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Preparation of dextran-bioactive compound adducts by the direct esterification of dextran with bioactive carboxylic acids

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The direct esterification reaction of dextran with α-naphthylacetic acid (a model bioactive carboxylic acid) is studied in homogeneous phase using pyridine-sulfonyl chloride as catalyst. The structure of the resulting adducts was determined by means of infra-red, ^{1}H nuclear magnetic resonance (n.m.r.) and ^{13}C n.m.r. spectroscopy. The influence of the pyridine concentration, the type of the sulfonyl chloride as well as the temperature was evaluated. Direct esterification reaction was also performed with other bioactive carboxylic acids, such as naproxen and nicotinic acid. ^{13}C n.m.r. spectra at 75.4 MHz of partially modified dextran with α-naphthylacetate groups were studied in order to evaluate the selectivity of the reaction between dextran and α-naphthylacetic acid. Analysis of the spectra of ring carbons in the anhydroglucose units shows that the reactivity of the individual hydroxyl groups decreases in the order C2 > C4 > C3. On the basis of these results a probable mechanism for the reaction is suggested. © 1997 Elsevier Science Ltd.

(Keywords: dextran; bioactive carboxylic acids; degree of substitution)

INTRODUCTION

The preparation of polymer-bioactive agent adducts, in which non-macromolecular biologically active substances are linked to the polymeric matrices by means of chemical bonds of limited stability to biological environments, is receiving the increasing attention of several groups[1,2], as an approach to solve the problems which accompany the use of biologically active agents, whether pharmaceutical or agrochemical. In this connection, dextran possesses several properties which would make it a feasible carrier candidate, and so has been selected for the preparation of polymer-bioactive agent adducts[3,4]. Unfortunately this polymer has little reactivity at low temperatures with the bioactive compound. To solve this problem, two main routes may be used: a) the creation of new, highly reactive functional groups into the carrier molecule or the bioactive agent, enabling the coupling through the covalent bonds to occur; b) the stimulation of the direct reaction by using specific activators.

With respect to the coupling of bioactive carboxylic acids to dextran, the direct esterification reaction in the presence of some specific catalyst, such as dicyclohexylcarbodiimide, proved to be partially successful[5]. On the other hand, the transformation of the bioactive carboxylic acid into a more reactive derivative is sometimes difficult, because of the formation of byproducts[6,7].

The successful direct acylation of cellulose with several carboxylic acids by using pyridine-*p*-toluensulfonyl chloride as catalyst has been reported recently[8]. Also, the tosyl chloride-*N*-methylimidazole has been successfully used as an appropriate catalytic system for the preparation of aromatic polyesters by the direct polycondensation of aromatic dicarboxylic acids and bisphenols[9].

In this article, it is shown that the direct esterification reaction of dextran with some model bioactive carboxylic acids (α-naphthylacetic, naproxen and nicotic acids) in the presence of pyridine-sulfonyl chloride as a catalyst, is an efficient method for the synthesis of dextran-bioactive carboxylic acid adducts. A ^{13}C nuclear magnetic resonance (n.m.r.) study for the quantitative assay of the distribution of α-naphthylacetate groups of some partially modified dextran polymers was also made, and the results were correlated with the probable mechanism of the esterification reaction.

EXPERIMENTAL

Materials

The dextran was a commercial product (T-70 from Pharmacia Fine Chemicals) with a linear structure (as revealed by ^{13}C n.m.r.) and a weight-average molecular weight (from light scattering) of $M_w = 70\,000$. The polymer was dried *in vacuo* for a few days at 80°C in the presence of phosphorus pentoxide to constant weight. *N*,*N*-Dimethylformamide (DMF) (from Panreac) and pyridine (from Panreac) were purified following one of the conventional methods[10,11]. LiCl (from Panreac) was dried *in vacuo* in the presence of phosphorus pentoxide. α-Naphthylacetic acid (from Fluka), 6-methoxy-α-methyl-2-naphthaleneacetic (naproxen) acid (from Sigma), nicotinic acid (from Ferosa), *p*-toluenesulfonyl chloride (tosyl chloride) (from Aldrich)

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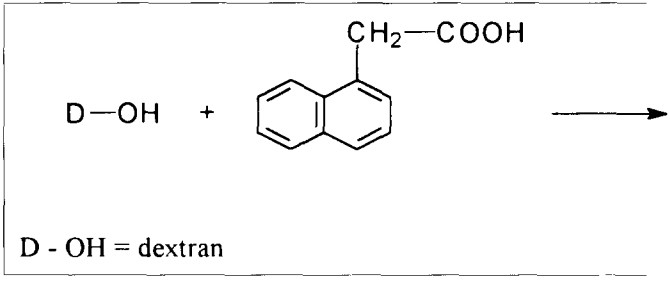
and methanesulfonyl chloride (mesyl chloride) (from Aldrich) were used without further purification.

