Polymer Bulletin 31, 145–149 (1993)

Polymer Bulletin

A new approach to aldehydic dextrans

Edith Dellacherie and François Bonneaux*,**

Laboratoire de Chimie-Physique Macromoléculaire, URA CNRS 494 ENSIC, BP 451,
F-54001 Nancy Cedex, France

SUMMARY

4-carboxybenzaldehyde as well as 3- and 4-hydroxybenzaldehyde can form adducts with dextran or its derivatives. Few easy reaction steps are necessary and even if moderate yields of aldehyde are obtained, the aldehydic dextrans thus prepared are suitable for use as drug carrying systems.

INTRODUCTION

Dextran is frequently used to immobilize active molecules (2) (3) but owing to its chemical structure mainly constituted of non-reactive hydroxylic functions, the use of a coupling agent (4) (5) and/or the introduction of more reactive functions are required (6). Among these modifications, the production of aldehydes through periodic oxidation is mostly used (7) (8). This method leads to glucosidic ring opening and affords high aldehydic contents with regard to other methods. Apart from this procedure, another method was described by CALLANT (9) and it consists in linking to dextran an aliphatic chain which is then selectively oxidised in an aldehydic derivative. On the other hand, the fixation of aromatic aldehydes to dextran has never been described and yet such polysaccharide-linked aldehydes could easily form imine linkages with amino groups.

This paper deals with the reaction of substituted benzaldehydes (4-carboxybenzaldehyde, 3- and 4-hydroxybenzaldehyde) with dextran containing or not, a hydroxypropylic spacer arm and gives the various characteristics of the aldehydic dextrans thus prepared..

EXPERIMENTAL PART

Abbreviations are listed in ref. (1).

Materials and Methods

Dextran T_{10} ($\overline{M}_w \approx 10{,}400$, $\overline{M}_n \approx 5700$) was obtained from Pharmacia (Sweden). 4-carboxybenzaldehyde (4-CBA), 3-hydroxybenzaldehyde (3-HBA), 4-hydroxybenzaldehyde (4-HBA) and 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDCI) were purchased from Aldrich (Belgium). All other reagents were of analytical grade. The low-pressure gel permeation chromatographic experiments were performed on AcA 202 Ultrogel (from Sepracor-IBF, France). Cellulosic dialysis tubes (molecular weight cut-off 6000-8000) were obtained from Poly-Labo (France).

---

*Corresponding author

**Present address: Faculté de Pharmacie, 7 rue Albert Lebrun, F-54000 Nancy, France

The aldehydic content of dextran derivatives was determined by iodine titration and/or by U.V. spectroscopy (257.8 nm for 4-carboxybenzaldehyde). Chlorinated dextran (**Dx-CHP**, see Fig.1, compound I) and aminated dextran (**Dx-NHP**, Fig.1, compound II) were prepared according to procedures described elsewhere (10).

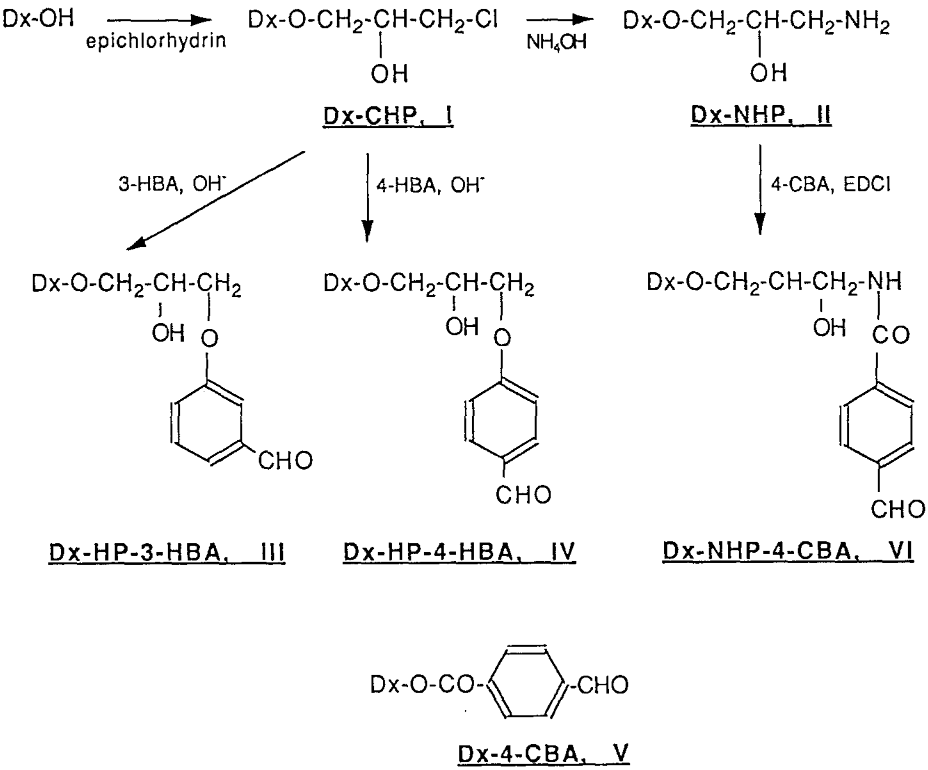

Figure 1 : Synthesis pathways and structures of aldehydic dextran derivatives

Synthesis of the aldehydic dextran derivatives

Derivatives with aromatic aldehydes were obtained according to the schemes represented in Figure 1, and according the following procedures :

Dx-HP-3-HBA and Dx-HP-4-HBA (Fig. 1, compounds III and IV).

