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Polymer 40 (1999) 6877–6881

polymer

Polymer Communication

Synthesis, characterization of 2-(methacryloyloxy)ethyl-(di-) L-lactate and their application in dextran-based hydrogels

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Received 22 January 1999; received in revised form 17 May 1999; accepted 21 May 1999

Abstract

This paper describes the synthesis and characterization of 2-(methacryloyloxy) ethyl-L-lactate and 2-(methacryloyloxy)ethyl-di-L-lactate. These compounds were obtained by reaction of L-lactide and 2-hydroxyethyl methacrylate (HEMA) at a molar ratio of 1:2 using stannous octoate as catalyst. After a purification step using preparative HPLC, the obtained products were characterized by ^{1}H-NMR, HPLC and mass spectrometry. 2-(Methacryloyloxy)ethyl-(di-)lactate as well as HEMA were grafted to dextran using 1,1′-carbonyldiimidazole as coupling agent resulting in dex–(lactate)$_{1,2}$–HEMA and dex–HEMA, respectively. Hydrogels were obtained by radical polymerization of aqueous solutions of the methacrylated dextrans. Due to the presence of hydrolytically sensitive groups in the crosslinks, these hydrogels degraded when exposed to an aqueous solution (pH 7.2, 37°C). For hydrogels with a fixed crosslink density, the degradation times were 30, 12 and 6 days for dex–HEMA, dex–lactate–HEMA and dex–(lactate)$_2$–HEMA hydrogels, respectively. This demonstrates that the degradation rate and time of dextran hydrogels can be tailored by the length of the spacer in the crosslink of the hydrogels.

Keywords: 2-(Methacryloyloxy)ethyl-(di-)L-lactate; Dextran; Hydrogel

1. Introduction

In recent years, there is a great interest in soluble polymers which contain hydroxy acids (e.g. lactic acid [1–4], glycolic acid [5], and ϵ-caprolacton [6]) oligomers in their side chains as well as in polymeric networks which contain such oligomers in their crosslinks [7–12]. These polymeric networks are gaining much attention as delivery system for pharmaceutically active proteins and peptides. Two different synthetic strategies are exploited to obtain these systems. Firstly, poly(hydroxy acids) macromers are synthesized by a ring opening oligomerization of e.g. glycolide or lactide initiated by hydroxyethyl methacrylate in the presence of a suitable catalyst[3,13]. Secondly, hydroxy acid oligomers are functionalized by reaction with acryolyl chloride [9,14].

It has been shown that hydroxy compounds in the presence of stannous octoate form very active initiating systems for the ring opening polymerization of lactide [15]. The average molecular weight of the lactic acid polymers/oligomers can be effectively controlled by the monomer/initiator ratio [12,16,17]. In order to control the properties of the hydroxy acid macromers as well as the polymers and networks derived hereof, it might be advantageous to use macromers with a fixed amount of hydroxy acid groups. In this paper, we report on the synthesis of HEMA esterified with one lactic acid group (2-(methacryloyloxy)ethyl-lactate) and two lactic acid groups (2-(methacryloyloxy)ethyl-di-lactate), respectively (Fig. 1). The potential application of these well defined compounds is demonstrated in biodegradable, dextran based hydrogels.

2. Materials and methods

2.1. Materials

Hydroxyethyl methacrylate (HEMA, 2-hydroxyethyl methylpropenoate, 95% by GC), hydroquinone monomethyl ether (>98% by HPLC), dextran (from Leuconostoc mesenteroides, T40) and dimethyl sulfoxide (DMSO,<0.01% water) were obtained from Fluka Chemie, AG, Buchs, Switzerland. L-lactide ((3S-*cis*)-3,6-dimethyl-1,4-dioxane-2,5-dione, >99.5%) was purchased from Purac Biochem BV

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0032-3861/99/$ - see front matter
PII: S0032-3861(99)00354-7

