UDC 541.64:547.455

DEXTRAN DERIVATIVES

I. SYNTHESIS OF O-AMINOACYL DERIVATIVES OF DEXTRAN

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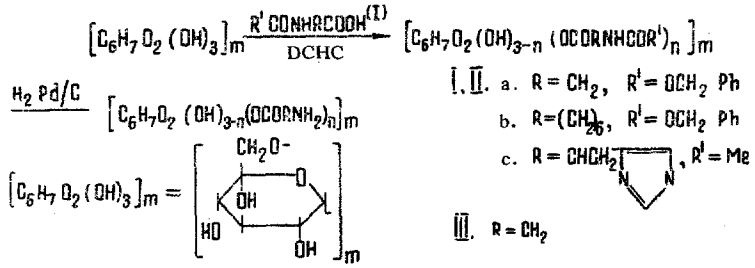
Khimiya Prirodnykh Soedinenii, Vol. 5, No. 5, pp. 427-432, 1969

Glycoproteins, mixed biopolymers, contain within themselves the elements of polysaccharide and polypeptide structures, and also fulful very diverse biological functions. However, the synthetic production of analogous structures or their simplified models has been little studied. In this paper we give the results of the synthesis of polysaccharide derivatives containing amino acid residues attached by ester bonds. The aim of the investigations was to obtain derivatives of dextran, which is an α-1,6-glucan produced by Leuconostoc mesenteroides and some other microorganisms. The glucan has a branched main chain involving 1,2-, 1,3-, and 1,4-bonds, the amount, length, and nature of the branching depending on the producing microorganism [1]. The starting material was the preparation "Polyglucin" [2]—the product of the partial acid hydrolysis of native dextran (mol. wt. 55 ± 15 thousand).

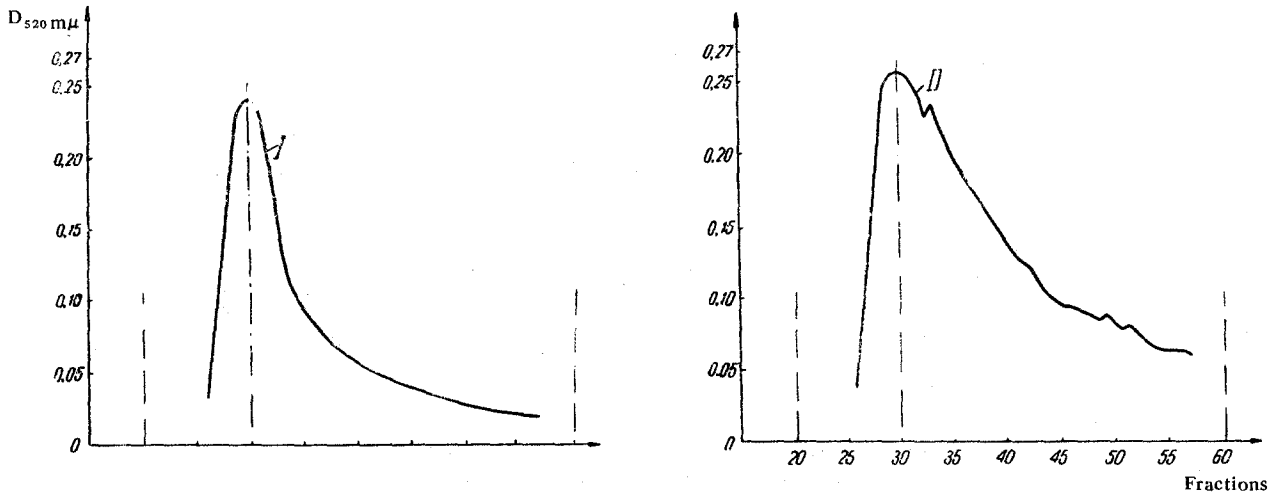
Aminoacyl derivatives of dextran have been obtained under very severe conditions in the presence of monochloroacetic acid and $Mg(ClO_4)_2$ at 100° C in a heterogeneous medium [3]. But under these conditions numerous degradation processes take place. Consequently we obtained the O-aminoacyl derivatives of polyglucin (dextran) by a method developed previously [4] which is based on the condensation of carbohydrates with N-benzyloxycarbonyl- (boc-) -amino acids in the presence of dicyclohexylcarbodiimide (DCHC). The performance of the condensation under the conditions described for the O-aminoacylation of monosaccharides (in pyridine) required a heterogeneous medium, and no clear result was obtained. Consequently, as solvent we used a mixture of pyridine and dimethyl sulfoxide in which not only the polysaccharide but also all the reaction products were fairly soluble. This variant of the carbodiimide condensation is obviously suitable for the aminoacylation of polysaccharides. Condensation was carried out at room temperature for 40—60 hr with the boc derivatives of glycine, ω-aminoenanthic acid, and N-acetylhistidine in accordance with the following scheme:



