Sugar-containing Polyamines Prepared Using Galactose Oxidase Coupled with Chemical Reduction

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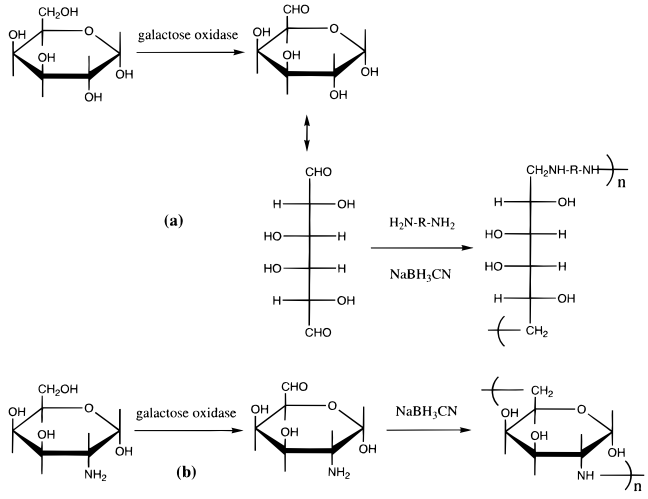
Received July 27, 1998

Synthetic sugar-based polymers are used or show promise in a wide range of applications including functional components of hydrogels,[1] adsorbents,[2] and biorecognition agents.[3] These materials may be polysaccharides[4] or can be polymers that are comprised of ester, amide, acrylate, or other covalent linkages.[5] Since there is a push toward environmentally benign syntheses,[6] the selective incorporation of sugars and their derivatives into polymeric materials is taking on increased importance, particularly in the preparation of biocompatible and biodegradable materials. Until now, the vast majority of in vitro preparation of sugar-containing polymers has utilized glycosidases for polysaccharide synthesis involving unnatural monosaccharide precursors[7] or lipases, esterases, and proteases for poly(sugar ester, amide, or acrylate) synthesis in organic solvents.[8] In both cases, easily hydrolyzable glycoside or ester/amide linkages, respectively, comprise the polymer, and this may limit the overall ruggedness of these materials to conditions where hydrolysis is suppressed.

An alternative synthetic strategy is to take advantage of selective oxidative transformations followed by chemical synthesis to generate sugar-based polymers comprising poorly hydrolyzable linkages. In the present work, we use a multistep chemoenzymatic approach to incorporate a sugar, galactose, into the backbone of a synthetic polymer without forming easily hydrolyzable linkages. The entire process, depicted in Scheme 1, can take place in aqueous media, thereby providing an environmentally benign alternative to solely chemical synthesis.

Numerous enzymes are capable of catalyzing sugar oxidation.[9] A synthetically interesting reaction is catalyzed by galactose oxidase (GO), such as the enzyme from *Dactylium dendroides*. GO has a broad substrate specificity for a variety of galactose- and nongalactose-based compounds.[10] The native reaction on galactose yields a C-6 aldehyde which has been shown to undergo spontaneous Schiff base formation in the presence of amines.[3a,11] We reasoned that the oxidation of galactose by GO actually results in a dialdehyde in the small fraction of the oxidized galactose product that exists in the ring opened hexose form. Thus, in the presence of a suitable diamine, an AA−BB polycondensation reaction is feasible, where AA represents the dialdehyde and BB represents the diamine. Reduction of the Schiff bases would yield a polymer with galactose units linked via stable secondary amine linkages of controllable lengths as shown in Scheme 1.

Scheme 1. Chemoenzymatic Synthesis of Poly(galactose amine)s[a]



[a] (a) Open-form AA−BB polymerization of galactose 1,6-dialdehyde prepared via galactose oxidase catalysis. R represents the alkyl chain length (= 4, 7, and 10 carbons). (b) Galactosamine as substrate for galactose oxidase for A−B type polymerization.