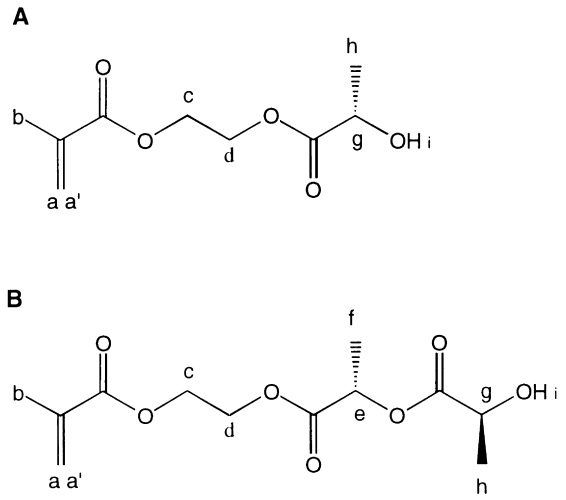

Fig. 1. Chemical structure of 2-(methacryloyloxy)ethyl-L-lactate (A) and 2-(methacryloyloxy)ethyl-di-L-lactate (B). H_a–H_b are described in Section 2.5.

(Gorinchem, The Netherlands) and used without pretreatment. Stannous octoate (stannous (II) bis(2-ethylhexanoate), $SnOct_2$, 95%) from Sigma Chemical Co., St. Louis, MO, USA was used as received. Toluene, 4-(*N*, *N*-dimethylamino) pyridine (DMAP, 99%) and 1,1′-carbonyldiimidazole (CDI, 98%) were purchased from Acros Chimica (Geel, Belgium). Tetrahydrofuran (THF) was distilled from $LiAlH_4$ immediately before use. Acetonitrile (HPLC-S, gradient grade) was obtained from BiosolveLTD (Valkenswaard, The Netherlands). Dialysis tubes (cellulose, molecular weight cut off (based on proteins) = 12 000–14 000 D) were purchased from Medicell International Ltd., London, UK.

2.2. NMR spectroscopy

NMR spectra were recorded with a Gemini 300 MHz spectrometer (Varian Associates Inc. NMR Instruments, Palo Alto, CA, USA). Approximately 30 mg of material was dissolved in 0.8 ml solvent. For measurements in deuteriochloroform (99.6 + % ^2H, Acros), the chloroform signal at 7.26 ppm was used as the reference line, whereas in DMSO-d_6 (99.8% ^2H, Merck) containing ^2H_2O, the central DMSO line was set at 2.50 ppm. A pulse length of 4.5 μs (PW90 = 12 μs) was used with a relaxation delay of 15 s.

2.3. Mass spectrometry

Electron spray (ESI) mass spectra were run with a VG-platform Benchtop LC-MS (Micromass, Altrichem, UK). An electron spray interface was used to ionize the molecules (positive ion mode). The nebulizing gas had a flow of 25 L/h; the flow of the drying gas was 300 L/h. The voltage applied to the capillary was 4.2 kV, and the cone voltage was 35 V.

2.4. High-performance liquid chromatography

2-(Methacryloyloxy)ethyl-oligolactate and 2-(methacryloyloxy)ethyl-lactate$_{1,2}$ were analyzed by reversed phase HPLC [16] (analytical column 100 RP-18, 5 μm, 125 mm × 4 mm i.d. including a RP-18 guard column (4 mm × 4 mm; Merck)) with a LC Module I plus system (Waters™). The samples (10 mg) were dissolved in acetonitrile (1 ml), diluted 10 times with water, and 25 μl of this solution was injected onto the column. A linear gradient was run from 100% A (water:acetonitrile 95:5 (w/w)) to 100% B (water:acetonitrile 5:95 (w/w)) in 30 min. The flow rate was 1.0 ml/min and the column oven was set at 30°C. UV detection at a wavelength of 220 nm was applied. Peak areas were determined with Millennium 2010 V. 2.15 software (Waters Associates Inc.). The calibration curves (peak area versus μmol) for the different compounds (HEMA, 2-(methacryloyloxy)ethyl-lactate and 2-(methacryloyloxy)-ethyl-di-lactate) were linear (up to 0.12 μmol) and not significantly different from each other. This can be explained by the fact that under the selected conditions the unsaturated methacrylate group is detected, assuming that the different compounds have the same molar extinction coefficient.

