Since *Anacystis nidulans* is capable of carrying out a normal photosynthesis despite its lack of α -tocopherolquinone, an obligatory role of α -tocopherolquinone in oxygen-producing photosynthesis must be seriously questioned. However, perhaps the function of α -tocopherolquinone in this organism is taken over by the hydroxyphyllloquinone, which is not present in the other species of blue-green algae examined.

The suggestion of Henninger *et al.* (1965) that the quinones and related compounds of the blue-green algae are different from those characteristic of higher plants appears now to be based on one species, *Anacystis nidulans*, which is probably atypical.

We thank the Science Research Council for financial support and for the award of a Research Fellowship to R. P. We are also indebted to Dr N. G. Carr for supplying some of the algal cultures, Dr J. F. Pennock for samples of tocopherols and Miss J. M. Farr for technical assistance.

Allen, C. F., Franke, H. & Hirayama, O. (1967). *Biochem. biophys. Res. Commun.* **26**, 562.

Carr, N. G. & Hallaway, H. M. (1966). In *Biochemistry of Chloroplasts*, vol. 1, p. 153. Ed. by Goodwin, T. W. New York: Academic Press Inc.

Henninger, M. D., Bhagavan, H. N. & Crane, F. L. (1965). *Arch. Biochem. Biophys.* **110**, 69.

Hirayama, O. (1967). *J. Biochem., Tokyo*, **61**, 179.

Kratz, W. A. & Myers, J. (1954). *Amer. J. Bot.* **42**, 282.

Pennock, J. F., Hemming, F. W. & Kerr, J. D. (1964). *Biochem. biophys. Res. Commun.* **17**, 542.

Biochem. J. (1967) **104**, 26c

A Hybrid Protein-Polysaccharide of Keratan Sulphate and Chondroitin Sulphate from Pig Laryngeal Cartilage

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(Received 15 May 1967)

Chondroitin sulphate-protein complexes from cartilage contain variable amounts of glucosamine and galactose attributable to keratan sulphate, which may be an integral part of the complex macromolecule (Partridge & Elsdon, 1961; Seno, Meyer, Anderson & Hoffman, 1965). The present paper describes the isolation of an antigenically distinct protein consisting of a single antigen that contained keratan sulphate together with considerable amounts of uronic acid and galactosamine that may represent a hybrid proteoglycan containing both keratan sulphate and chondroitin sulphate.

Proteoglycans of chondroitin 4-sulphate from pig laryngeal cartilage have been fractionated by gel filtration on 6% agarose (Tsiganos & Muir, 1965) and electrophoresis (Muir & Jacobs, 1967) into distinct antigenic components differing in size, amino acid composition and glucosamine and galactose contents. The largest constituents, possessing all the antigens of the starting material, contain considerably more glucosamine and galactose than the smallest, which possess a single-species cross-reacting antigen (C. P. Tsiganos & H. Muir, unpublished work). The moiety that contained glucosamine was therefore isolated after exhaustive

hyaluronidase digestion of a proteoglycan preparation obtained by extracting cartilage for a total of 72 hr. essentially as described by Muir (1958).

A 1.5 g. sample of the above preparation dissolved in 0.1 M-acetate buffer, pH 5, containing NaCl (0.15 M) (Hoffman, Meyer & Linker, 1956) was digested under toluene with 70 000 units of hyaluronidase (EC 3.2.1.35) added in three portions over 3 days. Insoluble material was removed by centrifuging and the supernatant treated with an equal volume of saturated aqueous 9-aminoacridine hydrochloride. After standing overnight at 4° the precipitated hyaluronidase-resistant material was centrifuged off, and the supernatant was saved and stored at 4°. The precipitate was converted into the soluble sodium salt by suspending it in acetate buffer and shaking with Zeo-Karb 225 8% DVB (Na⁺ form), which took up the aminoacridine. The hyaluronidase digestion and precipitation of resistant polyanionic material with aminoacridine was repeated first with 15 000 units of hyaluronidase for 24 hr. followed by 20 000 units of purified enzyme for 24 hr. It was re-isolated and further digested with 80 000 units of highly purified enzyme for 24 hr. After the final precipitation with amino-

Step (1) Exhaustive hyaluronidase digestion of chondroitin sulphate-protein complexes.

Step (2) Polyanions precipitated via 9-aminoacridine.

Step (3) Regeneration of sodium salt (see the text); resubmitted to steps (1)–(3) twice more.

Hyaluronidase-resistant
keratan sulphate-protein
complex

Composition (% of dry wt.)

