Notes

Reaction of Dextran with Glycidyl Methacrylate: An Unexpected Transesterification

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Received December 3, 1996
Revised Manuscript Received March 18, 1997

Introduction

Recently, we reported on the synthesis, characterization and polymerization of glycidyl methacrylate derivatized dextran (dex-GMA).[2] This polysaccharide can be polymerized to form hydrogels, which are under investigation for the delivery of pharmaceutically active peptides and proteins.[3,4] In a recent study,[5] we investigated the dextranase-catalyzed degradation of dextran and dex-GMA with mass spectrometry. The mass spectra of the obtained degradation products could be interpreted by assuming that the methacryloyl group was directly attached to a hydroxyl group of dextran, the glyceryl spacer being absent between the methacryloyl group and dextran. Also, when attempts were made to establish the position of the glyceryl group at the glucose ring by the routinely used alditol−acetate method for analysis of polysaccharides,[6] no glycerol-substituted glucose units could be detected. Therefore, we reinvestigated the reaction of GMA with dextran as well as the structure of the reaction product.

Experimental Section

1. Materials. Dextran (from *Leuconostoc mesenteroides*, T40, M_n = 15 000, M_w = 32 500, as determined by GPC analysis), dimethyl sulfoxide (DMSO, <0.01% water), glycidyl methacrylate (GMA, 95% by GC, 2,3-epoxypropyl methylpropenoate), and glycidol (GDOL, 90% by acidimetric titration, (±)-2,3-epoxypropanol) were obtained from Fluka Chemie AG, Buchs, Switzerland. 4-(*N*,*N*-Dimethylamino)pyridine (DMAP, 99%) was from Acros Chimica, Geel, Belgium. Dex-MA (DS 37) was synthesized as described previously.[2]

2. Methods. NMR. NMR spectra were recorded with a Gemini 300 MHz spectrometer (Varian Associates Inc. NMR Instruments, Palo Alto, CA). For ^{1}H-NMR in ^2H_2O (99.8% ^{2}H, Merck) ^{2}HOH at 4.8 ppm was used as the reference line, whereas in dimethyl sulfoxide (DMSO-d_6, 99.8% ^{2}H, Merck) the central DMSO resonance was set at 2.50 ppm. Approximately 30 mg of material was dissolved in 0.8 mL of solvent. The pulse length was 4.5 μs ($PW_{90} \approx 12$ μs) and the relaxation delay 15 s. For quantitative ^{13}C-NMR, the decoupler was gated on during acquisition and off during delay, to suppress the nuclear Overhauser effect. The relaxation delay was set at 5 s and the pulse length at 4.5 μs ($PW_{90} \approx 12$ μs). The $(CH_3)_3Si$ resonance (0 ppm) of sodium 2,2-dimethylsilapentanesulfonate (DSS) was used as the reference line.[7]

GC−MS. GC−MS was carried out with a JEOL AX505W mass spectrometer and a HP 5890 gas chromatograph with a BPX 70 capillary column (25 m × 0.32 mm i.d., film thickness 0.25 μm). The carrier gas was helium. The column was kept at an initial temperature of 50 °C for 2 min, and the temperature was raised at 5 °C/min to 250 °C. The accelerating voltage was 3 kV and the mass range 15−500.

Reaction of GMA with Dextran in DMSO-d_6. Dextran (254 mg) was dissolved in DMSO-d_6 (2.25 mL) in a 10 mL stoppered round-bottomed flask in a nitrogen atmosphere. After dissolution of DMAP (50 mg), 107 μL GMA was added. Immediately after the addition of GMA, part of the reaction mixture (800 μL) was transferred to an NMR tube, analyzed with ^{1}H-NMR, and returned to the flask. After being stirred at room temperature for 4 days, the reaction mixture was analyzed again with ^{1}H-NMR. Next, methanol was added to part of the reaction mixture, and the precipitated dextran was removed by centrifugation. The supernatant was analyzed for the presence of glycidol with GC−MS.