2.5. Synthesis and purification of 2-(methacryloyloxy)ethyl-oligolactate

2-(Methacryloyloxy)ethyl-oligolactate was synthesized using HEMA and L-lactide at a molar ratio of 2:1, essentially as described by Van Dijk-Wolthuis [13]. In brief, a mixture of HEMA (13.02 g, 100 mmol) and L-lactide (7.2 g, 50 mmol) was stirred at 110°C in a nitrogen atmosphere until the lactide was molten. Next, a catalytic amount of $SnOct_2$ (0.4 g, 1 mol% with respect to HEMA; diluted 1:1 with toluene) was added and the reaction mixture was stirred for 1 h. Samples were withdrawn from this reaction mixture at different time intervals, rapidly cooled with liquid nitrogen and analyzed with HPLC as described above. Some samples were also analyzed using ^1H-NMR. After 1 h, the reaction mixture was allowed to cool to room temperature and insoluble products, probably consisting of $SnOct_2$ complexes, were removed by centrifugation (16 000*g*, 5 min). Next, 1*g* of the clear, viscous mixture was dissolved in 1 ml acetonitrile and 500 μl of this solution was injected onto a preparative HPLC column (EconospherC8, 10 μm, 250 mm × 22 mm; Alltech, IL). An Äcta™ Purifier system (10XT, Pharmacia Biotech, Sweden) was used. Elution was done using a mobile phase consisting of 59% (w/w) water and 41% (w/w) acetonitrile with a flow rate of 5 ml/min (UV detection λ = 254 nm). The chromatograms were analyzed with Unicorn 2.30 software (Pharmacia Biotech, Sweden). Under these conditions HEMA, 2-(methacryloyloxy)ethyl-lactate and 2-(methacryloyloxy)ethyl-di-lactate had a retention time of approximately 16, 19 and 24 min, respectively. Corresponding fractions of different runs were collected, pooled and freeze dried.

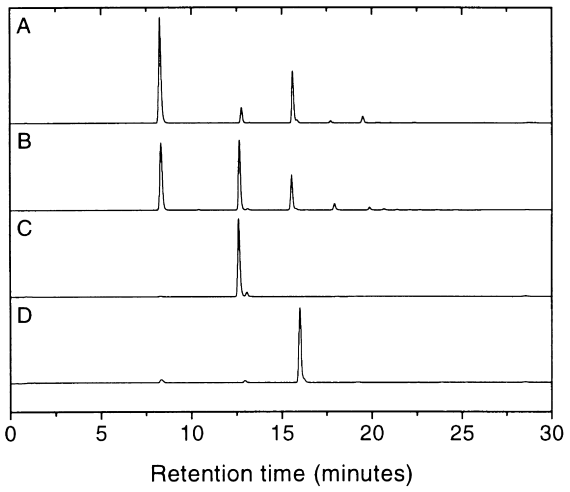

Fig. 2. HPLC chromatograms of the reaction mixture of HEMA/L-lactide/$SnOct_2$ after 1 min (A), 1 h (B) and of 2-(methacryloyloxy)ethyl-lactate (C) and 2-(methacryloyloxy)ethyl-di-lactate (D).

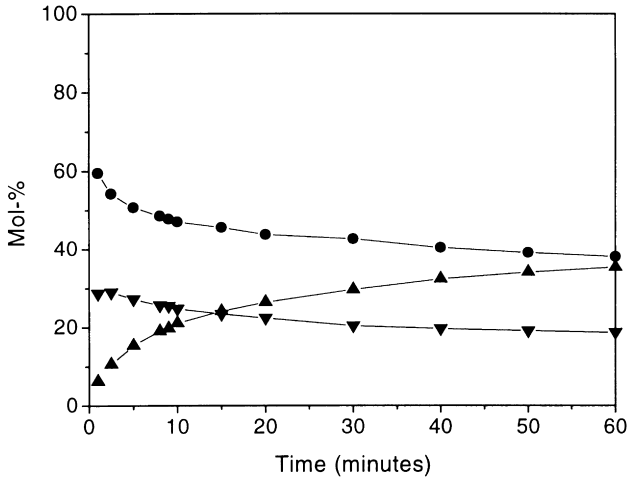

Fig. 3. Mol% of HEMA (●), 2-(methacryloyloxy)ethyl-lactate (▼) and 2-(methacryloyloxy)ethyl-di-lactate (▲) as a function of time (HEMA/L-lactide molar ratio of 2:1; $SnOct_2$ 1 mol% with respect to HEMA; reaction temperature 110°C).