Protein	15–20
Hexosamine	17.0
Galactose	20.2
Uronic acid	10.5
Xylose	3.2
Sulphate	8.1
Sialic acid	< 0.05
Fucose	Not detected

Glucosamine/galactosamine
molar ratio 1:3.1

$[\alpha]_D^{25} - 4.7^\circ$

Infrared spectrum
resembles that of corneal
keratan sulphate (920 cm.⁻¹
band absent)

Pooled supernatants from
step (2)

(oligosaccharides and all
precipitating protein
antigens observed in the
starting chondroitin
sulphate-protein
complexes)

Scheme 1. Isolation and composition of keratan sulphate-protein complex.

acridine, and regeneration of the sodium salt, the material was precipitated with ethanol containing sodium acetate, washed with ethanol and then acetone and dried (yield 60mg.). The supernatants from all three aminoacridine precipitations were pooled, and after removal of aminoacridine with Zeo-Karb 225 they were concentrated to 100ml. The isolation procedure and analysis of the hyaluronidase-resistant material are shown in Scheme 1. The main constituents were glucosamine and galactose, which together with the low optical rotation and the infrared spectrum indicated that it was keratan sulphate. Nevertheless, even after repeated digestion with hyaluronidase there remained 10% of uronic acid; the reaction product with carbazole had an absorption spectrum in the range 420–570m μ , identical with that given by alkali-prepared chondroitin sulphate. Moreover, galactosamine was present in amounts equivalent to the uronic acid. There was 15–20% of protein, but neither methylpentose nor sialic acid was detected. Mathews & Cifonelli (1965) also found that methylpentose was generally absent from skeletal keratan sulphate and that the presence of sialic acid was uncertain; however, Seno *et al.* (1965) and Gregory & Rodén (1961) found these sugars in skeletal keratan sulphate.

The keratan sulphate-protein complex moved as one spot on cellulose acetate electrophoresis at pH 4.5. Its mobility was lower than that of corneal keratan sulphate and it would separate from a

mixture with a chondroitin sulphate proteoglycan fraction that was retarded on agarose-gel filtration. It was reasonably homogeneous on sedimentation in 4M-guanidine, having $S_{25,w}$ approx. 3s.

A 3mg. sample of the keratan sulphate-protein complex in borate buffer, pH 8.2, containing CaCl₂ (0.02M) was digested for 24hr. at 60° under toluene with 3mg. of Pronase from *Streptomyces griseus*. The resulting keratan sulphate that was precipitated with 9-aminoacridine contained only traces of uronic acid and had lost much of the galactosamine, which now accounted for only 16% of the total hexosamine (cf. Scheme 1). Mathews & Cifonelli (1965) also found that keratan sulphate isolated after proteolysis contained some galactosamine. The uronic acid and hexosamine that were identified in the supernatant after Pronase digestion should consist of oligosaccharides because oligosaccharides from partial hyaluronidase digestion of chondroitin sulphate, obtained by gel filtration on Sephadex G-50, were not precipitated by 9-aminoacridine. This would imply that galactosamine and uronic acid, in the form of oligosaccharide, had been attached to the same protein as keratan sulphate, forming a hybrid molecule. Rosenblum & Cifonelli (1967) have evidence for such hybrids, and Seno *et al.* (1965) were unable to separate residual chondroitin sulphate from keratan sulphate prepared by proteolysis until treated with alkali.

If the galactosamine and uronic acid in the keratan sulphate-protein complex were derived from longer chondroitin sulphate chains that were largely digested by hyaluronidase, these chondroitin sulphate chains would be expected to be attached to protein by xylose-serine linkages (Lindahl & Rodén, 1964), which are labile to alkali (Muir, 1958), and undergo β -elimination with destruction of serine (Anderson, Hoffman & Meyer, 1965). A 3mg. sample of keratan sulphate-protein complex was therefore dissolved in 4ml. of 0.5N-KOH and left at room temperature under nitrogen for 24hr. (Seno *et al.* 1965). After hydrolysing in 6N-HCl at 105° for 24hr. under nitrogen, the amino acid composition was determined, and also that of the untreated keratan sulphate-protein complex, with a Technicon analyser. About 60% of serine and 35% of threonine were destroyed by alkali, whereas in control experiments, with ribonuclease (recrystallized four times), only 8% of each amino acid was destroyed. These losses of threonine, as well as serine, suggest that both hydroxyamino acids may be involved in glycosidic linkages in the keratan sulphate-protein complex. Seno *et al.* (1965) showed that there was destruction of both threonine and serine when skeletal keratan sulphate was treated with alkali, whereas the residual aspartic acid and glutamic acid in corneal keratan sulphate survived. There may thus be a difference

between corneal and skeletal keratan sulphates (Mathews & Cifonelli, 1965).