^{1}H-NMR. GDOL (DMSO-d_6): δ 4.82 (t, $^3J_{OH-Hc}$ = 5.9 Hz, 1H, O*H*), 3.60 (ddd, $^3J_{Hc1-OH}$ = 5.7 Hz , $^3J_{Hc1-Hd}$ = 3.1 Hz, $^2J_{Hc1-Hc2}$ = 12.3 Hz, 1H, H_{c1}), 3.34 (water), 3.32 (ddd, $^3J_{Hc2-OH}$ = 5.6 Hz, $^3J_{Hc2-Hd}$ = 5.6 Hz, $^2J_{Hc2-Hc1}$ = 12.2 Hz, 1H, H_{c2}), 2.98 (m, 1H, H_d), 2.67 (dd, $^3J_{He1-Hd}$ = 4.2 Hz, $^2J_{He1-He2}$ = 5.3 Hz, 1H, H_{e1}), 2.52 (dd, $^3J_{He2-Hd}$ = 2.7 Hz, $^2J_{He2-He1}$ = 5.3 Hz, 1H, H_{e2}), and 2.50 (DMSO).

GMA (DMSO-d_6): δ 6.06 (m, 1H, H_{a1}), 5.72 (m, 1H, H_{a2}), 4.46 (dd, $^3J_{Hc1-Hd}$ = 2.5 Hz, $^2J_{Hc1-Hc2}$ = 12.5 Hz, 1H, H_{c1}), 3.92 (dd, $^3J_{Hc2-Hd}$ = 6.3 Hz, $^2J_{Hc2-Hc1}$ = 12.5 Hz, 1H, H_{c2}), 3.26 (m, 1H, H_d), 2.80 (dd, $^3J_{He1-Hd}$ = 4.2 Hz, $^2J_{He1-He2}$ = 5.0 Hz, 1H, H_{e1}), 2.66 (dd, $^3J_{He2-Hd}$ = 2.6 Hz, $^2J_{He2-He1}$ = 5.1 Hz, 1H, H_{e2}), and 1.89 (m, 3H, H_b).

Dextran T-40 (DMSO-d_6): δ 4.90 (d, $^3J_{OH-H}$ = 4.8 Hz, 1H, OH), 4.83 (d, $^3J_{OH-H}$ = 4.4 Hz, 1H, OH), 4.68 (bs, 1H, H_1), 4.48 (d, $^3J_{OH-H}$ = 5.8 Hz, 1H, OH), 3.8−3.1 (m, 6H, H_2, H_3, H_4, H_5 and H_6), 3.35 (water), and 2.50 (DMSO).

Dextran T-40 (^2H_2O): δ 5.27 (m, H_1 in α-1,3 linkage), 5.02 (d, 1H, H_1), 4.0 (m, 1H, $H_{6'}$), 3.9 (m, 1H, H_5), 3.75 (m, 1H, $H_{6''}$), 3.70 (m, 1H, H_3), 3.55 (m, 1H, H_2), 3.50 (m, 1H, H_4).

^{13}C-NMR. Dextran T-40 (^2H_2O): δ 100.4 (C_1), 76.1 (C_3), 74.1 (C_2), 72.9 (C_5), 72.2 (C_4), 68.2 (C_6).[7]

Dex-MA DS 37 (^2H_2O): δ 171.9 and 171.2 (C=O), 138.3 and 138.0 (CH_2=*C*−CH_3), 130.8 and 130.0 (H_2C=C−CH_3), 100.4 (C_1), 97.8 (C_1−S_2), 78.7 (C_3−S_3), 76.1 (C_3), 75.8 (C_2−S_2), 74.1 (C_2), 73.7 (C_3−S_2), 72.9 (C_5), 72.4 (C_2−S_3), 72.2 (C_4), 70.2 (C_4−S_3), 68.2 (C_6) and 20.2 (H_2C=C−*C*H_3).

GC−MS. The retention time of glycidol was 6.8 min. Mass spectrum: parent peak *m*/*z* 74 (0.3%), *m*/*z* 73 (1.0%, $[M - H]^+$), *m*/*z* 56 (2.7%, $[M - H_2O]^{\bullet+}$), base peak *m*/*z* 44 (100%, $[M - CH_2O]^{\bullet+}$), 43 (89.4%; $[M - CH_3O]^{\bullet+}$), 31 (58.8%; $[M - C_2H_3O]^{\bullet+}$) and 29 (42.4%; $[M - C_2H_5O]^{\bullet+}$).

