DEXTRAN DERIVATIVES

IV. ACYLATION OF DEXTRAN WITH IMIDAZOLIDES OF N-PROTONATED AMINO ACIDS

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In preceding papers [1, 2] we have described various methods of acylating dextran (the domestic clinical preparation "Polyglucin") with N-substituted amino acids. Some of these methods (with dicyclohexylcarbodiimide and with benzenesulfonyl chloride) are fully satisfactory for preparative purposes. So far as concerns the elimination of the N-protective groups from the dextran derivatives obtained, the situation is more complex. Up to the present time, only the hydrogenolysis of the benzyloxycarbonyl group [1], taking place in quantitative yield, and the thiolysis of the o-nitrophenylsulfenyl group [2] can be considered to be applicable. The application of the method of acidolysis, which is widespread in peptide chemistry, to dextran derivatives, as to other polysaccharides, is complicated by the low stability of the glycosidic bonds in an acid medium. Consequently, the removal of even such an acid-labile N-protection as the tert-butoxycarbonyl (BOC) group in a O-(BOC-aminoacyl)dextran takes place to an insignificant extent or is accompanied by side reactions involving the decomposition of the polymer [2].

N-Protective groups that can be removed in an alkaline medium cannot be used for dextran esters because of the high lability of the ester bond. Furthermore, the N-substituted amino acids that are widely used in peptide chemistry are, as a rule, nonpolar. When nonpolar residues are introduced into the dextran macromolecule, the solubility of the modified polysaccharide in water and sometimes its solubility in dimethyl sulfoxide (DMSO) falls [2, 3], which also has an adverse effect on the possibility of eliminating the N-protective groups.

Consequently, it was necessary to find a protection for the amino group of the amino acid esterifying the dextran which could be eliminated easily and completely under mild conditions with the retention both of the glycosidic bonds of the dextran itself and of the ester bonds between the amino-acid residues and the

TABLE 1

Substance	Meth. of preparation	N	γ_{tot}	$\frac{\gamma_{tot}}{\gamma_{sud}}$	γNH_3^+	$\bar{n}$
		%				
Trifluoroacetate of Gly-dextran (Va)	A	0,230	2,70	5,40	0,66	4,10
	B	0,701	11,1	22,2	1,87	5,95
Trifluoroacetate of Aen*-dextran (Vb)	A	0,185	2,19	4,38	0,75	2,64
	B	0,132	1,49	2,98	0,58	2,57
Trifluoroacetate of L-Leu-dextran (Vc)	A	0,070	0,82	1,64	0,16	5,00
	B	0,139	1,64	3,28	0,74	2,21
Trifluoroacetate of L-Phe-dextran (Vd)	A	0,054	0,63	1,26	0,16	3,86
	B	0,290	3,53	7,06	2,13	1,65
Trifluoroacetate of L-His-dextran (Ve)	A	0,039	0,45	0,90	—	—
	B	0,161	1,91	3,82	—	—
Hydrochloride of L-His-dextran (Vf)	A	0,050	0,59	1,18	—	—
	B	0,267	3,17	6,34	—	—
Trifluoroacetate of L-Ala-L-His-dextran (Vg)	A	0,070	0,08	0,16	—	—
	B	0,778	0,82	1,64	—	—

*Aen – ω-aminoenanthyl.

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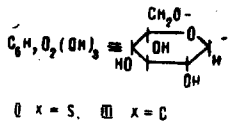
polysaccharide. Recently, A. A. Kraevskii, N. B. Tarusova, and B. P. Gottikh [4] have proposed to introduce aminoacyl residues into the carbohydrate moiety of nucleosides and nucleotides with the aid of imidazolides of trifluoroacetates of amino acids $[CF_3COO]^-[H_3NCHRCOJ_m]^+$, where J_m = N-imidazolyl. We decided to use a similar method to obtain esters of dextran with amino acids.

The dextran was aminoacylated by the following route:

$$[CF_3COO]^-[H_3NRCOOH]^+ \xrightarrow{J_{m_2}XO(II,\ III)} \underset{I}{\longrightarrow} [CF_3COO]^-[H_3NRCOJ_m]^+ \xrightarrow[IV]{[C_6H_7O_2(OH)_3]_m} \underset{V}{\longrightarrow} \{C_6H_7O_2(OH)_{3-n}[OCORNH_3]_n^+ [CF_3COO]_n^-\}_m$$

I, IV, V a. $R = CH_2$
b. $R = (CH_2)_6$
c. $R = CHCH_2CH(CH_3)_2$
d. $R = CHCH_2C_6H_5$
e. $R = CHCH_2C_3H_3N_2$
f. $R = CHCH_2C_3H_3N_2$ (Cl^- in place of CF_3COO^-)
g. $R = CH(CH_3)CONHCH(CH_2C_3H_3N_2)$.