Reaction of dextran with bioactive carboxylic acids

The dextran (20 g l^{-1}) was dissolved in DMF containing 2 g of LiCl per 100 ml at 90°C using a Pyrex double-walled reactor through which thermostatted water at the reaction temperature was circulated. The solution obtained was thermostatted at the reaction temperature and the calculated amounts of pyridine, bioactive carboxylic acid and sulfonyl chloride (see *Tables 1* and *2*) were added while stirring. The polymer remained soluble throughout the process. After 22 h, the modified polymer was isolated by precipitation. Different precipitants were used to isolate the polymer, depending on the degree of substitution (DS). All samples were purified by reprecipitation using tetrahydrofuran (THF) as solvent and propan-2-ol or propan-2-ol/diethyl ether mixtures as precipitants, and then dried *in vacuo* in the presence of phosphorus pentoxide.



Characterization of bioactive carboxylic acid-dextran adducts

The infra-red (i.r.) spectra were obtained on a Perkin-Elmer 457 spectrometer on KBr discs. The ^{1}H n.m.r. spectra were registered in DMSO-d_6 at 70°C using a 200 MHz Bruker AM-200 spectrometer. ^{13}C n.m.r. spectra were obtained in DMSO-d_6 at 70°C using a Varian XL-300 spectrometer operating at 75.4 MHz in the proton noise-decoupled mode. Chemical shifts were measured with respect to that of the central peak of the methyl carbon of DMSO, which was taken to be at 39.7 ppm downfield from tetramethylsilane. The spectral measurement conditions were similar to those of the structural analysis of cellulose derivatives[12]. The spectra were accumulated for about 16000 scans with a repetition time of 3 s. The signal areas of the spectra were determined by spectral integration as well as by tracing over the peaks with a planimeter. The DS was determined by means of alkaline hydrolysis at 85°C using an aqueous solution of sodium hydroxyde (0.5 M). The resulting homogeneous solution contained the released bioactive agent, which was quantitatively determined by ultraviolet (u.v.) spectroscopy at the absorption wavelength of α-naphthylacetic, naproxen or nicotinic acid, 281 nm ($\epsilon = 6.32 \times 10^3$ l mol^{-1} cm^{-1}), 271 nm ($\epsilon = 5.12 \times 10^3$ l mol^{-1} cm^{-1}) and 268 nm ($\epsilon = 2.35 \times 10^3$ l mol^{-1} cm^{-1}), respectively, using calibration curves (aqueous solution of sodium hydroxide as solvent) previously determined. The DS was also determined by ^{13}C n.m.r. The results by both methods proved to be in agreement.

RESULTS AND DISCUSSION

The covalent attachment of α-naphthylacetic acid to dextran through ester bonds was carried out by using pyridine-sulfonyl chloride as catalyst and DMF LiCl system as solvent according to the following scheme:

The structure of the resulting polymers was confirmed by i.r., and ^{1}H and ^{13}C n.m.r. spectroscopies. The i.r. spectra of partially modified dextran with α-naphthylacetate groups exhibit the characteristic carbonyl band at 1720 cm^{-1}. At 1590 and 1510 cm^{-1} two bands are observed which are assigned to the naphthalene ring[13]. The ^{1}H n.m.r. spectra of the same polymers show several signals between 3.6 and 5.4 ppm due to the protons linked to the sugar carbon atoms. In this interval a band at 4.1 ppm which corresponds to the methylene protons in the α-naphthylacetate groups can be also observed. The signals between 7.2 and 7.8 ppm are due to protons in the naphthalene ring[13]. The ^{13}C n.m.r. spectra exhibit a signal at 37.5 ppm due to the methylene carbons of the α-naphthylacetate groups. The peaks between 66.6 and 98.1 ppm are due to sugar carbons. The peaks that appear in the interval 123.4–133.0 ppm can be assigned to the carbons of the naphthalene ring[13]. The signal at 170.3 ppm corresponds to the carbonyl carbons from the grafted α-naphthylacetate groups.

