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On the use of watersoluble carbodiimides for binding carboxylic compounds to aminated dextrans

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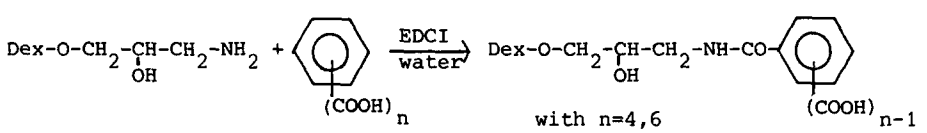
Summary

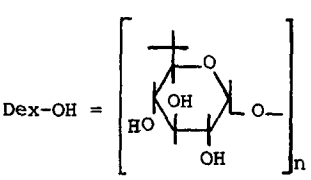
To prepare dextran-linked benzene polycarboxylic acids which are stable in-vivo, the acids were allowed to react with aminated dextrans in water, in the presence of a water-soluble carbodiimide. Under these conditions, ester linkages are formed preferentially to amide ones, probably because of the formation of intramolecular cyclic anhydrides promoted by the carbodiimide. Thus, with benzoic acid, esterification also took place, but to a much lesser extent.

Introduction

Because of their versatile chemical properties, carbodiimides rank as one of the most important classes of compounds in organic chemistry. They are particularly important as condensing agents, not only in the field of peptide and nucleotide synthesis (2), but also in that of the immobilization of drugs, proteins and enzymes on polymeric materials (3,4). In the latter case, carbodiimides are used for promoting amide linkages between polymers bearing amine or carboxyl groups, and drugs or proteins containing carboxyl or amine functions. As the reagents are often hydrophilic, watersoluble carbodiimides such as, for example, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) (5,6) are mainly used.

In order to prepare functionalized dextrans capable of reacting specifically in the allosteric site of hemoglobin to create stable polymeric conjugates for blood substitution (7,8), we planned to bind benzene polycarboxylates to aminated dextrans by means of EDCI, according to the following scheme :





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In the present communication, we report on the results of this type of condensation reaction, obtained under various conditions. *(For abbreviations see ref. (1).)*

Experimental part

Dextran T10 ($\bar{M}_w \simeq 9900$, $\bar{M}_n \simeq 5400$) was obtained from Pharmacia (Sweden). BHC, BTC, BTCA, benzoic acid and EDCI were purchased from Aldrich (Belgium).

^{13}C NMR spectra were run in D_2O on a Brüker AM 200 MHz spectrometer (concentration : 200 mg/ml), with DSS as internal reference. The low pressure gel permeation chromatographic experiments were performed on AcA 202 Ultrogel (from IBF, France). Cellulosic dialysis tubes (molecular weight cut-off 6000-8000) were obtained from Poly Labo (France). The benzene carboxylate content of dextran derivatives was measured by U.V. spectroscopy.

The condensation reaction between benzene polycarboxylate and aminated dextran synthesized according to a procedure described elsewhere (9) was carried out as follows : 1 g of aminated dextran (0.6 mmol amine/g) and 6 mmol of a benzene polycarboxylate (BHC or BTC) were dissolved in several ml of 1N NaOH ; the pH of the solution was adjusted to 9.2 or 6.5 with 0.1N NaOH and the final volume was increased to 20 ml with water. Then 0.6 or 6 mmol of EDCI were added and the mixture stirred at room temperature for 15h. To eliminate the free benzene polycarboxylate, the solution was eluted on AcA 202 Ultrogel (0.05N NaCl, pH 9). The eluate was then dialyzed against water and freeze-dried.

The reactions of BHC, BTC or benzoic acid with dextran in presence of EDCI, and of BTCA with dextran, were carried out in water under the same conditions as described above, with regard to pH, temperature and benzene carboxylate/dextran ratio. The products were purified in the same way.

Results and Discussion

The characteristics of the various compounds obtained by reaction of benzene polycarboxylate with the aminated dextran (0.6 mmol NH_2/g) are shown in Table 1 :

Conditions of reaction[a]		Content in benzene polycarboxylate (mmol/g polymer)[c]
pH	r[b]	
9.2	0.1	0.36 (A1)
9.2	1	0.67 (A2)
6.5	0.1	0.35 (A3)
9.2	0.1	0.40 (B1)
9.2	1	0.98 (B2)

Table 1 : [a] reactions performed with 6 mmol of benzene polycarboxylate per g of dextran ; [b] EDCI/benzene polycarboxylate molar ratio used for the condensation ; [c] A refers to the reaction of BHC,B to that of BTC.

The ^{13}C NMR spectra of compounds A2, A3 and B2 are reported in Fig. 1. By comparison with the ^{13}C NMR spectrum of free BHC and BTC we easily assigned the peaks centered around 178 ppm to the carboxylate functions, and those situated around 140 ppm to the -C= (A) or -CH= (B) groups of the aromatic ring. The peaks at 174 ppm and 133 ppm, observed for all the compounds (the spectra of A1 and B1 were quite similar to that of A3), were assigned to the expected groups, respectively -CO-NH- and =C-CO-NH- (or =CH-CO-NH-).

