A SYNTHETIC HEPARINOID FROM AMYLOSE

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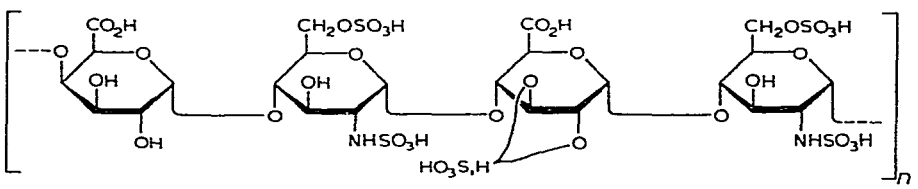
ABSTRACT

Aminated (mainly on C-2; d.s. 0.6) 6-*O*-tritylamylose was selectively *N*-(trifluoroacetyl)ated, and the product detritylated; the product was re-*N*-(trifluoroacetyl)ated, and the primary alcohol group on C-6 was oxidized to the carboxyl group (d.s. 0.2) with nitrogen dioxide. The product was de-*N*-(trifluoroacetyl)ated, and the non-dialyzable fraction of the modified amylose was sulfated and re-*N*-sulfated, to give a white powder (11.5% S) which had negligible activity as a blood anticoagulant.

INTRODUCTION

Numerous attempts have been made to produce a substitute for heparin by the sulfation of a variety of readily available polymers[1]. However, the sulfated products, although showing moderate blood-anticoagulant activities, all lower than that of heparin, were found to be pathologically unacceptable for clinical use because of delayed toxicities. It was considered possible that these unacceptable biological activities might be due to dissimilarity of the polysaccharide structures to that of the heparin molecule. At the time that this work was initiated, it was supposed that the heparin molecule is a structural analog of amylose of the structure[2–5] shown in **1**. It



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was deemed feasible to operate chemically on amylose, prepared commercially from potato starch, to produce a modified polysaccharide which, after sulfation, would be rather similar in structure to **1**.

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RESULTS AND DISCUSSION

The preparation of an aminated 6-*O*-tritylamylose has been described[6]. 6-*O*-Tritylamylose[7,8] had been aminated (replacement of hydroxyl group by amino group), mainly on C-2, to a degree of substitution (d.s.) of 0.6 in respect to the amino group. The amino groups in this polysaccharide were then protected by selective *N*-(trifluoroacetyl)ation[9–11]. Detritylation of the product was effected with methanolic hydrochloric acid, and the product was subjected to a second *N*-(trifluoroacetyl)ation with *S*-ethyl trifluorothioacetate[12,13] to ensure complete protection of the amino groups. The *N*-(trifluoroacetyl)ated aminoamylose was then subjected to preferential oxidation with nitrogen dioxide in carbon tetrachloride[14] in order to introduce carboxyl groups involving C-6. This oxidation procedure, with some variation thereof, was capable of introducing only 4–7% of carboxyl groups into the *N*-(trifluoroacetyl)ated aminoamylose, a value much less than the 11% required for a completely desulfated heparin. As amylose can, under like conditions, be oxidized to a carboxyl content of 17%, as shown by Kenyon and co-workers[14], it would seem that the presence of the *N*-(trifluoroacetyl) groups in the (trifluoroacetamido)amylose interferes with the functioning of nitrogen dioxide as an oxidant.