GO catalyzed the facile oxidation of galactose in aqueous buffer,[12] yielding the galactose 6-aldehyde (72% conversion). No reaction was observed in the absence of GO or in the presence of GO heat-inactivated by boiling in water for 30 min. We then added an equimolar concentration of 1,4-diaminobutane to the aqueous solution followed by addition of $NaBH_3CN$ to reduce the Schiff bases formed upon reaction of the diamine with the galactose 6-aldehyde. Following dialysis (1000 MWCO), the retentate was lyophilized to give a white solid (polymer **I**) with an isolated yield of 7.7%.[13] No polymer formation was observed in the absence of GO. GPC of the retentate material showed an $M_n = 40\,600$ and an $M_w = 69\,000$ (polydispersity = 1.7) (Table 1) and structural analysis by FT-IR (see Supporting Information) and ^{13}C NMR provided evidence of alkyl chain incorporation into the polymer network and formation of C−N linkages.[14] Thermal analysis[14] indicated a glass transition temperature nearly 100 °C lower than the thermal decomposition temperature, suggesting that the material can be processed without fear of decomposition.

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(12) The reaction consisted of 1.6 mmol (288 mg) of β-D-galactopyranoside, 1.0 mg (250 Units) GO, and 0.8 mg (2080 Units) bovine catalase (added to remove H_2O_2 produced in the GO-catalyzed oxidation reaction) dissolved in aqueous buffer (0.1 M PBS, pH 6.5), and the reaction mixture was stirred gently at 50 rpm at 25 °C for 30 min resulting in 72% conversion. Solution-phase ^{13}C NMR spectra were recorded on a Brüker MW360 NMR spectrometer, using TMS as internal standard. ^{13}C NMR (DMSO-d_6 δ ppm) 63.5 (C2), 71.5 (C5), 74.5 (C3), 78 (C4), 99 (C1), 204 (C6). After 30 min, 1.6 mmol (141 mg) of 1,4-diaminobutane was added and the mixture agitated at 50 rpm at 25 °C. Following complete consumption of the galactose 6-aldehyde, 2.4 mmol (150 mg) $NaBH_3CN$ was added and the mixture maintained at 25 °C for 3 days.

(13) The relatively low yield was most likely due to the formation of a large amount of oligomers with molecular weights <1000. These components consisted of the 6-aldehyde monomer (as determined by its characteristic NMR spectrum) as well as a likely series of dimers, trimers, and tetramers that may be terminated at both ends with either galactose residues or the diamine, thereby resulting in premature chain termination. Work is underway to further characterize these low molecular weight products as well as to improve the yield of higher molecular weight polymers.

10.1021/ja9826439 CCC: $18.00
Published on Web 12/29/1998

Table 1. Properties of Poly(sugar amines) Generated Chemoenzymatically[a]

polymer	$H_2N-R-NH_2$	M_w	M_n	T_g (°C)	$T_{decomposition}$ (°C)	isolated yield (%)	
						H_2O-soluble	H_2O-insoluble
I	$H_2N-(CH_2)_4-NH_2$	69 000	40 600	59	150[b]	7.7	0
II	$H_2N-(CH_2)_7-NH_2$	140 000	117 000	55	184	4.7	15.5
III	$H_2N-(CH_2)_{10}-NH_2$	403 000	212 000	42	195	1.0	20.9
IV	galactosamine[a]	49 000	35 000	95	98	19.4	0

[a] Polymers **I**−**III** used β-D-galactopyranoside as sugar, while β-D-galactosamine was used as the substrate for the generation of polymer **IV** without addition of external diamine. [b] Broad decomposition temperature ranging from ca. 120−180 °C with an average of ca. 150 °C.

To generalize this approach, we incorporated diamines with different chain lengths into poly(galactose amine)s; 1,7-diaminoheptane and 1,10-diaminodecane, the former in a one-pot synthesis and the latter in two separate reaction solutions because of the poor solubility of the diamine in water.[15] Incorporation of the alkyl chains into the respective polymers (polymers **II** and **III**) is shown by FT-IR (see Supporting Information) which depicts the clear increase in the intensity of C−H bond stretching at 2800 cm^{-1} as the length of the alkyl chain increases.

As summarized in Table 1, the longer alkyl chains resulted in a lower T_g and higher thermal decomposition temperatures, consistent with increased hydrophobicity and flexibility of the polymer backbone.[16] Longer chain alkyl groups also led to a higher molecular weight of the water-soluble polymer fractions. Longer alkyl chains, however, resulted in a smaller fraction of polymer that was water soluble, consistent with the more hydrophobic character of the polymer and the potential for increased chain entanglements and/or covalent cross-linking of the polymer as the molecular weight increases. The water-insoluble fractions of **II** and **III** were found, on the basis of FT-IR and ^{13}C NMR analysis, to be structurally similar to the respective water-soluble polymers.