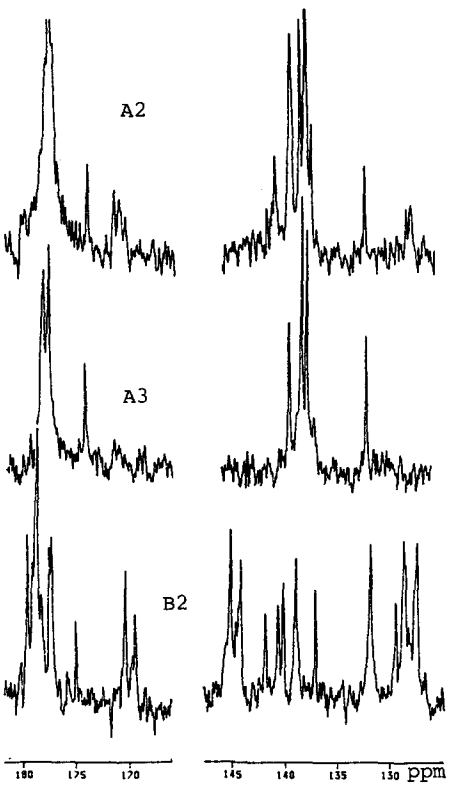


Fig. 1. ^{13}C NMR spectra of compounds A2, A3, B2 in D_2O.

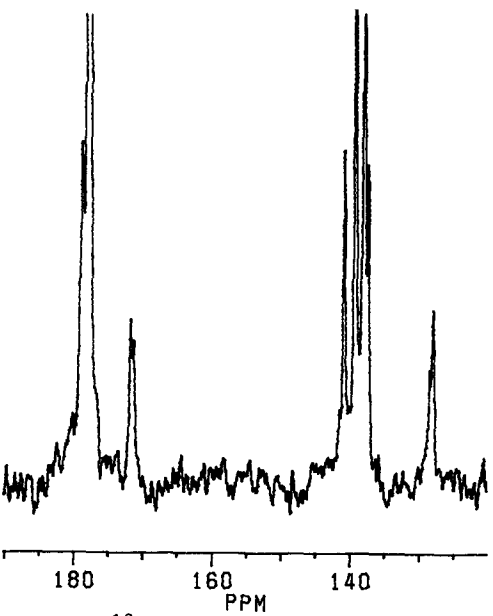
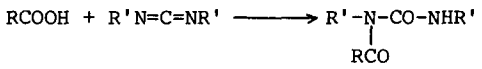


Fig. 2. ^{13}C NMR spectrum of the ester of BHC with dextran in D_2O.

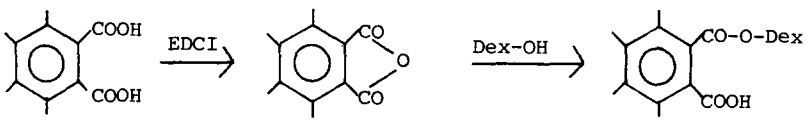
To assign the peaks centered around 171 ppm and 128 ppm, which were only observed for compounds A2 and B2, we assumed that EDCI could favor the reaction of the carboxylate compounds on the dextran hydroxyl groups ; to check this assumption, we investigated the reaction of BHC with dextran in presence of EDCI under the same conditions as those used with the aminated dextran (6 mmol BHC/g dextran, pH 9.2). The dextran derivatives obtained with r = 0.1 and 1 (r = molar ratio EDCI/BHC) contained respectively 0.15 and 0.63 mmol of linked BHC/g of polymer. The ^{13}C NMR spectrum of the second one is shown in Fig. 2, and it can be seen that it does in fact exhibit peaks around 171 ppm and 128 ppm, which proves that EDCI is capable of promoting ester linkages between polycarboxylic acids and hydroxylic functions. For compounds A2 and B2 obtained with r = 1, the ester function content, evaluated from the NMR spectra, is about 2 to 2.5 times higher than that of amide groups.

The formation of esters by means of carbodiimides is not an easy reaction to carry out, since very often N-acyl-N,N'-disubstituted urea is obtained as the predominant product (10) :

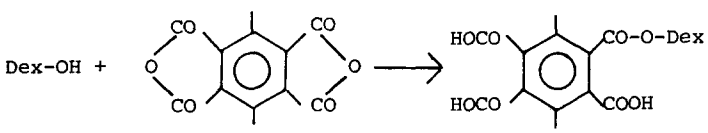


To obtain high yields of ester, it is generally recommended to carry out the reaction in organic solvents and in the presence of N,N-dialkyl-aminopyridine and dicyclohexylcarbodiimide (11). The esterification of carboxylic acids by the carbodiimide method can also be achieved through the isourea procedure (12) but only in organic media and in the presence of catalysts.

In the present case it should therefore be assumed that the reaction of EDCI with the benzene polycarboxylates results in the formation of cyclic anhydrides which are capable of acylating the hydroxylic groups of dextran in water, according to the following mechanism :



When the reaction is carried out with an aminated dextran in presence of an excess of EDCI (r = 1), as the amine content is about 20 to 30 times smaller than the hydroxyl one, and although the amine group is more nucleophilic than the hydroxyl one, it is normal that the process yields a greater number of ester functions than amide linkages. On the other hand, we have shown that BTCA easily reacts with dextran in water (pH 6.5 to 9.2) to give the corresponding ester, as follows :



BTCA

which can confirm the mechanism proposed for esterification by the carbodiimide method.

To evaluate the general character of this process, we investigated the reaction of benzoic acid with dextran in water, in the presence of EDCI and under the same conditions as those used for the benzene polycarboxylates (6 mmol of benzoic acid/g of dextran ; r = 1 mol EDCI/mol benzoic acid ; pH 9.2). The derivative thus obtained only contained about 3.10^{-5} mol of benzoic ester/g of polymer ; this proves that if the esterification reaction is greatly favored with aromatic polycarboxylic acids, this probably results from the better stability of intramolecular cyclic anhydrides, compared with that of the linear anhydride of benzoic acid.

References

1. Abbreviations : EDCI = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide ; Dex OH = dextran ; BHC = benzene hexacarboxylic acid ; BTC = 1,2,4,5-benzene tetracarboxylic acid ; BTCA = 1,2,4,5-benzene tetracarboxylic anhydride ; DSS = 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt.
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