Results and Discussion

1. Reinvestigation of the Reaction. In our previous study, we assumed that the reaction between GMA (Figure 1-**1**) and dextran (Figure 1-**2**) proceeds by a nucleophilic attack of a hydroxyl group of dextran at the methylene carbon of the epoxy group of GMA, which is a common reaction for an epoxide with an alcohol

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S0024-9297(96)01764-0 CCC: $14.00

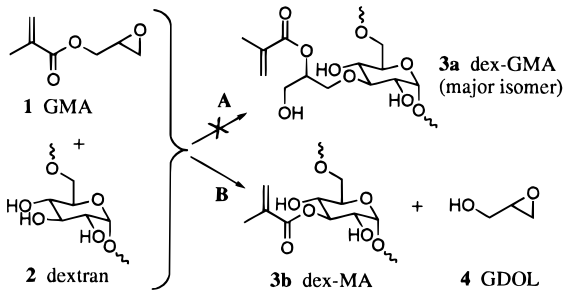

Figure 1. Reaction of dextran with GMA, based on former (A), and current insights (B).

(Figure 1, path A). On the basis of 2D-NMR analysis of dex-GMA and a model compound, glyceryl methacrylate, we concluded that part of the methacryloyl groups moved from position 3 to 2 of the glyceryl spacer, yielding 2-methacryloylglyceryldextran (Figure 1-**3a**). The latter product was thought to be responsible for the signal at 5.2 ppm, which was not present in the ^{1}H-NMR of either dextran or GMA. However, absence of the glyceryl spacer[5] invalidates the proposed reaction mechanism for the formation of dex-GMA, as well as the interpretation of the ^{1}H-NMR spectrum of dex-GMA.

A reaction which results in direct attachment of methacrylate esters to dextran is transesterification of the methacryloyl group from GMA to dextran, yielding dex-MA (Figure 1, path B). This reaction should result in the concomitant formation of glycidol (GDOL, Figure 1-**4**), which indeed was shown to be a reaction product. Monitoring the reaction in deuterated DMSO with ^{1}H-NMR showed that GDOL is formed during the reaction (appearance of signal at 2.98 ppm), with a concomitant decrease in the amount of GMA (decrease in signal at 2.80 ppm). Moreover, in the reaction mixture the presence of a compound with a retention time and mass spectrum identical with those of GDOL could be detected by GC−MS. This proves that methacrylation of dextran by GMA indeed occurs via a transesterification reaction, yielding dex-MA with the methacryloyl group directly attached to dextran (Figure 1-**3b**). To our knowledge, this type of reaction between GMA and alcohols has not yet been reported in the literature. An attempt to synthesize dex-MA by reaction of dextran with methacryloyl chloride instead of GMA was unsuccessful. This might be due to side reactions e.g. oxidation of the hydroxyl groups (similar to the Swern oxidation).[8]

Using hydroxyethyl methacrylate (HEMA) as the methacrylating agent did also not result in a detectable degree of substitution (DS < 0.5). Obviously, glycidol is a better leaving group than hydroxyethanol. This can be attributed to stabilization of the glycidol anion through delocalization of the negative charge over the two oxygen atoms, which does not occur in hydroxyethanol (Figure 2).

2. Position of Methacryloyl Group on the Glucopyranose Ring. Knowing that the methacryloyl group is directly attached to the glucose unit in dextran enables the determination of the position of this group at the glucopyranose ring.[9] It has been reported[10] that esterification of a hydroxyl group of glucopyranosyl compounds, including dextran, causes a downfield shift (+0.9 to +1.9 ppm) of the resonance of the carbon directly attached to the ester, whereas the resonance of adjacent carbons is shifted upfield (−1.8 to −3.3 ppm). The chemical shift of the other carbon atoms is hardly affected. A universal set of ^{13}C-shift parameters was

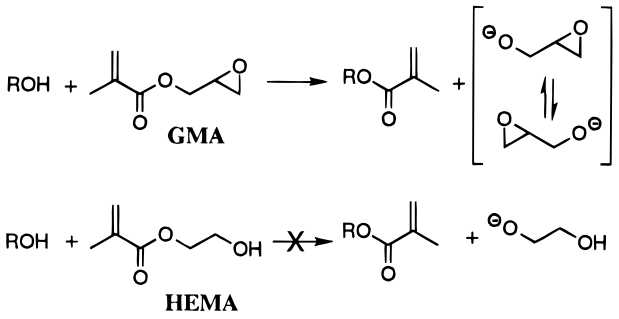

Figure 2. Comparison of glycidol and hydroxyethanol as leaving group.