The obtained products were characterized by ^{1}H-NMR, HPLC and mass spectrometry.

^{1}H-NMR 2-(Methacryloyloxy)ethyl-lactate (Fig. 4(a); $CDCl_3$): δ 6.11 (s, 1H, H_a), 5.59 (s, 1H, $H_{a'}$), 4.50–4.26 (m, 4H, H_c, H_d), 4.42 (q, $J_{gh} = 6.9$ Hz, 1H, H_g), 2.80 (bs, OH_i), 1.94 (s, 3H, H_b), 1.40 (d, $J_{gh} = 6.9$ Hz, 3H, H_h).

^{1}H-NMR 2-(Methacryloyloxy)ethyl-di-lactate (Fig. 4(b); $CDCl_3$): δ 6.11 (s, 1H, H_a), 5.59 (s, 1H, $H_{a'}$), 5.19 (q, 1H, H_e), 4.50–4.26 (m, 4H, H_c, H_d), 4.42 (q, $J_{gh} = 6.9$ Hz, 1H, H_g), 2.80 (bs, OH_i), 1.94 (s, 3H, H_b), 1.55 (d, 3H, H_f), 1.45 (d, $J_{gh} = 6.9$ Hz, 3H, H_h).

2.6. Preparation of dextran hydrogels

Dex–$lactate_{1,2}$–HEMA and dex–HEMA with a degree of substitution (DS, the number of methacryloyl groups per 100 glucose units) of approximately 6 were prepared as previously described. In brief, the hydroxyl group of monodisperse HEMA-lactate with a DP of 1 or 2 was activated with 1,1′-carbonyl-diimidazole (CDI, molar ratio 1:1) yielding HEMA–$(lactate)_{1,2}$–CI. Coupling of these activated compounds to dextran in DMSO in the presence of DMAP resulted in dex–$lactate_{1,2}$–HEMA. In the same way, dex–HEMA was synthesized by the coupling of CDI-activated HEMA to dextran [13].

Hydrogels were obtained by radical polymerization of aqueous solutions of dex–HEMA or dex–$lactate_{1,2}$–HEMA according to the following general procedure [8]. For a hydrogel with an initial water content of 80% (w/w), 200 mg of dex–lactate–HEMA was dissolved in 730 μl phosphate-buffer (PB; 100 mM, pH 7.2), in a 2 ml Eppendorf cup. To this solution, 50 μl of potassium peroxodisulfate (KPS; 20 mg/ml) in 100 mM phosphate-buffer (PB, pH 7.2) was added and mixed well. Subsequently, 20 μl of a 20% (v/v) *N*, *N*, *N′*, *N′*-tetramethylethylenediamine (TEMED; adjusted with 4 N HCl to pH 7) solution in PB was added. After mixing, the resulting solution was allowed to polymerize for 1 h at room temperature.

2.7. Degradation of hydrogels

After polymerization, the dex–$(lactate\text{-})_{1,2}$–HEMA hydrogels were removed from the cups, cut into a cylindrical shape (length 1.3 cm, radius 0.45 cm), and accurately weighed (W_0). The hydrogels were transferred into glass vials containing 15 ml of phosphate-buffered saline (100 mM PB, 0.9% NaCl, pH 7.2), and incubated in a water bath at 37°C. At regular time points, the weight of the hydrogels was determined and used to calculate the swelling ratio (defined as the ratio of the weight of the hydrogel at time t (W_t) and the initial weight of the hydrogel (W_0)).

