis & Macmillan, 1971).

All polymeric collagen samples were more resistant to thermal denaturation after Pronase treatment than after treatment with cold alkali, but there were differences in response to these treatments according to the age of the individual from which the polymeric collagen was obtained. After Pronase treatment, the

stabilities of the polymeric collagen increased up to the age of 25–30 years. After treatment with cold alkali, however, the stability of the polymeric collagen appeared to increase up to the age of 60 years. The trends were similar whether values from normal individuals alone were taken or whether the whole group was considered, including patients with various connective-tissue disorders. There is thus evidence for two distinct biochemical processes leading to an increased stability of polymeric collagen; one of these may continue throughout life. The relation of the changes to the known inter- and intramolecular cross-links present in collagen fibres is being investigated.

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- Bailey, A. J. (1969) *Gerontologia* **15**, 65
 Bornstein, P. (1970) *Amer. J. Med.* **49**, 429
 Francis, M. J. O. & Macmillan, D. C. (1971) *Biochim. Biophys. Acta* **251**, 236
 Hamlin, C. R. & Kohn, R. R. (1971) *Biochim. Biophys. Acta* **236**, 458
 Jackson, D. S. (1965) *Advan. Biol. Skin* **6**, 216
 Steven, F. S. (1966a) *Biochim. Biophys. Acta* **130**, 196
 Steven, F. S. (1966b) *Biochim. Biophys. Acta* **130**, 202

Fractionation of Water-Soluble Pig Gastric Mucus by Equilibrium Density-Gradient Centrifugation in Caesium Chloride

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The application of the technique of equilibrium density-gradient centrifugation in CsCl (Creeth & Denborough, 1970) to the separation of protein from mucoprotein blood-group substances has been followed to fractionate further the water-soluble gastric mucus preparation (Snary & Allen, 1971). This water-soluble mucus has previously been shown from gel filtration, ultracentrifugation and electrophoretic studies to consist of two apparently homogeneous, though polydisperse, mucoprotein components. Because these two mucoprotein components have very similar chemical, physical, biosynthetic and immunological properties, the high-molecular-weight component A is thought to be a polymer of the lower-molecular-weight component B/C.

Equilibrium density-gradient centrifugation in CsCl of the unfractionated water-soluble mucus produced a clear separation between a protein fraction at the top of the tube ($\rho = 1.38 \text{ g} \cdot \text{ml}^{-1}$) and the mucoprotein fraction, containing all the bound hexose and

fucose, at the bottom of the tube ($\rho = 1.56 \text{ g} \cdot \text{ml}^{-1}$). Sedimentation analysis of this mucoprotein fraction showed two components of $s_{25,w}^0$ 33S and 5.7S that are shown to correspond to the high-molecular-weight and low-molecular-weight components A and B/C ($s_{25,w}^0$ 18.7S and 4.3S respectively) of the unfractionated water-soluble mucus. The two mucoprotein components from the equilibrium density-gradient centrifugation were separated in the excluded and included volumes respectively of a Sepharose 4B column. The carbohydrate analysis of the high-molecular-weight 33S component was hexose 26%, fucose 11.3%, glucosamine 19.5% and galactosamine 8.3%. Purified 18.7S mucoprotein A isolated by gel filtration of the water-soluble mucus, which contained 21.7% protein, was fractionated by equilibrium density-gradient centrifugation, producing a protein fraction and the 33S mucoprotein containing 16.5% protein. Amino acid analysis of this 33S mucoprotein showed an enriched serine, threonine and proline content and a lower aspartic acid and glutamic acid content compared with the parent 18.7S mucoprotein fraction A. The protein content, however, is still higher than blood-group substance isolated by proteolytic digestion of pig stomach (Pusztai & Morgan, 1963). These results show that equilibrium density-gradient fractionation in CsCl removes non-covalently bound protein from gastric mucoprotein that was judged to be homogeneous by other methods.

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- Creeth, J. M. & Denborough, M. A. (1970) *Biochem. J.* **117**, 879
 Pusztai, A. & Morgan, W. T. J. (1963) *Biochem. J.* **88**, 546
 Snary, D. & Allen, A. (1971) *Biochem. J.* **123**, 845

The Effect of Caesium Chloride on the Tertiary Structure of the Water-Soluble Pig Gastric Mucus

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The two mucoproteins isolated from water-soluble pig gastric mucus by using equilibrium density-gradient centrifugation in CsCl (Starkey *et al.*, 1972) have $s_{25,w}^0$ values of 33S and 5.7S respectively in 0.2M-KCl buffer, pH 5.5. These values compare with 18.7S and 4.3S for the same mucoproteins (A and B/C) isolated by gel filtration (Snary & Allen, 1971). The viscosity of the water-soluble mucus decreased 63% between 1mM-CsCl and 0.2M-CsCl but then remained constant up to 3.5M-CsCl, the concentration