

The Protein-Polysaccharide Linkages in Some Proteoglycans¹

Acid mucopolysaccharides are usually linked to proteins in protein-polysaccharide complexes. The linkages between the carbohydrate and the protein moieties in some of these complexes have recently been studied (MUIR², ANDERSON et al.³, GREGORY et al.⁴) and found similar, in some regards, to those observed in submaxillary mucins (TANAKA et al.⁵, CARUBELLI et al.⁶, HARBON et al.⁷).

In previous papers^{8,9} we studied the protein-polysaccharide linkage present in a protein-polysaccharide complex (PP-L) isolated in our laboratory¹⁰ from pig epiphyseal plate, and we observed that serine was the amino acid which linked, by its hydroxyl, the carbohydrate to the protein moiety of the complex. In another paper¹¹, in which a keratosulphatepeptide from bovine cornea was studied, we observed that aspartic acid was involved in the linkage as in ovalbumin¹².

In the present note we briefly collected our results on protein-polysaccharide linkages in some proteoglycans which are at present the object of our research: a keratosulphate-peptide isolated from tracheal cartilage of a 50-year-old man, a similar fraction isolated from the urine of patients with Morquio-Ullrich disease, a proteoglycan prepared by papain digestion from dog submaxillary gland, and another one isolated from human submaxillary gland collected by autopsy 24 h after death.

The preparation and composition of these proteoglycans will be reported in detail in a more extensive paper. The carbohydrate moiety of the compound isolated from human tracheal cartilage was mainly composed of keratosulphate, and contained chondroitin sulphate C as minor component, the protein moiety was 9% of the total and contained many amino acids, with a prevalence of serine, threonine, glutamic acid and proline.

The fraction isolated from the urine of patients with Morquio-Ullrich disease was composed of keratosulphate and chondroitin sulphate in an equimolar ratio, and contained about 3% protein.

The proteoglycan prepared from dog submaxillary gland¹³, contained 80% carbohydrate and 20% protein, while that from human submaxillary mucin contained about 18% protein.

The study of the protein-polysaccharide linkage in these proteoglycans was carried out according to the suggestion of ANDERSON et al.³: the product was dissolved in 0.5N NaOH and maintained under continuous stirring for 48 h at room temperature; in some cases hydrogenation in the presence of Adams catalyst was associated with the alkali treatment. Before and after the treatment, the amino acids were determined by a Spinco-Beckman Analyzer after hydrolysis of the product for 24 h at 100°C under nitrogen with a large volume (1:2500) of 6N HCl.

In all the proteoglycans studied, a strong decrease of threonine and serine (Table) was observed after the alkali treatment. This decrease may be explained according to ANDERSON et al.³ and CARUBELLI et al.⁶, assuming that these amino acids are linked by their hydroxyls to the carbohydrate moiety of the molecule. In this situation the influence of a near electrophilic group increases the mobility of the proton in the α -position and facilitates the nucleophilic attack of the alkali, with the production of an unstable carbanion and a consequent formation of α -aminoacrylic acid from serine and α -aminocrotonic acid from threonine. Catalytic hydrogenation associated with the alkali treatment produced formation of alanine and α -aminobutyric acid.

In conclusion, our results show that in all the proteoglycans studied in the present paper, serine and threonine are connected with the protein-polysaccharide linkage by links which involve the hydroxyls of these hydroxyamino acids.

Content of threonine and serine before and after the alkali treatment of proteoglycans (% values)

Proteoglycans isolated from	Threonine		Serine	
	before	after	before	after
1. Urine (Morquio-Ullrich)	0.44	0.18 (- 59%)	0.51	0.24 (- 53%)
2. Human tracheal cartilage	1.15	0.36 (- 68%)	1.03	0.46 (- 55%)
3. Dog submaxillary gland	4.15	0.81 (- 80%)	2.12	1.08 (- 49%)
4. Human submaxillary gland	3.12	0.98 (- 68%)	1.48	0.78 (- 47%)

Riassunto. È stato studiato il legame proteina-polisaccharide di alcuni proteoglicani preparati da urina di pazienti con malattia di Morquio-Ullrich, da cartilagine tracheale umana, e da ghiandole sottomascellari di cane e di uomo. In tutti questi complessi, la serina e la treonina legano, mediante il loro ossidrilico, la parte glucidica alla parte proteica della macromolecola.

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¹ The name proteoglycans, recently proposed by E. BALAZS, includes all the complexes containing carbohydrate and protein moieties and it may be used for both mucoproteins and mucins.

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