Table 1 shows the effect of the pyridine concentration,

Table 1 Reaction of dextran ([OH] = 0.37 mol l^{-1}) with α-naphthylacetic acid using pyridine–sulfonyl chloride as catalyst and DMF containing 2 g of LiCl per 100 ml as solvent[a]

[Pyridine] (mol l^{-1})	Sulfonyl chloride	Temperature (°C)	Degree of modification (mol%)
–	Tosyl	50	40.0
0.37	Tosyl	50	45.1
0.74	Tosyl	50	56.2
1.48	Tosyl	50	68.2
0.74	Tosyl	30	37.7
0.74	Tosyl	60	65.2
0.74	Tosyl	70	70.6
0.74	Mesyl	50	55.4

[a] [α-Naphthylacetic] = [sulfonyl chloride] = 0.37 mol l^{-1}; time = 22 h

Table 2 Reaction of dextran ([OH] = 0.37 mol l^{-1}) with bioactive carboxylic acids using pyridine (0.74 mol l^{-1})–tosyl chloride (0.37 mol l^{-1}) as catalyst and DMF containing 2 g of LiCl per 100 ml as solvent[a]

Bioactive carboxylic acid	Degree of modification (mol%)
α-Naphthylacetic acid	56.2
Naproxen	66.0
Nicotinic acid	52.1

[a] [Bioactive carboxylic acid] = 0.37 mol l^{-1}; temperature = 50°C; time = 22 h

the type of the sulfonyl chloride and the temperature on the reaction of dextran with α-naphthylacetic acid using pyridine–sulfonyl chloride as catalyst. The results obtained show that, as could be expected, the degree of modification increases as pyridine concentration or temperature increases. The reaction of dextran with α-naphthylacetic acid takes place to a noticeable extent in the absence of pyridine. According to the literature[8], this result can be explained by admitting the catalytic action of the DMF, which promotes the esterification reaction. As shown in *Table 1*, a similar yield was obtained when mesyl chloride was used instead of tosyl chloride.

Table 2 shows that the direct esterification reaction was also effective with other bioactive carboxylic acids, such as naproxen or nicotinic acid, and the yields are nearly comparable to that obtained with α-naphthylacetic acid.

It may be noted that a kinetic study of the reaction of dextran ([OH] = 0.37 mol l^{-1}) with α-naphthylacetic acid (0.37 mol l^{-1}) in the presence of pyridine (0.74 mol l^{-1})–tosyl chloride (0.37 mol l^{-1}) as catalyst and a DMF/LiCl mixture (2% w/v) as solvent, was carried out at 50°C. The DS was shown to approach a limit (55.0 mol% of α-naphthylacetate groups) after approximately 2.5 h. It is quite probable that this result may be explained to some extent by the steric effects of α-naphthylacetate groups previously incorporated into the polymeric chain.

On the other hand, several studies have shown that the three secondary hydroxyl groups at C2, C3 and C4 positions of the anhydroglucose (AHG) units of dextran (*Figure 1*) show significant differences in reactivity[14,15]. A special feature of polysaccharide derivatization is that, in many cases, only the partial substitution of its three alcoholic groups is desired or achieved. The DS and also the partial DS in the different positions of the AHG units are of great importance for determining physical, chemical and biochemical properties. In this work, the relative reactivity of hydroxyl groups in the AHG units of dextran in the reaction with α-naphthylacetic acid was evaluated by ^{13}C n.m.r., which has been suggested as an adequate method to determine the relative reactivity of each hydroxyl group in polysaccharides[12,16].