The reaction product, after the usual working up, was freed from salts and low-molecular-weight impurities by gel filtration on Sephadex G-50 and was reprecipitated from water with ethanol. The modified dextrans obtained contained a definite amount of nitrogen, which permitted their degree of substitution (O-aminoacylation) to be established. For boc-glycine it was 11.1 residues per 100 anhydroglucose units, for boc-ω-aminoenanthic acid 2.2, and for N^{α}-acetylhistidine 1.05. The degree of substitution found in the O-aminoacyldextrans was always lower than was to be expected from the ratio of the substances used in the reaction. The presence of an ester bond in the derivatives obtained was confirmed by their IR spectra. Absorption at 1738-1742 cm^{-1} was ascribed to the carbonyl of an ester group, and that at 1638-1644 and 1534-1543 cm^{-1} to the carbonyl of a urethane or N-acetyl group. At the present time, the distribution of O-aminoacyl groups in the polysaccharide chain cannot be established; from information on the aminoacylation of glucose under the given conditions it must be regarded as probable that they are attached to the free terminal primary hydroxyl groups of the dextran and to the secondary hydroxyls at $C_{(3)}$ of the units present in the chain [5].

To decide the question of possible degradation of the polysaccharide on condensation, comparative gel filtration of the initial and the modified polymers on Sephadex G-75 was carried out.

The close similarity of the elution curves of the preparations (see figure) shows the absence of substantial destruction of the polysaccharide chain, although isolated random cleavages of the molecule are not excluded. This is also confirmed by the results of sedimentary analysis: the sedimentation coefficients for dextran and the modified polymer are similar. To obtain a modified polysaccharide with a free amino group, hydrogenation was carried out over Pd/C in the presence of two equivalents of oxalic acid, as has been described for aminoacyl derivatives of the monosaccharides [4]. We have carried out this conversion for the glycine derivatives. The degree of hydrogenolysis was checked by titrating the amount of CO_2 liberated. The oxalate of O-glycldextran (III) that was formed was converted via the base into the



Fraction curves on Sephadex G-75 in water: I) dextran ("polyglucin"); II) N-boc-glycyldextran.

hydrochloride, which is stable under ordinary conditions. Titration of the hydrochloride of III with alkali in the presence of phenolphthalein showed the hydrogenolysis had taken place quantitatively and gave a degree of substitution of the dextran with glycyl residues agreeing with that determined by elementary analysis for nitrogen of the O-(N-boc-glycyl)dextran (IIa) and also with the amount of CO_2 liberated on hydrogenation.

The structures of substances II and III were confirmed by comparing their IR spectra with that of the initial dextran, and also by their sedimentation constants.

Experimental

Dry "polyglucin" (dextran) with 2.31% of moisture was dried further at 80° C/1 mm to constant weight before use in the reaction. The preparation was homogeneous on sedimentation analysis ($S_{19} = 2.14 \times 10^{-13}$ in water at 49200 rpm). The molecular-weight distribution, according to the technical specifications, was $M_W = 55 \pm 15$ thousand, the fraction with $M_W \leq 150$ thousand being 5–10%, and that with $M_W \geq 15$ thousand 5–10%. N-boc-Glycine with mp 118–119° C and N-boc-aminoenanthic acid with mp 88–89° C (from chloroform), obtained by the usual method, and N^{α}-acetyl-L-histidine with mp 145° C of the firm "Reanal" (Hungary) were used. The dimethyl sulfoxide was purified by vacuum distillation. Gel filtration through Sephadex G-50 (coarse) and fractionation on Sephadex G-75 were carried out in water. The concentration and evaporation of the solutions were done in vacuum in a rotary evaporator at ≤ 50°C. The solid substances were dried at 40° C/1 mm over P_2O_5 to constant weight.

The UV spectra were taken in water on an SF-4[a] instrument, and the IR spectra in KBr tablets on a UR-10 instrument. The sedimentation analysis was carried out in a "Five" ultracentrifuge at 49,200 rpm in aqueous solutions*. The dextran and its derivatives were determined quantitatively in the fractions by a color reaction with sulfuric acid in the following way**. To 1 ml of a solution of dextran or a dextran derivative containing 50-750 μg of dextran was added 3.5 ml of concentrated sulfuric acid, the mixture was heated at 100° C for 30 min and cooled to room temperature, and its optical density was measured at 520 mμ in the SF-4[a] spectrophotometer. The concentration was determined from a calibration curve. There is a linear relationship between the optical density and the concentration of solutions of dextran within the limits mentioned.

