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Short Communications

The Influence of Glycoprotein on Collagen Fibril Formation in the Presence of Chondroitin Sulphate Proteoglycan

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Tropocollagen molecules can aggregate under physiological conditions *in vitro* to form gels consisting of native collagen fibrils (Wood, 1964). *In vivo*, collagen fibrillogenesis takes place in the extracellular matrix (Goldberg & Green, 1964), which consists of sulphated proteoglycans and glycoproteins together with variable amounts of serum proteins. Some of the sulphated proteoglycans in cartilage, skin and heart valves appear to be tightly bound to the insoluble fibrous network (Schubert, 1966; Toole & Lowther, 1968*a*), and have been observed by electron microscopy of suitably stained preparations. (Serafini-Fracassini & Smith, 1966; M. Natarajan & D. A. Lowther, unpublished work). The addition of dermatan sulphate proteoglycan (Toole & Lowther, 1968*b*) or chondroitin sulphate proteoglycan (Toole & Lowther, 1968*a*) to solutions of tropocollagen has been shown to modify the kinetics of collagen fibril formation from these molecules. It seems likely therefore that the interactions of sulphated proteoglycans with tropocollagen may play a role in collagen fibrillogenesis both *in vitro* and *in vivo*.

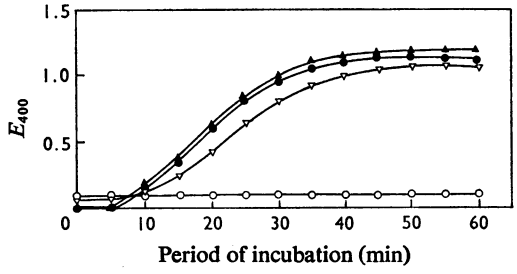

Fig. 1. *Influence of glycoprotein on collagen fibril formation at* 37°C *in the presence of chondroitin sulphate proteoglycan*

All cuvettes contained 1000μg of tropocollagen/ml. ●, Tropocollagen only; ▲, tropocollagen + glycoprotein (16μg/ml); ○, tropocollagen + chondroitin sulphate proteoglycan (160μg/ml); ▽, tropocollagen + aggregated chondroitin sulphate proteoglycan (160μg/ml) + glycoprotein (16μg/ml).

There is considerable evidence that sulphated proteoglycan solutions are polydisperse with molecular weights varying from 1×10^6 to 5×10^6, but aggregates up to 10 times as large as this were also shown to be present (Barrett, 1968). Hascall & Sajdera (1969) have shown that the formation of proteoglycan aggregates may depend on the interaction of chondroitin sulphate proteoglycan subunits with a glycoprotein. We have found not only that the removal of glycoprotein 'bridges' from proteoglycan aggregates diminishes polydispersity but that such preparations show a very different effect on the kinetics of fibril formation.

Acid-soluble tropocollagen was prepared as described by Toole & Lowther (1968*b*). Chondroitin sulphate proteoglycan and a glycoprotein were isolated from bovine nasal cartilage by extraction with 4M-guanidinium chloride as described by Hascall & Sajdera (1969) and Sajdera *et al.* (1970). Aggregated chondroitin sulphate proteoglycan was prepared by adding the glycoprotein to chondroitin sulphate proteoglycan in the manner described by these authors. The proteoglycan, glycoprotein and tropocollagen preparations were dialysed for 24h against 8mM-Na_2HPO_4–KH_2PO_4 buffer, pH7.3, containing 0.14M-NaCl at 4°C. Collagen fibril formation was measured by following changes in extinction at 400nm of tropocollagen solutions incubated at 37°C as described by Toole & Lowther (1968*b*).

The curves shown in Fig. 1 indicate the effect of proteoglycan and glycoprotein on the kinetics of collagen fibril formation. Unaggregated proteoglycan inhibits collagen fibril formation *in vitro* for several hours. When aggregated chondroitin sulphate proteoglycan containing the glycoprotein is added to tropocollagen solutions no inhibition of fibril formation is observed. The glycoprotein alone has no effect on collagen fibril formation.

Thus marked differences are seen in the effects produced by chondroitin sulphate proteoglycan and aggregated chondroitin sulphate proteoglycan on the kinetics of collagen fibrillogenesis. However, collagen

fibrils could be formed *in vitro* in the presence of chondroitin sulphate proteoglycan after a prolonged lag period of several hours. No significant differences are observed between the two proteoglycan preparations with respect to their binding to collagen fibrils (0.19mg of chondroitin sulphate proteoglycan and 0.15mg of aggregated chondroitin sulphate proteoglycan are bound to 1mg of tropocollagen respectively) and the thermal stability of the fibrils tested as described by Toole (1969), indicating that the physical state of the proteoglycans is important in controlling only the initial stages of collagen fibril formation.

Robert & Robert (1968) suggested that glycoproteins play some role in the organization of the highly ordered collagen fibre lattice in the cornea, and it seems likely that such effects may depend on their interaction with sulphated proteoglycans. There seems to be little doubt that both proteoglycan and glycoprotein become firmly bound to collagen fibres during fibrogenesis *in vivo*. It is suggested that these, in part, represent aggregates of sulphated proteoglycans that are bridged by glycoproteins.

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