The immunological characteristics of the keratan sulphate-protein complex and of the pooled supernatants from the hyaluronidase digestion were entirely different. With antisera to the starting proteoglycan preparation (Loewi & Muir, 1965), only the pooled supernatants contained the several antigens of the original material, none of which was revealed in the keratan sulphate-protein by double-diffusion at various dilutions. Nevertheless, antisera to the keratan sulphate-protein complex were successfully raised in rabbits, and showed no cross-reaction with any of the antigens in the pooled supernatants. The keratan sulphate-protein complex was thus immunologically distinct from these antigens, and furthermore appeared to be a single antigen on double-diffusion and immunoelectrophoresis. In the original complex, this protein to which both keratan sulphate and some chondroitin sulphate is attached is therefore different from the proteins to which most of the chondroitin sulphate is bound. Since the latter comprise the greater part of the protein in the whole complex, they are responsible for its overt antigenic properties. The keratan sulphate-protein complex, although a minor component, appears to be more prevalent in larger complexes since these contain more glucosamine than smaller ones. The

keratan sulphate-protein complex may therefore either vary greatly in size or be in some way involved building in up larger molecules.

We are grateful to Dr B. Högborg, A. B. Leo, Halsingborg, Sweden, and Professor A. G. Lloyd for purified hyaluronidase, and to Dr R. Pain for sedimentation experiments. The competent technical assistance of Mr R. Ewins and the support of C. P. T. by the Nuffield Foundation is gratefully acknowledged.

- Anderson, B., Hoffman, P. & Meyer, K. (1965). *J. biol. Chem.* **240**, 156.
 Gregory, J. D. & Rodén, L. (1961). *Biochem. biophys. Res. Commun.* **5**, 430.
 Hoffman, P., Meyer, K. & Linker, A. (1956). *J. biol. Chem.* **219**, 653.
 Lindahl, U. & Rodén, L. (1964). *Biochem. biophys. Res. Commun.* **17**, 254.
 Loewi, G. & Muir, H. (1965). *Immunology*, **9**, 119.
 Mathews, M. B. & Cifonelli, J. A. (1965). *J. biol. Chem.* **240**, 4140.
 Muir, H. (1958). *Biochem. J.* **69**, 195.
 Muir, H. & Jacobs, S. (1967). *Biochem. J.* **103**, 367.
 Partridge, S. M. & Elsdon, D. F. (1961). *Biochem. J.* **79**, 26.
 Rosenblum, E. L. & Cifonelli, J. A. (1967). *Fed. Proc.* **26**, 282.
 Seno, N., Meyer, K., Anderson, B. & Hoffman, P. (1965). *J. biol. Chem.* **240**, 1005.
 Tsiganos, C. P. & Muir, H. (1965). *Proc. int. Symp. Biochimie et Physiologie du Tissu Conjonctif, Lyon*, p. 27. Ed. by Comte, P.

Biochem. J. (1967) **104**, 28c

Variations in the C-Terminal Half of Immunoglobulin λ -Chains

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(Received 17 May 1967)

The immunoglobulin light chains contain multiple variations of amino acid sequence localized in the N-terminal half of the molecule, whereas the C-terminal half is essentially invariant. This is true for κ -chains (Hiltschmann & Craig, 1965; Titani, Whitley, Avogardo & Putnam, 1965; Milstein, 1966a) and also for λ -chains (Milstein, Clegg & Jarvis, 1967). In κ -chains, however, one variant occurs at position 191 that can be correlated with the allotypic marker Inv. Thus Inv *a* chains contain leucine whereas Inv *b* chains contain valine in that position (Milstein, 1966a,b; Baglioni, Zonta, Cioli & Carbonara, 1966). Position 122 is still controversial

(discussed by Milstein, 1966c). The invariancy of the C-terminal half of light chains has not been surveyed very extensively. In κ -chains the C-terminal half of three proteins (Hiltschmann & Craig, 1965; Titani *et al.* 1965) and stretches around the cysteine residues of five more (Milstein, 1966c) have been studied in detail. 'Fingerprints' of a larger number of proteins have not established any other variation (Baglioni & Cioli, 1966). The sequences of the C-terminal half of two λ -proteins have been established independently in two different Laboratories and there is complete agreement in the two reported sequences with the exception of the