The ^{13}C n.m.r. spectra of the ring carbon region of dextran and several samples of partially modified dextran with α-naphthylacetate groups (DS ranging from 0.58 to 2.12) are shown in *Figure 2*. The ^{13}C n.m.r. spectra of modified polymers show a decrease in the intensity of the signals assigned to C1 (98.4 ppm), C2 (71.8 ppm), C3 (73.3 ppm), C4 (70.4 ppm) and C5 (70.4 ppm) carbon atoms in the original dextran, together with the appearance of several new peaks at 95.3, 76.3, 73.9, 72.6, 71.3 and 68.1–67.7 ppm. The presence of these peaks is consistent with the fact that esterification reaction of a hydroxyl group of glucopyranosic compounds involves an upfield shift of the resonance of the adjacent carbons and that the resonance of the carbon directly linked to a modified hydroxyl group is shifted downfield with respect to the chemical shift of the carbon bearing an unsubstituted hydroxyl group[17]. The assignments of these new signals were made as described previously for other esters of dextran[15]. The peak at 95.3 ppm may be ascribed to C1 carbons adjacent to C2 carbons bearing a substituted hydroxyl group. The signal at 76.3 ppm may be attributed to C3 carbons bearing a substituted hydroxyl group in monosubstituted AHG units. The peak 73.9 ppm corresponds to C2 carbons bearing a substituted hydroxyl group in monosubstituted AHG units. The C3 carbons bearing a substituted hydroxyl group in 2,3-disubstituted AHG units are assumed to give a peak at 73.3 ppm, which appears at the same position as that of the C3 carbons of the original dextran. The signal at 72.6 ppm is due to C2 carbons bearing a substituted hydroxyl group in 2,4-disubstituted AHG units. The peak at 71.3 ppm may be assigned to C4 carbons bearing a substituted hydroxyl group in monosubstituted AHG units, as well as to C4 carbons in 2,4-disubstituted AHG units. The peaks at 68.1–67.7 ppm are considered to belong to C5 carbons adjacent to C4 carbons bearing a substituted hydroxyl group as well as to C4 carbons adjacent to C3 carbons bearing a substituted hydroxyl group. These assignments allow one to estimate the relative reactivities of the three hydroxyl groups. In this sense, according to several authors[12,18,19], it may be considered that under the experimental conditions used in the present work, the spin-lattice relaxation times and the nuclear Overhauser effect factors of all six carbons of the AHG units must be very similar. Therefore, peak area measurements can be used for evaluation of the relative DS values for individual hydroxyl groups attached to C2, C3 and C4 carbons.

In the present study, the mole fractions (A) of monosubstituted AHG units at C2 (A_2), C3 (A_3) and C4 (A_4), of disubstituted AHG units at C2, C3 ($A_{2,3}$), C2,

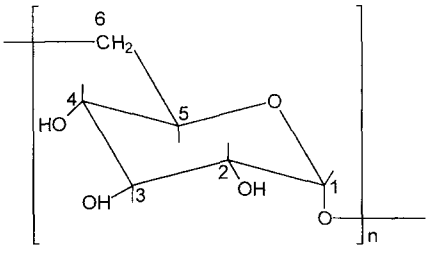


Figure 1 Basic structural unit of dextran

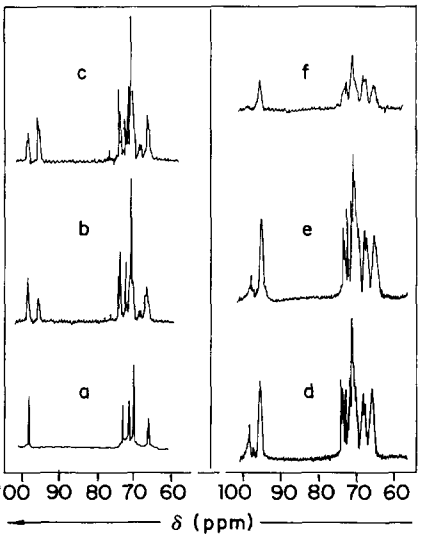


Figure 2 ^{13}C n.m.r. spectra of the ring carbon region of some partially modified dextrans with α-naphthylacetate groups measured in DMSO-d_6: (a) DS = 0.00; (b) DS = 0.58; (c) DS = 0.98; (d) DS = 1.50; (e) DS = 1.73; (f) DS = 2.12

Table 3 Variation of mole fraction of mono-, di- and trisubstituted anhydroglucose units with the total DS in modified dextrans with α-naphthylacetate groups

Mole fraction	DS[a]				
A_i	0.58	0.98	1.50	1.73	2.12
A_2	0.35	0.41	0.22	0.21	0.04
A_3	0.08	0.05	0.02	0.00	0.00
A_4	0.04	0.01	0.04	0.02	0.00
$A_{2,3}$	0.00	0.03	0.09	0.15	0.28
$A_{2,4}$	0.04	0.14	0.25	0.32	0.41
$A_{3,4}$	0.00	0.00	0.00	0.00	0.00
$A_{2,3,4}$	0.00	0.06	0.19	0.20	0.22