The partially carboxylated, *N*-(trifluoroacetyl)ated, partially aminated amylose was treated with sodium borohydride in water, in order to reduce any keto groups generated as a result of oxidation by the nitrogen dioxide; at the same time, the alkalinity of the aqueous sodium borohydride solution was sufficient to remove all the *N*-(trifluoroacetyl) groups within three hours. The resulting partially carboxylated, partially aminated amylose (sodium salt) gave positive tests for free amino (ninhydrin) and carboxyl groups (naphthoresorcinol), and was non-dialyzable. This modified amylose was dissolved in the minimal volume of M hydrochloric acid, and precipitated with ethanol in order to remove the sodium ions. The precipitate was centrifuged off and made into a paste with triethylamine, and further amounts of ethanol were added directly to the centrifuge tube. The suspension was well stirred, and centrifuged, and the precipitate was washed with ether, and dried exhaustively. The treatment with triethylamine effectively regenerated the amino group from its corresponding hydrochloride (salt) and simultaneously converted the free carboxyl groups into their organic amine salts. This material was readily soluble in anhydrous *N*,*N*-dimethylformamide containing sulfur trioxide–pyridine complex as the sulfating agent. The sulfated polysaccharide resulting was subjected to several further *N*-sulfations with sulfur trioxide–pyridine complex in aqueous sodium carbonate solution, according to the general procedure described by Nominé and associates[15].

The final product, as the sodium salt, had a sulfur content of 11.5%, a value normal for commercial heparin. The infrared spectrum of the final product resembled that of Upjohn beef-lung heparin (110 I.U./mg) in every respect, and yet the anticoagulant potency was only about 1/9th that of the latter. The low anticoagulant activity of the synthetic compound is probably due to one or more of four factors: (1) the sequence and regularity of carboxyl and amino groups in the modified amylose;

(2) the low carboxyl content as compared to that of heparin; (3) the lack of L-iduronic acid residues in the synthetic material; and (4) the molecular size (~0.5 that of commercial heparin[16]). The presence of L-iduronic acid residues in heparin[17] was not definitely established until after the present work was near completion. To prepare a synthetic heparinoid that further simulates the structure of heparin, based upon data at present available, L-iduronic acid residues should, presumably, be incorporated in the modified amylose.

EXPERIMENTAL

N-(Trifluoroacetyl)ation of aminated 6-O-tritylamylose. — Aminated 6-*O*-tritylamylose[6] (40 g, d.s. 0.6 with respect to amino groups) was dissolved in dichloromethane (400 ml) and pyridine (40 ml). The solution was cooled to 0°, and trifluoroacetic anhydride (45 g) was added, with stirring, during 2 h. The reaction was continued for a further hour at room temperature, and the solution was then concentrated under diminished pressure to ~100 ml. Pyridine (100 ml) was added, and the brown solution was poured slowly into 1:9 (v/v) methanol–water (3 liters) containing 5 g of sodium hydrogen carbonate. The white precipitate was washed thrice with water in a blender, and air dried; yield 43 g. This *N*-(trifluoroacetyl)ated, aminated 6-*O*-tritylamylose showed a negative ninhydrin test.

Anal. Calc. for $[C_6H_7O_2(OC_{19}H_{15})(OH)_{1.4}(NHCOCF_3)_{0.6}]_m$: N, 1.57. Found: N, 1.47.

Detritylation of N-*(trifluoroacetyl)ated, aminated, 6-O-tritylamylose.* — The acylated material just described (42 g) was suspended in methanol (1 liter) containing 1% of concentrated hydrochloric acid, and the suspension was stirred for 3 h. The acid was neutralized with Dowex-1 (CO_3^{2-}) anion-exchange resin, and the suspension was filtered through a coarse, sintered-glass funnel. The filtrate was concentrated under diminished pressure to ~100 ml, and poured into ether (6 liters). The precipitate was collected by filtration, washed exhaustively with ether, and dried under diminished pressure to a white powder; yield 16 g. This product exhibited a faint ninhydrin test, probably because some *N*-(trifluoroacetyl) groups had been removed during the detritylation. It was therefore dissolved in methanol (50 ml) and *S*-ethyl trifluorothioacetate (25 g) was added. The solution was well agitated, and sodium hydrogen carbonate was continuously added to maintain the pH at ~8–9 during 4 h The completely *N*-(trifluoroacetyl)ated aminoamylose was recovered by dilution of the methanol solution with water (700 ml), and was dialyzed for 3 days in suspension, followed by freeze-drying; yield 9.0 g. The resultant *N*-(trifluoroacetyl)ated, aminated amylose did not respond to the ninhydrin test.