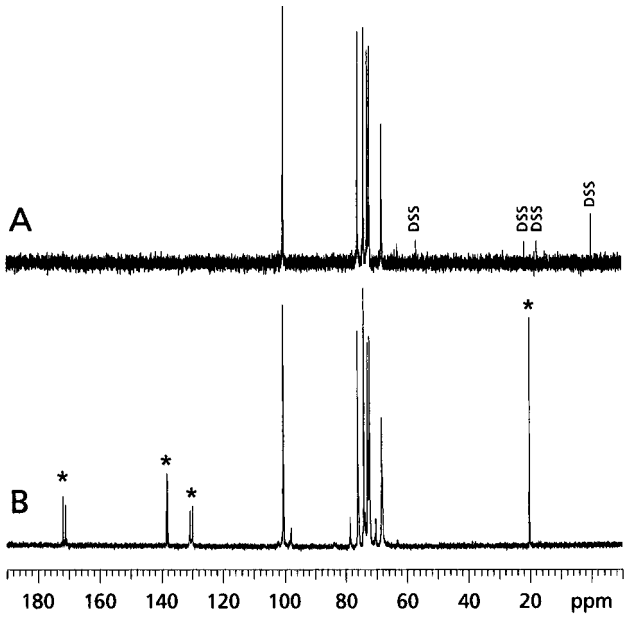

Figure 3. ^{13}C-NMR spectrum of dextran (A), and dex-MA DS 37 (B) in ^2H_2O, containing 2,2-dimethylsilapentanesulfonate (DSS). The signals from the methacryloyl group are designated by an asterisk.

deduced[10] and was used successfully for determining the overall distribution of esters on polysaccharides, such as acetylated cellulose[11] and acetylated dextran.[12] The inductive effect of methacryloyl groups on the ^{13}C-shifts is comparable with that of acetate groups,[13] which means that these shift parameters can also be used for the analysis of methacrylated dextran.

The ^{13}C-NMR of dextran (Figure 3A) shows the glucopyranose carbons between 68 and 101 ppm. The ^{13}C-NMR of dex-MA DS 37 (Figure 3B) gives, in addition, the signals for the methacryloyl group. The ratio of the integrals of a methacrylate carbon and a glucose carbon is in good agreement with the degree of substitution calculated from the ^{1}H-NMR spectrum. Except for the methyl signal at 20.2 ppm, all signals from the methacryloyl group are double: 171.9 and 171.1 ppm for the carbonyl carbon, 138.3 and 138.0 ppm for the carbon atom α with respect to the carbonyl group, and 130.7 and 130.0 ppm for the double bond carbon β to the carbonyl group. This indicates that there are two different positional isomers present in dex-MA. The integrals of the two ^{13}C-resonances are approximately equal. This means that both isomers are present to about the same extent.

Analysis of the ring carbon region of dex-MA (Figure 4B) shows, apart from the signals of the six dextran carbons (Figure 4A), additional signals at 97.8, 78.7, 75.8, 73.7, 72.4, and 70.2 ppm. These signals can be

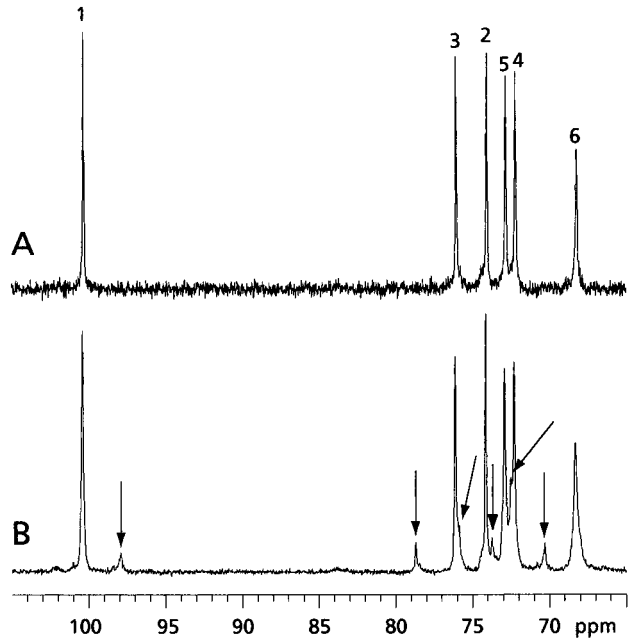

Figure 4. Ring carbon region of the ^{13}C-NMR spectra of dextran (A) and dex-MA DS 37 (B) in $^{2}H_2O$. The latter is taken from a nonquantitative ^{13}C-NMR with a better signal to noise ratio. The additional signals in dex-MA are designated by an arrow.