[a] Determined by chemical analysis

Table 4 Quantitative structural analysis of substitution at individual hydroxyl groups in the esterification of dextran with α-naphthylacetic acid

DS determined by chemical analysis	DS at position			
	C2	C3	C4	Total DS
0.58	0.39	0.08	0.08	0.55
0.98	0.64	0.14	0.21	0.99
1.50	0.75	0.30	0.48	1.53
1.73	0.88	0.35	0.54	1.77
2.12	0.95	0.50	0.63	2.08

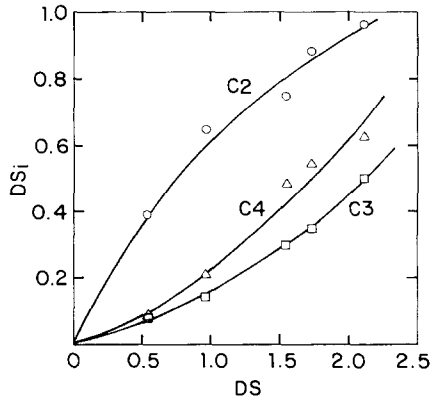


Figure 3 Variation of the degree of substitution at individual hydroxyl groups (DS_i) with the total DS in modified dextran with α-naphthylacetate groups

C4 ($A_{2,4}$) and C3, C4 ($A_{3,4}$), and of trisubstituted AHG units ($A_{2,3,4}$) were calculated (*Table 3*) from the expanded forms of the spectral ranges at 100–67 ppm, using in each case an adequate combination of the relative intensities of the signals at 98.1, 95.3, 76.3, 73.9, 73.3, 72.6, 71.3 and 68.1–67.7 ppm.

The relative DS values (DS_i) of individual hydroxyl groups attached to C2, C3 and C4 carbons have been estimated from the following equations:

$$DS_2 = A_2 + A_{2,3} + A_{2,4} + A_{2,3,4}$$

$$DS_3 = A_3 + A_{2,3} + A_{3,4} + A_{2,3,4}$$

$$DS_4 = A_4 + A_{2,4} + A_{3,4} + A_{2,3,4}$$

The average DS of each polymer sample was calculated from:

$$DS = DS_2 + DS_3 + DS_4$$

The values obtained from the above equations are summarized in *Table 4*. It may be noted that in all cases the magnitude of the total DS determined by chemical analysis and those obtained from the sum of the partial DS values of individual hydroxyl groups attached to C2, C3 and C4 carbons are in good agreement.

Figure 3 shows the variation of the relative DS_i of individual hydroxyl groups in the reaction of dextran with α-naphthylacetic acid, as a function of the total DS value. The analysis of data shown in *Figure 3* clearly indicates that the relative reactivities of the three hydroxyl groups of AHG units decrease in the order: C2 > C4 > C3. The substitution pattern shown in *Figure 3* is very similar to the one that we have previously established for the reaction of dextran with acetyl chloride using pyridine as catalyst[15]. Bearing in mind the similitude of these results, it seems reasonable to accept that both reactions follow the same mechanism, which implies the participation of acylpyridinium ions as reactive intermediates. This mechanism was proposed previously by Shimizu *et al.*[8] in the direct esterification of cellulose with acetic acid using pyridine–tosyl chloride as catalyst, as well as by Higashi *et al.*[9] in the synthesis of aromatic polyesters and polyamides in the presence of *N*-methylimidazole–tosyl chloride as catalyst. Consequently, the mechanism of the reaction of dextran with α-naphthylacetic acid presumably involves the following steps:

$$R{-}COOH + TsCl \xrightarrow{\text{pyridine}} [C_6H_5N{-}CO{-}R]^{\oplus}Cl^{\ominus} + C_6H_5N{\cdot}TsOH$$

$$D{-}OH + [C_6H_5N{-}CO{-}R]^{\oplus}Cl^{\ominus} \longrightarrow D{-}O{-}CO{-}R + C_6H_5N{\cdot}HCl$$

R–COOH = α-naphthylacetic acid

TsCl = Tosyl chloride

It is interesting to note that no presence of tosyldextran groups in the synthesized dextran-α-naphthylacetic adducts was detected from the ^{13}C n.m.r. spectra as well as by elemental and u.v. analysis. These results indicate that there was no possibility of the esterification proceeding via tosyldextran, which could be formed by the reaction between dextran and tosyl chloride.

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