The trifluoroacetate of an amino acid (Ia-e) or of a peptide (Ig) was treated in dimethylformamide solution of sulfinyldiimidazole (II) [5] in tetrahydrofuran. The imidazolide of the trifluoroacetate (IVa-e, g) so formed was caused to react with a solution of dextran in water (method A) or in DMSO (method B). The reaction with histidine hydrochloride (Ie) was carried out in the same way as with the trifluoroacetate (Id). The reaction products were freed from low-molecular-weight impurities by gel filtration on Sephadex G-50. The macromolecular structure of the dextran in the aminoacyl derivatives obtained was confirmed by the results of comparative gel chromatography on Sephadex G-75, as we have described previously [1]. Table 1 gives the characteristics of the aminoacyl derivatives of dextran (V) obtained via the imidazolides of the protonated amino acids (IV).

The number of amino-acid residues introduced per 100 anhydroglucose units of dextran (γ_{tot}) was determined from the nitrogen contents of the derivative (V) found by the Kjeldahl method. The efficiency of the aminoacylation reaction was judged from the ratio $\gamma_{tot}/\gamma_{sud}$, where γ_{sud} is the degree of substitution of the dextran by amino-acid residues (per 100 anhydroglucose units) that could be achieved if the acylation reaction took place quantitatively (for all the compounds (V) given in Table 1, $\gamma_{sud} = 50$). It can be seen from Table 1 that in an anhydrous medium acylation usually takes place more effectively than in an aqueous organic medium. Furthermore the efficiency depends strongly on the structure of the acylating amino acid or peptide, although the reaction takes place both for di- and trifunctional α-amino acids (glycine, L-phenylalanine–L-leucine, L-histidine) and for ω-amino acids (ω-aminoenanthic acid), and also for peptides (L-alanyl–L-histidine). Acylation is possible not only with trifluoroacetates but also with hydrochlorides (exemplified by L-histidine), with approximately the same effect. All the dextran derivatives shown in Table 1 are soluble in water.

We obtained interesting results by the mild alkaline hydrolysis of the aminoacyldextrans (V). When the hydrolysates were subjected to amino-acid analysis, the amount of amino acid or peptide liberated on hydrolysis was in all cases less than that determined by the nitrogen-content analysis of the corresponding aminoacyldextran. The chromatograms of the hydrolysates analyzed showed, in addition to the peaks corresponding to the amino acids, additional peaks which, in the case of (Va) were identified as glycine, diglycine, and polyglycines. Under the conditions of alkaline hydrolysis, only the ester bonds between the amino acid and the dextran are cleaved. Consequently, the results of the amino-acid analysis can be explained by the presence in (V) of not only single amino-acid residues but also a certain number of poly(amino acid) chains:

$$\{C_6H_7O_2/OH-_{3-n}[OCO(RNHCO)_pNH_3]_n^+ \cdot [CF_3COO]_n^-\}_m \quad (VI)$$
$$(p = 1, 2, 3\ldots)$$

TABLE 2

Solvent	γ_{sud}	N cont.	γ_{tot}	$\frac{\gamma_{tot}}{\gamma_{sud}}$	$\gamma_{NH_3^+}$	$\bar{n}$
	%					
DMSO – DMFA	50	1,32	19,81	38,1	—*	—
DMSO – DMFA	20	0,25	3,03	15,2	0,48	6,32
DMSO – DMFA	10	0,17	2,00	20,0	0,36	6,25
DMSO – DMFA†	5	0,12	1,41	28,2	0,15	9,40
Water – DMFA	50	0,87	11,9	23,8	—*	—
1 M imidazole buffer – DMFA	50	0,38	4,70	9,4	0,73	6,44
1 M imidazole buffer – DMFA	20	0,20	2,41	12,1	0,42	5,73
1 M imidazole buffer – DMFA†	10	0,23	2,80	28,0	0,28	10,00

*Substance insoluble in water.

†Time of obtaining the imidazolide 10 min.

The presence of poly(amino acid) chains in (V) is confirmed by their IR spectra (–CONH groups) and by the results of the titration of the trifluoroacetate groups in these substances by alkali in the presence of phenolphthalein. The number of protonated amino groups in compounds (V) determined by titration $\gamma_{NH_3^+}$ per 100 anhydroglucose units of the dextran) was less than the total number of amino-acid residues γ_{tot} [titration was not performed on compounds (Ve-g) containing histidine].

From the values of γ_{tot} and $\gamma_{NH_3^+}$ found, we calculated the mean degree of polymerization of the amino-acid residues in the chains:

$$\bar{n} = \frac{\gamma_{tot}}{\gamma_{NH_3^+}}.$$

To determine the influence of the reaction conditions and the ratio of the reagents (γ_{sud}) on the structures of the aminoacyldextrans (V) formed, we carried out a series of experiments on the acylation of dextran with the imidazolide of the trifluoroacetate of L-phenylalanine (IVd). In contrast to the preceding experiments, the imidazolide (IVd) was obtained with the aid of carbonyldiimidazole (III) [6]. Acylation was effected by adding the imidazolide in dimethylformamide (DMFA) to a solution of dextran in DMSO, water, or 1 M imidazole buffer; the subsequent procedure was as described in [1].