HBA was dissolved in 1M NaOH ; Dx-CHP bearing 11 mmol Cl/mol glucose unit was added so that the HBA/Cl molar ratio was 11. pH was adjusted at a given value with 1M NaOH and the volume was adjusted with water in such a manner that the final concentration was 0.2 M to 0.7 M in glucose units (i.e. about 34 to 120 g of dextran/l).

The mixture was stirred at room temperature for 24 h in order to remove the unreacted aldehyde and the solution was eluted on AcA 202 Ultrogel (0.05 M NaCl, pH 9). The eluate was then dialysed against distilled water and freeze-dried.

Dx-4-CBA (Fig. 1, compound V)

4-CBA was dissolved in 1M NaOH ; then dextran and EDCI were added so that the CBA/glu and EDCI/glu molar ratios were 0.8. pH was adjusted at a given value with 1M NaOH or 1M HCl and the volume was adjusted in such a manner that the final concentration was 0.3 M in glucose units (i.e. about 49 g of dextran/l). The mixture was stirred at room temperature for 16 h and purification was performed as above.

Dx-NHP-4-CBA (Fig.1, compound VI)

4-CBA was dissolved in 1M NaOH ; then Dx-NHP bearing 85 mmol amine/mol glucose unit and EDCI were added so that the CBA/amine and EDCI/amine molar ratios were 9.7. pH was adjusted at 6.5 with 1M HCl and the volume was adjusted in such a manner that the final concentration was 0.3 M in glucose units. Then reaction and purification were carried out as above.

RESULTS AND DISCUSSION

The characteristics of the various compounds resulting of the reaction of both 3 and 4 isomers of hydroxybenzaldehyde with chlorinated dextran (Dx-CHP) are gathered in Table 1.

Conditions of reaction[a]			content in linked aldehydes (mol/100 mol glu)[b]
Type of isomer	pH	[glu]	
3	10	0.2	1.5
3	11	0.2	2
3	11.5	0.2	3
3	12	0.2	3
3	13	0.2	0
4	11.5	0.2	<1
3	11.5	0.5	4
3	11.5	0.7	5

Table 1 : Reaction of the hydroxybenzaldehydes with chlorinated dextran (Dx-CHP)

[a] 3 = 3-HBA ; 4 = 4-HBA ; [glu] : molar concentration in glucose units ; Dx-CHP contains 0.11 mol Cl/mol glu ; CHO/glu molar ratio = 1.2. Other conditions as described in the experimental part.

[b] : iodine titration.

In fact the reaction proceeds via two steps, which can explain the various obtained results. As shown in Figure 2, the first step is the transformation of Dx-CHP into an epoxy form. Then this epoxide is opened by the action of a nucleophilic species. Between pH 10 and pH 11.5, the yield of the expected reaction, i.e. of the fixation of HBA onto dextran (Fig. 2, compound a), increases with pH. Then above pH 11.5, it dramatically decreases probably because of competitive reactions due to the high pH which corresponds to high OH^- concentrations and which leads to a partial ionisation of dextran hydroxylic groups. Thus the epoxy form may be hydrolysed into a glycolic dextran (Fig. 2, compound b) and, in an alkaline enough medium, the hydroxylic groups of dextran can provoke intermolecular or intramolecular cross-linking (Fig. 2,compound c).

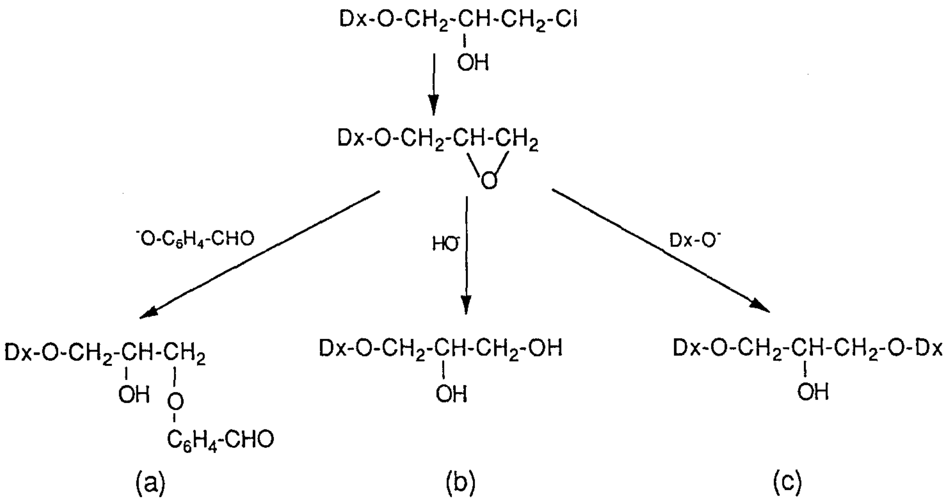

Figure 2 : Possible by-products formed during the reaction of chlorinated dextran (Dx-CHP) with hydroxybenzaldehyde (HBA)