Table 1. ^{13}C-NMR Assignment of the Glucopyranose Ring Carbons in Dex-MA with DS 37 for 2- and 3-Substituted and 2- and 4-Substituted Dex-MA

obsd signal (ppm)	2- and 3-subst	shift value exptl	shift value theor[10]	2- and 4-subst	shift value exptl	shift value theor[10]
100.4[a]	C_1			C_1		
97.8	C_1-S_2[b]	−2.6	-3.3	C_1-S_2	−2.6	−3.3
78.7	C_3-S_3	+2.6	+1.9	C_2-S_2	+4.6	+1.1
76.1[a]	C_3			C_3		
75.8	C_2-S_2	+1.7	+1.1	C_4-S_4	+3.6	+0.9
74.1[a]	C_2			C_2		
73.7	C_3-S_2	−2.4	−3.1	C_3-S_4	−2.4	−2.6
72.9[a]	C_5			C_5		
72.4	C_2-S_3	−1.7	−1.8	C_3-S_2	−3.7	−3.1
72.2[a]	C_4			C_4		
70.2	C_4-S_3	−2.0	−2.4	C_5-S_4	−2.7	−2.1
68.2[a]	C_6			C_6		

[a] Assignment based on literature. [b] C_1-S_2 means the C-1 carbon in dextran adjacent to a substituted C-2. Underlining indicates a considerable deviation from the literature.

attributed to the carbon atoms in a methacrylated glucopyranose unit. The integral of these new signals corresponds well with that of a methacrylate signal of either one of the isomers. The signal at 97.8 ppm can only be attributed to a C-1 carbon adjacent to a substituted C-2 carbon. Therefore, one of the isomers must have the methacryloyl group at position 2. Table 1 gives an overview of ^{13}C-resonances of the ring carbon region of dex-MA and possible assignment of the signals based on either 2- and 3-substituted or 2- and 4-substituted isomers. This table shows that the position of the signals agrees fully with the presence of 2- and 3-substituted glucopyranose units, whereas for the 2- and 4-substituted isomer certain shift values deviate considerably from the literature data. We therefore conclude that in dex-MA part of the methacryloyl groups are attached to the hydroxyl group at position 2 and part to the hydroxyl at position 3, in approximately a 1:1 ratio. This implies that the order of reactivity for the three secondary alcohols in dex-MA (DS < 50) is OH-2 ≈ OH-3 > OH-4. The same order was reported

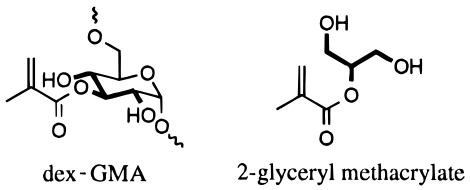

Figure 5. Structural similarity between dex-MA and the model compound 2-glyceryl methacrylate.

for partially acetylated dextrans, derivatized with acetic anhydride in lithium chloride-containing DMF.[12] On the basis of basicity, the order of reactivity is OH-2 > OH-3 > OH-4.[14] However, steric hindrance at position 2 might induce substitution of the hydroxyl group at position 3.[12]

In dex-MA DS 37 double substitution at one glucopyranose residue is not likely, but could not be excluded.

The signal at 78.7 ppm (C_3−S_3) in the ^{13}C-NMR spectrum of dex-MA was shown to correlate with the 5.2 ppm signal in the ^{1}H-NMR in water, formerly assigned to a proton of the glyceryl spacer in dex-GMA.[2] This means that the signal at 5.2 ppm can now be attributed to the H-3 in the 3-substituted glucose ring. The model compound glyceryl methacrylate is still useful in the assignment of the signals, since there is a strong structural similarity between 2-glyceryl methacrylate and dextran with directly coupled methacryloyl groups (Figure 5). Esterification causes a downfield shift of +1.5 ppm for the proton H-3 at the substituted C-3. H-2, normally at 3.55 ppm, can then, in the 2-substituted glucose ring, be expected to appear around 4.8 to 5.0 ppm, *i.e.* under the water signal.

In conclusion, the reaction of GMA with dextran proceeds via transesterification and results in the direct attachment of methacryloyl groups at the 2- and 3-hydroxyl group of the glucopyranose ring, in a 1:1 ratio.

References and Notes

(1) The degree of substitution (DS) is defined as the amount of methacryloyl groups per 100 dextran glucopyranose residues.
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MA961764V