3. Results and discussion

In a previous paper, we demonstrated that the average molecular weight of HEMA-lactate oligomers could be controlled by the HEMA/lactide ratio in the feed [13]. In order to favor the formation of HEMA with one or two lactate groups, we selected a HEMA/lactide ratio of 2:1 (mol/mol). The kinetics of this reaction catalyzed by $SnOct_2$ (1 mol% with respect to HEMA) was followed by HPLC (Fig. 2(A) and (B)). Fig. 2(A) shows that under the selected conditions, the reaction of HEMA and lactide proceeded rather rapidly: after 1 min the reaction mixture consisted of HEMA (retention time 8 min; 60 mol%), 2-(methacryloyloxy)ethyl-lactate (retention time 13 min; 30 mol%) and 2-(methacryloyloxy)ethyl-di-lactate (retention time 17 min; 5 mol%) with some traces of 2-(methacryloyloxy)ethyl-$lactate_{n>2}$ (retention times 18–22 min; ±5 mol%) also present; lactide could not be detected

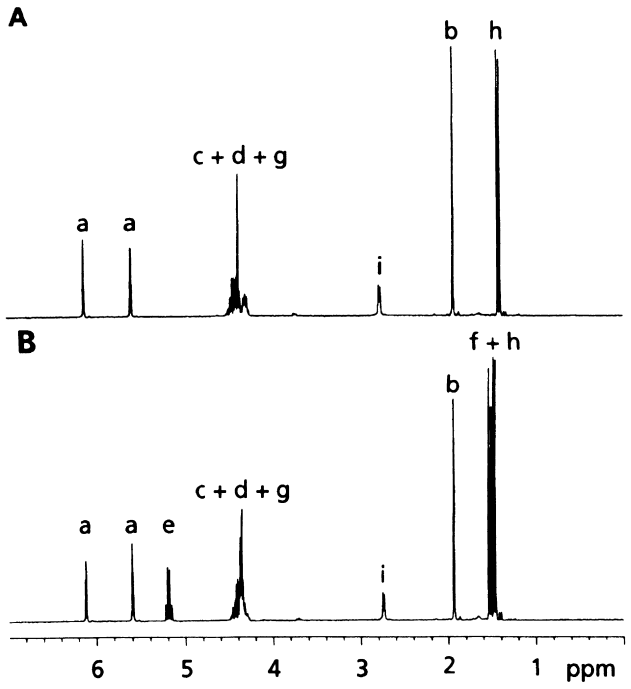

Fig. 4. ^{1}H-NMR in $CDCl_3$ of 2-(methacryloyloxy)ethyl-lactate (A) and 2-(methacryloyloxy)ethyl-di-lactate (B). H_a–H_b are described in Section 2.5.

(NMR, HPLC) in the reaction mixture. After 60 min, the mol% of HEMA, 2-(methacryloyloxy)ethyl-lactate and 2-(methacryloyloxy)-ethyl-di-lactate was 38, 35 and 19%, respectively (Fig. 2(B)).

Fig. 3 gives the concentration–time relations for HEMA and 2-(methacryloyloxy)ethyl-lactate$_{n=1,2}$. It appeared that both the amount of HEMA and 2-(methacryloyloxy)ethyl-di-lactate decreased in time, which was associated with a concomitant increase in the amount of 2-(methacryloyloxy)ethyl-lactate. This can most likely be explained by transesterification reactions which have been reported to occur during the ring opening polymerization of lactide catalyzed by $SnOct_2$ [16,18].

Using preparative HPLC, 2-(methacryloyloxy)ethyl-lactate and 2-(methacryloyloxy)ethyl-di-lactate were obtained with a yield of 43% (w/w) and 23% (w/w), respectively. Fig. 4(A) and (B) show the ^{1}H-NMR spectra of both compounds; the spectra could be fully assigned (see Section 2.5). Fig. 2(C) and (D) shows the HPLC chromatograms of the purified compounds. Both techniques showed that overall purity of the compounds was >95%; both compounds were only slightly contaminated with HEMA (peak at 8.3 min, <2 mol%), whereas 2-(methacryloyloxy)ethyl-lactate contains a minor fraction of 2-(methacryloyloxy)ethyl-di-lactate, and vice versa. ESI-MS analysis confirmed the molecular mass for both compounds: m/z 225 ($M–Na^+$), m/z 241 ($M–K^+$) for 2-(methacryloyloxy)ethyl-lactate; m/z 275 ($M–H^+$), m/z 297 ($M–Na^+$) for 2-(methacryloyloxy)ethyl-di-lactate.

