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Short Communications

Structural Studies on a Sulfated Fucan from Early Sea Urchin Embryos

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Several authors [1-5] have reported the presence of sulfated glycosaminoglycans, dermatan- and heparan sulfate, in the sea urchin embryo. A third sulfated polysaccharide, resistent to enzymic and nitrous acid treatment, has also been found in early embryos [2] and was assumed to represent fucan sulfate.[6] This substance has now been further characterized.

The material remaining after chondritinase ABC digestion and nitrous acid treatment of the glycosaminoglycans isolated from *P. lividus* embryos, contained 6.6 % S as analyzed by elemental analysis and 11.5 % protein.[7] The carbohydrate material was acidic and had $[\alpha]_{578}$ −44.7° (*c* 0.2, H_2O). Sugar analysis [8] revealed that the sample consisted mainly of fucose (88 %) and contained fucose, arabinose, galactose and glucose in the proportions 44:1:2:3. ^1H NMR showed *inter alia* a signal at δ 1.91, which was attributed to the CH_3-groups of the fucose residues. A comparison of the integrated signal for the protons of the methyl groups of the fucose residues with all other signals in the ^1H NMR spectrum, revealed that fucose accounted for more than 90 % of the material.

Methylation analysis [9,10] showed that the fucose residues were 1,2-linked. From amino acid analysis and ^{13}C NMR spectroscopy it was deduced that no glucosamine, galactosamine or uronic acid were present in the sample. The low optical rotation, together with data from NMR spectroscopy, sugar methylation and elemental analysis demonstrate that the carbohydrate material consists of a sulfated polysaccharide built up by 1,2-linked α-L-fucopyranosyl residues, thus confirming earlier results. The undegradable material, which on a weight basis consists of 72 % fucose, is composed primarily of a polymer of fucose with 2 sulfate groups per 5 monosaccharide, *i.e.*, of polyfucose sulfate.

Previous investigations [2] revealed that when sea urchin embryos were reared in the presence of radioactive glucose or glucosamine, 65 % of the activity incorporated was bound to the residue. In the presence of radioactive sulfur or fucose, as much as 74 and 93 %, respectively, of the label was found in this fraction. This quantitatively important polysaccharide is thus actively synthesized during early development.

Fucan sulfate has previously been found in the jelly coat of *P. lividus* and a variety of other sea urchins [11−14] The substance presently investigated cannot represent a contamination by jelly, since the embryos are collected several hours after hatching. While the jelly from certain species like *Strongylocentrotus pulcherrimus* or *Pseudocentrotus depressus* only contains fucose, the jelly of for instance *Strongylocentrotus droebachiensis* or *Paracentrotus lividus* contains minor amounts of galactose and glucose besides fucose. Small amounts of galactose, glucose and arabinose were detected in the material investigated here, indicating that the sulfated polysaccharides located within the embryo and those of the jelly are of very similar composition, and are in both cases most certainly part of larger glycoprotein complexes.

Fucan sulfate occurs also in the body-wall connective tissue of at least three exhinoderm species [6,15,16] while it was absent in the connective tissue of lower invertebrates like coelenterates and of poriferans.[6] Polyfucose sulfate may thus be specific for echinoderms. Polyglucose sulfate is another sulfated polysaccharide which as yet has been detected in only one phylum.[17]

General methods. Concentrations were carried out under reduced pressure at bath temperatures not exceeding 40 °C. For GLC, a Packard 427 instrument fitted with a flame-ionization detector was used. Separations were performed on a Sp 1000 glass capillary column at 220 °C (for partial-

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ly methylated alditol acetates), or on a glass column (180×0.15 cm) containing 3 % Sp 2430 on Supelcoport (100/120 mesh) at 170 °C (for alditol acetates). Peak areas were measured with an Autolab Minigrator. For GLC–MS, a Finnigan 4021 gas chromatographmass spectrometer was used. NMR spectra were recorded on a Jeol FX 90Q instrument. The ^{1}H NMR spectra were recorded at 90 MHz at 80 °C. The samples were dissolved in D_2O and external tetramethylsilane (^{13}C NMR) or internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulfonate (^{1}H NMR) were used as references. Optical rotations were determined with a Perkin-Elmer 141 instrument. Methylation was performed by the Hakamori method with sodium methylsulfinylmethanide-methyl iodide in dimethyl sulfoxide.[9] Hydrolysis and analysis of the products were performed according to Jansson *et al.*[10]

Isolation of polysaccharide material. Eggs from *Paracentrotus lividus* were collected from excised ovaries, washed several times and fertilized *in vitro*. Fertilization was controlled under the microscope. The eggs were rinsed by several washes with sterile-filtered sea water. The embryos were collected by centrifugation after about 24 h when development had reached the prism stage, and dried with acetone. All dry material was pooled, providing a total of 25 g dry powder from 40–50 females.

