Preliminary Notes

PN 1265

A serine-linked peptide of chondroitin sulfate

For many years the presence of excess nitrogen in mucopolysaccharides has indicated the necessity for further purification. However, in recent years, with the elucidation of the structures of the carbohydrate components[1], interest has turned to the components other than carbohydrate and their manner of attachment[2–8].

In our studies of the chondroitin sulfates, the 4 amino acids—serine, glutamic acid, proline, and glycine—have been found to be present to the greatest extent and often in equimolar quantities in samples containing fewest amino acids, suggesting that they are an integral part of a peptide linked directly to a sugar moiety. The disappearance of serine, and only serine, during alkali treatment of chondroitin 4-sulfate strongly indicates that the β-carbonyl elimination of an alkoxide from an *O*-substituted serine is occurring and, since this treatment results in the removal of all amino acids from the polysaccharides, that the substituent is a sugar component of the mucopolysaccharide.

The data in Columns 1, 2, and 3 of Table I show that when chondroitin 6-sulfate is treated with papain the amount of every amino acid decreases significantly except serine. This indicates that it is linked to the carbohydrate, as previously assumed by MUIR[2] with a preparation of much greater amino acid content.

The finding that serine, glutamic acid, proline, and glycine predominate and occur in most cases in equimolar amounts can be seen in all the columns except Column 11. This is a striking result, considering the various treatments to which the samples were subjected—especially the presence of amino acids in isolated oligosaccharide fractions (Sample 4) and in samples subjected to the desulfation procedure (Sample 8). Even in Sample 6 after 80% of the amino acids have been cleaved by alkali, the same four predominate in relatively equimolar quantities within the accuracy of the determination. There can be a linkage to serine every 67 repeating units (33000 formula wt.) for chondroitin 6-sulfate and every 42 repeating units (21000 formula wt.) for chondroitin 4-sulfate.

It has long been known that the linkage of amino acids to chondroitin sulfate is alkali labile[13], and the linkage has been assumed to be ester[2]. The data presented in Columns 10 and 11 were obtained from the same substrate. Using conditions which completely removed amino acids from chondromucoprotein, Sample 11 was treated with alkali prior to acid hydrolysis for amino acid analysis. The data show that at least two-thirds of the serine is chemically changed by the elimination reaction occurring in alkali. This is the first concrete evidence that the hydroxyl of serine is involved in the linkage and that the carbohydrate is eliminated from the serine residue as an alkoxide moiety. It is extremely unlikely that the result is due simply to the alkali lability of serine in peptide linkage[14]. This is indicated by the fact that threonine was not changed. Furthermore, edestin was treated in exactly the same way and showed no loss of serine or of threonine. Hydrazinolysis[15] of the same

TABLE I

ANALYSES OF CHONDROITIN SULFATES

Amino acids[9] in μmoles/g; other components in per cent[10]. Most of the amino acids not listed were present in much smaller amounts than valine. The treatment and source of the chondroitin sulfates were as follows: Sample 1: panprotease-treated shark cartilage; Samples 2 and 3: Fractions from papain-treated Sample 1; Sample 4: Tetrasaccharide from hyaluronidase-digested Sample 1; Sample 5: Same treatment as Sample 1 on different shark; Sample 6: Solution in 0.5 N NaOH at 2° for 7 h under N_2 followed by reisolation of sample 5; Sample 7: Alcohol-fractioned chondromucoprotein from nasal septa of 8-year-old cows, further fractioned on a DEAE-cellulose column with a linear NaCl gradient; Sample 8: Desulfation by the modification of HIRANO *et al.*[11] of Sample 7 followed by fractionation on a DEAE-cellulose column; Sample 9: Panprotease digestion of chondromucoprotein from nasal septa of calves; Sample 10: Panprotease digestion of residue from preparation of Sample 7; Sample 11: Solution in 0.5 N NaOH at 2° for 19 h of Sample 10. The solution was acidified and hydrolysed directly for amino acid analysis.

Components	*Chondroitin 6-sulfate*						*Chondroitin 4-sulfate*				
Sample No.	*1**	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Aspartic acid	8.0	1	2	0	3.6	0	53.6	58.8	9.5	18	16
Threonine	4.0	tr	tr	0	2.9	0	40.7	43.8	7.3	9.8	9.5
Serine§	31	27.5	32	9.2	29	4.5	90.8	164	47.8	47.9	16
Glutamic acid***	35	17	21	11	29	6.2	112	171	34.8	42.7	42
Proline***	55	22	27	17	32	~4	87	157	35.6	38	43
Glycine	48	22	25	14	31	8.0	86.8	279	43.8	45.9	49.0
Alanine	7.0	tr	tr	0	3	0	46	92.6	14	17	14
Valine***	13	tr	6.4	0	9.9	0	56	70	—	18	17
Total amino acids in %	3.0	1.1	1.5	0.6	2.0	0.5	10.1	13.7	—	3.3	—
$[\alpha]_D$	−20°	−16°	−16°	−10°	−15°	−16°	−22°	−21°	−28°	−33°	
Uronic acid (carbazole)	31.0	31.8	31.2	26.6	30.6	38.0	34.7	28.8	32.9	30.6	
Hexosamine	23.0	23.3	22.9	23.5	—	23.2	—	—	24.6	—	
Sulfate	19.8	19.2	18.6	15.5**	—	—	16.8	1.3	—	12.4	

* We thank Dr. R. CANFIELD for this amino acid analysis.
** as NH_4^+ salt. Other analyses for this sample after conversion to K^+ salt.
*** Corrected for destruction due to hydrolysis in presence of mucopolysaccharide.
§ Corrected for destruction due to acid hydrolysis[12].

chondromucoprotein used to prepare Sample 9 showed the incorporation of only 7 μmoles of bound hydrazine per gram. If this is corrected for protein content, it would represent at the most 10 μmoles of hydrazine per gram of chondroitin sulfate. These results are incompatible with an ester bond.

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Further investigations on the carbohydrate moiety of egg albumin*

It has been suggested that the bond linking carbohydrate to protein in egg albumin is that of an *N*-(β-aspartyl)glycosylamine[1,2] of *N*-acetylglucosamine[3–5]. Partial acid hydrolysis of a purified glycopeptide from the protein gave rise to small amounts of a substance, containing glucosamine and aspartic acid but no mannose, which behaved electrophoretically and chromatographically like 2-acetamido-1-β-(L-β-aspartamido)-1,2-dideoxy-D-glucose[6]. Further high-tension paper-electrophoretic studies (Whatman 3 MM, 40 V/cm, 60 min, pH 1.87) of partial acid hydrolysates (2 N HCl, 12 min, 100°) of the latter compound and of the glycopeptide from egg albumin have revealed a great similarity in the "fingerprint" obtained by staining the paper strip with the ninhydrin reagent described previously[7] (Fig. 1). In this Figure, Spot 2 in each case (brown with ninhydrin) was given by a substance which had the same mobility as 2-acetamido-1-β-(L-β-aspartamido)-1,2-dideoxy-D-glucose; Spot 3 (blue-purple) the same mobility as aspartic acid and Spot 8 (purple) the same mobility as glucosamine. Spot 8 was also Elson–Morgan positive in each case. The identity of the remainder

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