Anal. Calc. for $[C_6H_7O_2(OH)_{2.4}(NHCOCF_3)_{0.6}]_m$: N, 3.84. Found: N, 3.74

Oxidation of N-*(trifluoroacetyl)ated, aminated amylose, and de-*N-*(trifluoroacetyl)ation.* — The *N*-(trifluoroacetyl)ated, aminated amylose just described (3 g) was mixed with an equal weight of Celite, and the mixture was dried over phosphorus pentaoxide under diminished pressure The dried mixture was suspended in carbon

tetrachloride (50 g) at 0°, with stirring, and dried nitrogen dioxide was introduced from a cylinder. When 50 g of the oxidant had been absorbed, the temperature was raised to 16–20°. The oxidized material was then filtered off, washed exhaustively with carbon tetrachloride, and added to water (200 ml) containing sufficient sodium carbonate to neutralize any acid derived from the nitrogen dioxide. Sodium borohydride (1 g) was then added and the alkaline solution was stirred for 3 h to remove the *N*-(trifluoroacetyl) groups. The excess of borohydride was decomposed by the addition of dilute hydrochloric acid, the pH of the solution was adjusted to 3, and the solution was dialyzed for 3 days. The nondialyzable fraction was filtered through a Millipore filter and freeze-dried; yield 800 mg.

Anal. Found: CO_2H (ref. 18); 5.04 (d.s. 0.2).

Increased reaction times (up to 40 h) increased the carboxyl content to only 7%.

Sulfation. — The modified amylose just described (720 mg) was dissolved in the minimal volume (~10 ml) of M hydrochloric acid, and precipitated with ethanol (400 ml). After centrifugation, and washing with ethanol (60 ml), the precipitate was made into a paste with triethylamine (~8 ml). Ethanol was added directly to the centrifuge tube (to remove excess of triethylamine and its hydrochloride). The centrifuged precipitate was successively washed with ethanol (40 ml) and ether (40 ml), and dried under diminished pressure over phosphorus pentaoxide for 24 h. This material (470 mg) was dissolved in *N*,*N*-dimethylformamide (20 ml) containing the 1:1 sulfur trioxide–pyridine complex (5 g). Sulfation was continued for 2 days at room temperature, and the solution was then poured into 5% aqueous sodium hydrogen carbonate solution (200 ml). The sulfation product was dialyzed for 2 days, and freeze-dried to give a white powder; yield 510 mg. This material was further *N*-sulfated with 1:1 sulfur trioxide–pyridine complex (2 g) in 30% sodium carbonate solution (5 ml) for 4 h. Addition of the sulfating agent (1 g) and sodium carbonate (1.5 g) was repeated twice more. The solution was then diluted to 100 ml with water, and the sulfation product was precipitated with 20% aqueous solution of Cetavlon. The Cetavlon salt of the sulfated polysaccharide was dissolved in butyl alcohol (10 ml), and the yellow solution was extracted with a 10% solution of sodium acetate (10 ml). The aqueous layer was separated and poured into ethanol (150 ml), and the resulting precipitate was centrifuged off and washed successively with ethanol and ether, to give the sulfated, partially carboxylated, partially aminated amylose as a water-soluble, white powder; yield 350 mg, $[\alpha]_D^{24}$ +66° (*c* 3.89, water). The i.r spectrum of the product was essentially the same as that of commercial heparin (sodium salt, Upjohn); the optical rotation was more dextrorotatory.

Anal. Found: N, 1.8; S, 11.5; mol. wt., 6760 (ultracentrifuge–sedimentation equilibrium, uncorrected for effect of ionic charges); A. C. U.*, ~9 I. U./mg.

*Blood-anticoagulant activity expressed in international units per mg, performed according to the instructions in the United States Pharmacopeia (XVI, 317), by using sheep plasma supplied by the Wilson Laboratories, Chicago, Illinois.

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