The dry powder, in fractions of 3–4 g, was suspended in 50 ml of 0.1 M Tris–HCl buffer, pH 8.0, containing 5 mM $CaCl_2$, and digested in the presence of 1 mg pronase (Boehringer) per ml for 20 h at 50 °C. The digest was centrifuged at 400 g for 15 min and the pellet digested a second time under the same conditions. TCA was added to the combined supernatants to a final concentration of 10 % and maintained in the cold for 1 h. The precipitate was removed by centrifugation at 25,000 *g* for 15 min and the supernatant freed from TCA by ether extraction, followed by exhaustive dialysis against distilled water. The dialysate was concentrated by ultrafiltration on a YM5 filter (Amicon) and freeze-dried.

The dry material (about 0.75 g) was then dissolved in 5 ml of 50 mM sodium acetate buffer, pH 4.0, containing 0.2 M NaCl and applied to a DE-52 cellulose column (1.6×2.0 cm). Glycosaminoglycans were eluted in one step with 1.5 M NaCl in the same buffer, and concentrated by ultrafiltration. The concentrated fluid was desalted by passage through a disposable Sephadex G-25M column (1.5×5.0 cm) (PD-10, Pharmacia) and freeze-dried.

Degradation of glycosaminoglycans. The dry material (about 50 mg) was dissolved in 1 ml of 0.05 M Tris–HCl buffer, pH 8.0, containing 0.06 M sodium acetate, 0.05 M NaCl and 0.01 % bovine serum albumin, and digested in the presence of 2 U chondroitinase ABC (Sigma) for 20 h at 37 °C.[18] The undegraded material was separated from digestion products by gel filtration through a Sephadex G-50 column (0.9×65 cm) and freeze-dried.

The dry residue was treated with 5 % sodium nitrite and 33 % acetic acid in equal amounts for 30 min at room temperature. Undegraded material was purified on Sephadex G-50 as above and freeze-dried. A total amount of 12 mg was thus obtained and used for structural analysis.

Structural analysis. Part of the sample (7.6 mg) was used for determination of optical rotation, elemental analysis and amino acid analysis.[7] Part of the material (1 mg) was hydrolysed and the resulting sugars were analysed as their alditol acetates.[8] Uronic acid residues were investigated by ^{13}C–NMR spectroscopy and sugar analysis after reduction.[19]

Another part of the sample (4 mg) was desulfated in DMSO[20], dialysed, concentrated and freeze-dried. The polysaccharide material was methylated, hydrolysed, and analysed as alditol acetates by GLC–MS.[10] One major peak, with the relative retention time of 1.05 (1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol has 1.00) was identified as 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-fucitol. The mass spectrum had, *inter alia*, peaks at *m/e* 43 (100), 85 (10), 99 (12), 115 (13), 129 (12), 131 (13), 141 (5), 189 (6) and 233 (5) which is in accordance with that from a 1,2,5-tri-*O*-acetyl-6-deoxy-3,4-di-*O*-methyl-hexitol.[10]

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1. Kinoshita, S. *Exp. Cell Res. 56* (1969) 39.
2. Løvtrup-Rein, H. and Løvtrup, S. *Exp. Cell Biol.* (1984). *In press.*
3. Nakatsuji, N. and Løvtrup, S. *Arch. Anat. Microsc. Morphol. Exp. 67* (1978) 185.
4. Sugiyama, K. *Dev. Growth Differ. 14* (1972) 63.
5. Yamagata, T. and Okasaki, K. *Biochim. Biophys. Acta 372* (1974) 469.
6. Katzman, R. L. and Jeanloz, R. W. *Science 166* (1969) 758.
7. Spackman, D. H., Stein, W. H. and Moore, S. *Anal. Chem. 30* (1958) 1190.

8. Sawardeker, J. S., Sloneker, J. H. and Jeanes, A. *Anal. Chem. 37* (1956) 1602.
9. Hakomori, S. *J. Biochem. (Tokyo) 55* (1964) 205.
10. Jansson, P. E., Kenne, L., Liedgren, H., Lindberg, B. and Lönngren, J. *Chem. Commun. Univ. Stockholm* (1976) No. 8.
11. Tshihara, K., Oguri, K. and Taniguchi, H. *Biochim. Biophys. Acta 320* (1973) 628.
12. Katzman, R. L. and Jeanloz, R. W. *J. Biol. Chem. 248* (1973) 50.
13. Vasseur, E. and Immers, J. *Ark. Kemi 1* (1949) 39.
14. Muramatsu, T. *J. Biochem. 57* (1965) 223.
15. Motohiro, T. *Bull. Jpn. Soc. Sci. Fish. 26* (1960) 1175.
16. Tanaka, K., Nishi, C., Takaya, M. and Uchiyama, T. *J. Biochem. 72* (1972) 1265.
17. Lash, J. W. and Whitehouse, M. W. *Biochem. J. 74* (1960) 351.
18. Saito, H., Yamagata, T. and Suzuki, S. *J. Biol. Chem. 243* (1968) 1536.
19. Taylor, R. L. and Conrad, H. E. *Biochemistry 11* (1972) 1383.
20. Inoue, Y. and Nagasawa, K. *Carbohydr. Res. 46* (1976) 87.

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