O-(N-boc-Glycyl)dextran (IIa). To a solution of 1.55 g of dextran in 90 ml of dimethyl sulfoxide were added 30 ml of dry pyridine, 2 g of N-boc-glycine (Ia), and 7.88 g of dicyclohexylcarbodiimide (DCHC), and the mixture was stirred at 20° C for 48 hr and evaporated in vacuum. The residue was dissolved in the minimum amount of water, the dicyclohexylurea was filtered off, and the polymer was precipitated completely with ethanol. The solution was decanted off and the residue was triturated repeatedly with fresh portions of ethanol, dissolved in a small amount of water, and subjected to gel filtration through Sephadex G-50. The fractions containing the polymer were combined, concentrated

*The IR spectra were recorded by M. M. Ushakova and interpreted by O. S. Chizhov; the untracentrifuge investigations were performed by V. M. Shlimak.

**The color reaction was developed by A. A. Khachatur'yan and A. B. Livshits.

in vacuum to small volume, and the polymer was precipitated with ethanol and dried to constant weight. The yield was 1.24 g. White hygroscopic powder, readily soluble in water, and insoluble in organic solvents.

$[\alpha]_D^{20}$ +177.9° (c 0.95; water). Found, %: N 0.86; degree of substitution γ = 11.1. UV spectrum: $\lambda_{H_2O}^{max}$ 287 mμ (aromatic ring). IR spectrum, cm^{-1}: 1543, 1644 (broad bands), 1741. Sedimentation coefficient, $S_{19} = 1.66 \times 10^{-13}$.

Similarly, 5 g of dextran, 0.65 g of boc-glycine, and 0.76 g of DCHC gave O-(N-benzyloxycarbonylglycyl)dextran with γ = 2.8 $[\alpha]_D^{20}$ +149.2° (c 0.982; water).

O-(N-boc-Aminoenanthyl)dextran (IIb). To a solution of 8 g of dextran in 200 ml of dimethyl sulfoxide were added 20 ml of dry pyridine, 1.38 g of N-boc-aminoenanthic acid, and 1.2 g of DCHC and the mixture was stirred at 20° C for 48 hr. The pyridine was driven off in vacuum and the residue worked up. Yield 6.63 g. Found, %: N 0.18; γ = 2.2. UV spectrum: $\lambda_{H_2O}^{max}$ 287 mμ (aromatic ring). IR spectrum, cm^{-1}: 1548, 1644, 1738 (all broad).

O-(N^{α}-Acetyl-L-histidyl)dextran (IIc). To a solution of 7.92 g of dextran in 20 ml of dimethyl sulfoxide were added 20 ml of dry pyridine, 1.05 g of N^{α}-acetyl-L-histidine, and 1.2 g of DCHC, and the mixture was stirred at 20° C for 60 hr and treated as described above. Yield 6.25 g. White hygroscopic powder, soluble in water and insoluble in organic solvents, $[\alpha]_D^{20}$ +167.7° (c 0.975; water). An aqueous solution had pH 6.44, and a hydrolyzate gave the Pauly reaction. Found, %: N 0.17; γ = 1.05. Sedimentation coefficient, $S_{19} = 2.13 \times 10^{-13}$. The ratio $S/S_{dextran}$ was 1.0. IR spectrum, cm^{-1}: 1555, 1644, 1742.

Hydrochloride of O-glycyldextran (III). A solution of 184.3 mg of O-(N-boc-glycyl)dextran (IIa) with a degree of substitution γ of 11.1 in 35 ml of water was treated with 63 mg of oxalic acid and 200 mg of 5% Pd/C. The suspension was hydrogenated with stirring in a current of hydrogen for 2.5 hr. The issuing gases were passed through a standardized solution of $Ba(OH)_2$. A total of 0.0055 M of CO_2 was liberated. The catalyst was filtered off and the filtrate was treated with aqueous ammonia to pH 5.5–6.0, concentrated to small volume, and subjected to gel filtration through Sephadex G-50. From the fractions containing the polymer the substance was isolated by precipitation with ethanol. Yield 92.9 mg. White powder soluble in water and insoluble in organic solvents, γ = 11.0 from the CO_2 liberated, and γ = 11.6 from the results of the titration of the hydrochloride with alkali. The sedimentation coefficient was 1.17×10^{-13} and the IR spectra, cm^{-1}: 1741, 1640.

Conclusions

1. A method for the O-aminoacylation of dextran ("polyglucin") has been proposed.

2. The synthesis of O-aminoacyl derivatives of dextran containing residues of glycine, ω-aminoenanthic acid and N^{α}-acetyl-L-histidine has been effected.

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11 April 1968

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