

## SULFOSIALOPOLYSACCHARIDE-PEPTIDE FROM DOG SUBMAXILLARY GLAND

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Comparative histochemical studies, carried out by Bignardi (Bignardi et al., 1960, 1962; Aureli et al., 1960, 1964) on mammal salivary glands, suggested the presence of a sulfated mucopolysaccharide in the dog submaxillary gland.

In order to isolate the sulfate material, homogenates of this gland were partially digested by papain, according to Scott (1960), and the mucopeptide was isolated, as the sodium salt, with ethyl alcohol. With this method a degraded mucin was obtained which was useful for the study of its carbohydrate moiety.

The mucopeptide was purified by DEAE-Sephadex chromatography according to Schmidt (1962). Analysis of fractions, by the anthrone method of Trevelyan and Harrison (1952), showed galactose-containing material only in the step eluted by 1,25 N NaCl plus 0,01 N HCl. These fractions were collected, dialyzed 48 hr against distilled water, and concentrated; the mucopeptide was precipitated with 3 volumes of ethyl alcohol in the presence of 5% sodium acetate and 0,5 N acetic acid. The precipitation was carried out twice, the mucopeptide washed with absolute ethyl alcohol and ethyl ether and dried in an Abderhalden drying pistol.

Paper chromatography, with the solvent ethyl acetate-pyridine-water (10:4:3), of the neutral sugars obtained after hydrolysis with 1N HCl, showed the presence of galactose and fucose

Fucose was determined by the method of Dische et al. (1948); sialic acid according to Svennerholm (1958), and galactose by the method of Trevelyan and Harrison (1952) with correction for fucose interference.

Amino acids were determined with a Spinco-Beckman Analyzer, model 120 B, after hydrolysis for 24 hr, under nitrogen in a sealed tube, using a large volume of 6 N HCl. Amino sugars were analyzed after hydrolysis with 4N HCl for 7 hr in the above conditions.

Sulfur was determined according to Wagner (1957).

The carbohydrate components and amino acids found in our mucopeptide are reported in Table 1.

Table 1

COMPOSITION OF THE MUCOPEPTIDE, ISOLATED FROM DOG SUBMAXILLARY GLAND (GRAMS PER 100 GRAMS DRY WEIGHT)

		Aspartic acid	0,70
Galactose	19,5	Threonine	4,15
		Serine	2,12
Fucose	15,5	Glutamic acid	1,44
		Proline	2,91
Acetylglucosamine	16,0	Glycine	2,70
		Alanine	2,17
Acetylgalactosamine	15,0	Valine	0,64
		Isoleucine	0,33
Sialic acid	7,5	Leucine	1,23
		Tyrosine	0,03
Sulfate	6,1	Phenylalanine	0,16
		Lysine	0,15
		Histidine	0,32
		Arginine	0,67

The results obtained with the degraded mucin are in

agreement with the basic work of Hashimoto and Pigman (1962) on dog submaxillary mucin. Furthermore, the histochemical evidence of Bignardi et al. (1962) led to the chemical demonstration in the mucopeptide of sulfate ions.

Although the presence of sulfate in a sialopolysaccharide was very surprising, it should be noticed that a sialoglycoprotein-containing sulfate was also found by Kent and Marsden (1963) in sheep colonic mucin. Furthermore, infrared analysis of our peptide confirmed the presence of sulfur, as sulfate. In fact, a  $1240\text{ cm}^{-1}$  peak, arising from a S=O bond and a  $820\text{ cm}^{-1}$  peak, deriving from a C-O-S linkage, according to Lloyd et al. (1961) were present.

Attempts to fractionate the mucopeptide by column electrophoresis (LKB 3340, with cellulose powder and phosphate buffer at pH 5,6) were unsuccessful.

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