On the other hand, at pH 11.5, the meta isomer affords a larger substitution of the polysaccharide than the para isomer, which can be assigned to a stronger nucleophilicity of the phenolate in this case.

Moreover, in the case of 3-HBA, higher yields are obtained with increasing reagent concentrations. Anyway, less than half of chlorinated spacer arms can be substituted with the HBA.

The properties of the compounds obtained by reactions of 4-carboxybenzaldehyde with dextran or aminated dextran in the presence of EDCI are shown in Table 2.

Type of polymer	reaction pH[b]	content in linked in aldehydes (mol/100 mol glu)[c]
dextran	6.5	≈ 0
dextran	9	2.3
aminated dextran[a]	6.5	7.5

Table 2 : Reaction of 4-carboxybenzaldehyde with dextran and aminated dextran in the presence of EDCI

[a] 0.09 mol N/mol glu

[b] EDCI/glu molar ratio used for the condensation : 0.8
CBA/glu molar ratio used for the condensation : 0.8

[c] iodine and spectroscopic titrations

When substitution of dextran is carried out under mild acidic conditions (pH 6.5), 4-carboxybenzaldehyde does not react with it, while the use of an alkaline medium leads to a limited esterification. Nevertheless, the yield obtained in this case is significantly higher, about five fold than when benzoïc acid is used instead of carboxybenzaldehyde (5 mmol linked benzoïc acid/mol glu). The better reactivity of 4-carboxybenzaldehyde can be assigned to the presence of the electroattractive aldehyde on the aromatic ring.

On the other hand, when aminated dextran is used, the substitution by 4-carboxybenzaldehyde is far higher. In fact, almost all the amine functions of the polysaccharide are substituted (about 90 %) under our conditions. The comparison of results obtained at pH 6.5 with unmodified dextran and aminated dextran, shows that the fixation of 4-carboxybenzaldehyde onto the second one, proceeds only through the formation of amide bonds.

In conclusion, aromatic aldehydes can be introduced onto dextran by means of 3-HBA and 4-CBA. Various procedures were investigated involving a direct reaction of the aromatic aldehydes with dextran or a reaction with spacer arm-substituted dextran. The better results were obtained by reaction of 4-CBA with aminated dextran in the presence of a water-soluble carbodiimide. In any case, few preparation steps are required and the purified products are obtained with good yields (> 60 %).

The content in linked aldehydes appears to be limited (not greater than 7 aldehydes /100 glucose units) but should be sufficient for some uses, especially those concerning the modification of large macromolecules such as for example proteins, enzymes, antibodies... for which too much linkages with the polymer have to be avoided.

ACKNOWLEDGEMENTS

The authors thank Mrs T. GEOFFROY for her helpful technical assistance.

REFERENCES

(1) Abbreviations :
Dx-OH : dextran
Dx-CHP : chlorohydroxypropyl dextran
Dx-NHP : aminohydroxypropyl dextran
4-CBA : 4-carboxybenzaldehyde
3-HBA : 3-hydroxybenzaldehyde
4-HBA : 4-hydroxybenzaldehyde
EDCI : 1-ethyl-3-(3 -dimethyl - aminopropyl) carbodiimide hydrochloride
glu : glucose unit

(2) E.P. GOLDBERG, in "Polymeric Drugs", ed. by L.G. DONARUMA, O. VOGL, Academic Press New York, 239 (1978)

(3) H. SEZAKI, M. HASHIDA, in "CRC Crit. Rev. Ther. Drug Carrier Systems, 1, 1 (1984)

(4) N. IZUMIYA, M. MURAOKA, J. Am. Chem. Soc., 91, 2391 (1969)

(5) J.C. SHEEHAN, J.J. HLAVKA, J. Org. Chem., 21, 439 (1956)

(6) R. AXEN, J. PORATH, S. ERNBACK, Nature, 214, 1302 (1967)

(7) A. JEANES, C.A. WILHAM, J. Am. Chem. Soc., 72, 2655 (1950)

(8) E. SCHACHT, L. RUYS, J. VERMEERSCH, J. Controlled Release, 1, 33, (1984)

(9) D. CALLANT, F. VANDOORNE, E. SCHACHT, Reactive Polymers, 8, 129 (1988)

(10) F. BONNEAUX, Ph D, University of Nancy I, France (1992).

Accepted May 21, 1993 C