Table 2 gives the conditions of the aminoacylation reaction and the characteristics of the trifluoroacetates of the L-phenylalanyldextrans (Vd). The replacement of sulfinyldiimidazole (II) by carbonyldiimidazole (III) increases the efficiency of the reaction ($\gamma_{tot}/\gamma_{sud}$) and, as in the experiments shown in Table 1, the efficiency is higher in an anhydrous medium. In all the experiments, a considerable part of the phenylalanine entering the polysaccharide was present in poly(amino acid) chains. The solvent, the presence of imidazole, and the ratio of the reactants affect the mean length of the polyphenylalanine chains, but it is impossible to avoid the formation of such chains.

It has been established that the partial polymerization of the imidazolides (IV) takes place even during their formation. Thus, the hydrolysis of the imidazolide (IVa) in water for 24 h gave a hydrolysate containing not only glycine but also polyglycines.

However, the proposed method is convenient for the introduction of amino-acid residues into dextran in that case where it is necessary to introduce some total amount of amino acids, and also to obtain dextran derivatives with free amino groups.

EXPERIMENTAL

The general methods of working have been described previously [1]. The alkaline hydrolysis of the aminoacyldextrans was performed in 1 N NaOH (1 h at 20°C), i.e., under the conditions used in peptide synthesis for the hydrolysis of the ester group. The amino-acid analysis was performed on a Hitachi KLA-3B instrument by a known method [7].

Synthesis of Aminoacyl Derivatives of Dextran (V) with the Aid of Sulfinyldiimidazole (II)

Sulfinyldiimidazole (II). At 0°C, 1.35 ml (18.5 mmoles) of thionyl chloride in 5 ml of absolute tetrahydrofuran was added to a solution of 5.54 g (81.4 mmoles) of imidazole in 25 ml of absolute tetrahydrofuran.

The mixture was heated to 20°C, and after 30 min the imidazole hydrochloride was filtered off without the access of moisture and was washed with 10 ml of tetrahydrofuran. The filtrates were combined and were used in the following stage as a solution of sulfinyldiimidazole.

Imidazolides of Protonated Amino Acids and Peptides (IV). The solution of sulfinyldiimidazole (II) in tetrahydrofuran was added to a suspension of 18.5 mmoles of the trifluoroacetate (or hydrochloride) of the amino acid (or peptide) (I) in 25 ml of absolute DMFA, the mixture was stirred for 30 min, and the resulting clear solution of imidazolide was divided into two equal parts, which were used as described below.

Synthesis of the Aminoacyldextrans (V) in an Aqueous Organic Medium (Method A). The solution of the imidazolide of the protonated amino acid or peptide (IV) (half of the solution obtained above) was added dropwise to a solution of 3.0 g (18.5 mmoles) of dextran in 25 ml of water, the mixture was stirred for 1 h, and the polymer was precipitated with ethanol. To free it from low-molecular-weight impurities, the polymer was subjected to gel filtration on Sephadex G-50 and worked up as described previously [1].

Synthesis of the Aminoacyldextrans (V) in an Anhydrous Medium (Method B). The solution of the imidazolide of the protonated amino acid or peptide (IV) (the second half of the solution obtained above) was added to a solution of 3 g (18.5 mmoles) of dextran in 25 ml of absolute DMSO, and the mixture was stirred for 12 h and precipitated with ethanol. The remainder of the process was analogous to the preceding case. The properties of the aminoacyldextrans obtained are given in Table 1.

Synthesis of Derivatives of L-Phenylalanine with Dextran (Vd) with the Aid of Carbonyldiimidazole (III) (see Table 2)

Imidazolide of the Trifluoroacetate of L-Phenylalanine (IVd). A 10% molar excess of carbonyldiimidazole was added to a solution of the trifluoroacetate of L-phenylalanine (Id) in absolute DMFA, and after the evolution of gas had ceased (not more than 5 min) the product was used in the reaction with dextran.

Trifluoroacetates of L-Phenylalanyldextrans (Vd). The solution of the imidazolide of the trifluoroacetate of L-phenylalanine (IVd) obtained above was added to a solution of 2 g (12.35 mmoles) of dextran in 20 ml of DMSO, or water, or 1 M imidazole buffer (pH 7.08). The mixture was stirred for 1-1.5 h (18 h when the reaction was performed in DMSO), and the polymer was precipitated with ethanol and worked up as described above. The ratio of the reagents and the properties of the compounds (Vd) are given in Table 2.

SUMMARY

1. A method is proposed for obtaining O-aminoacyldextrans by the acylation of dextran with imidazolides of N-protonated amino acids and peptides in aqueous organic and anhydrous media.

2. On the basis of the results of a determination of total nitrogen by the Kjehldahl method, the titration of the protonated terminal amino groups, amino-acid analysis, and the IR spectra of the products of aminoacylation, it has been established that not only single amino acids but also poly(amino acid) chains add to dextran.

3. Derivatives of dextran with glycine, L-phenylalanine, L-leucine, L-histidine, ω-aminoenanthic acid, and L-alanyl-L-histidine have been obtained.

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