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