Both 2-(methacryloyloxy)ethyl-lactate and 2-(methacryloyloxy)ethyl-di-lactate were coupled to dextran after activation of the hydroxyl group with 1,1′-carbonyldiimidazole using a previously established method [13]. The degree of substitution for both compounds was around 6; as a control dex–HEMA with DS 6 was also synthesized. The methacrylated dextrans can be converted into hydrogels by a radical polymerization of aqueous solutions of these dextran derivatives using a suitable initiator system. Although the methacrylate esters can be hydrolyzed under physiological conditions (pH 7.2; 37°C), we demonstrated that after polymerization these groups were resistant against hydrolysis, even under extreme (pH, temperature) conditions [19]. The dex–HEMA hydrogel contains carbonate esters in its crosslinks, whereas the dex–lactate–HEMA hydrogels contain apart from a carbonate ester, also lactate esters in the crosslinks. In a previous paper we have demonstrated that both esters are susceptible for hydrolysis under physiological conditions resulting in dissolution of the hydrogels in time; the lactate esters are more sensitive for hydrolysis than the carbonate esters [8]. Fig. 5 shows the swelling characteristics of dex–HEMA and dex–(lactate)$_{1,2}$–HEMA hydrogels with comparable initial crosslink densities (initial water content of the hydrogels was 80%; DS 6). All hydrogels showed a progressive swelling in time, which is caused by hydrolysis of the crosslinks, followed by a dissolution phase. Interestingly, the degradation time of the hydrogel derived of dex–lactate$_2$-HEMA is shorter than for dex–lactate–HEMA as well as dex–HEMA hydrogels (6, 12 and 30 days, respectively). This can be ascribed to the fact that the more hydrolytically sensitive groups are present in one crosslink, the greater the probability that one crosslink hydrolyzes [20] and the shorter the degradation time of the hydrogel. This demonstrates that the degradation time of dextran based hydrogels can be tailored by both the crosslink density [8] (DS and initial water content) and the number of hydrolyzable groups in the crosslinks.

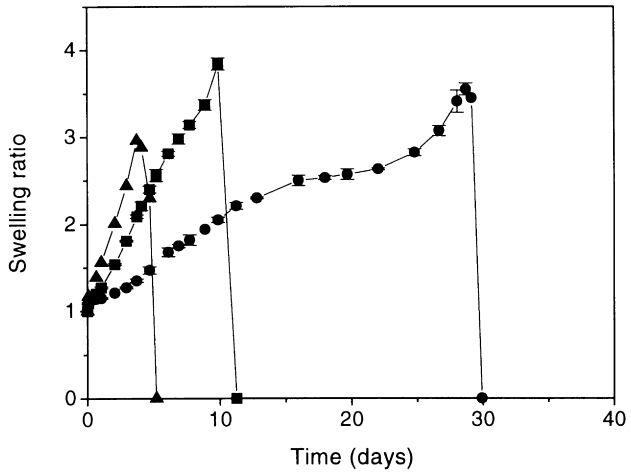

Fig. 5. Swelling behavior of dex–HEMA (●), dex–lactate–HEMA (■) and dex–lactate$_2$–HEMA (▲) hydrogels in aqueous solution (pH 7.2, 37°C). The initial water content of the hydrogels was 80%, the degree of methacryloyl substitution was approximately 6.

4. Conclusions

By reaction of HEMA and lactide at a ratio of 2:1 (mol/mol) in the presence of $SnOct_2$, predominantly 2-(methacryloyloxy)ethyl-lactate and 2-(methacryloyloxy)ethyl-dilactate were formed. These products were isolated in high yield and purity by preparative HPLC. Coupling of these compounds to dextran and subsequent polymerization of the methacrylate groups yielded biodegradable hydrogels of which the degradation time was dependent on the number of hydrolyzable groups in the crosslinks.

Acknowledgements

This investigation was supported by STW, research grant UFA55.3931. The authors wish to thank J.M.A. Damen for recording the ESI mass spectra and Dr J.J. Kettenes-van den Bosch for valuable discussions, both from the Department of Pharmaceutical Analysis, University Utrecht.

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