GO is also capable of catalyzing the oxidation of β-D-galactosamine, and this provided us with an opportunity to prepare an A−B type polycondensation reaction (where the amine and aldehyde are on the same monomer) as well as to generate a poly(galactose amine) material that does not contain a hydrocarbon linker. To that end, we dissolved 0.5 mmol galactosamine in the presence of 1.0 mg GO and 0.8 mg catalase and let the reaction proceed at 25 °C for 6 h at which point gas chromatographic analysis indicated complete conversion of galactosamine. The Schiff bases were reduced by $NaBH_3CN$ and the product (polymer **IV**, water-soluble) was dialyzed (1000 MWCO) and the retentate lyophilized to give a white solid. GPC analysis (Table 1) indicated an $M_n = 35\,000$ and thermal analysis indicated a higher T_g and lower thermal decomposition temperature than that of the diamine-containing polymers, consistent with the lack of an alkyl chain linker.

In summary, new sugar-containing polymers have been prepared by a chemoenzymatic strategy that involves an oxidative enzymatic step with high regioselectivity along with a nonselective reductive chemical step. These materials may find particular use as components in hydrogels or water-soluble emulsifiers and stabilizers. The strategy employed herein is likely to be expanded through the use of other oxidative enzymes along with different chemistries of the linker molecules including aromatic and charged moieties, and these studies are underway.

Acknowledgment. This work was supported by the Biotechnology Research and Development Corporation.

Supporting Information Available: ^{13}C NMR, DSC, and TGA of polymers **I**−**IV** (PDF). See any current masthead page for ordering information and Web access instructions.

JA9826439

(14) Gel permeation chromatography (GPC) was performed with a Waters Ultrahydrogel 1000 column (M_w range 5×10^3 to 1×10^6 with water as eluant) with a Wyatt miniDawn (Wyatt Technology, Santa Barbara, CA) light-scattering detector and a Waters 410 refractive index detector. GPC calibration was performed using the known AUX calibration constant and 100% mass analysis. Average standard errors were <5%. FT-IR spectra (KBr pellets) were recorded on a Mattson Cygnus 25 FT-IR spectrometer from 400 to 4 000 cm^{-1}. ^{13}C NMR (D_2O δ ppm) 27 (C2′, alkyl chain), 28 (C3′), 42 (C1′), 46 (C4′), 50 (C1, C6 sugar moiety), 66 (C2), 72 (C3), 73 (C4). Thermal analyses were performed on a differential scanning calorimeter (DSC 2910) (3.0 mg sample amount with a heating rate was 10 °C/min) and thermal gravimetric analyzer (TGA 1950) from DuPont Instruments (Wilmington, DE).

(15) The synthesis of the poly(galactose amine) with 1,7-diaminoheptane was performed in a single pot with 2.6 mmol (467 mg) β-D-galactopyranoside, 2.2 mg (529 Units) GO, and 1.3 mg (3467 Units) bovine catalase in 0.1 M PBS, pH 6.5, followed by addition of 2.6 mmol (337 mg) 1,7-diaminoheptane after 30 min. Reduction of the Schiff bases was accomplished by addition of 3.2 mmol (200 mg) $NaBH_3CN$ and the mixture kept at 25 °C for 3 days to yield both water-soluble and -insoluble fractions. Both solution- and solid-state ^{13} C NMR were performed (see Supporting Informaton). Solution-state ^{13}C NMR (D_2O δ ppm) 19(C4′, alkyl chain), 30 (multiple C2′, 3′, 5′, 6′), 42 (C1′, C7′), 52 (C1, C6 sugar moiety), 66 (C2), 72 (C5), 75 (C3), 78 (C4). Solid-state ^{13}C NMR (δ ppm) 30 (broad, alkyl chain carbons), 75 (broad, sugar carbons). Synthesis with 1,10-diaminodecane required a two-pot sequence because of the poor aqueous solubility of the diamine. In the first step, galactose 6-aldehyde was synthesized as described above[12] resulting in 1.87 mmol (332 mg) galactose 6-aldehyde. The aldehyde intermediate was then diluted with MeOH to give a 1:1 methanol:water mixture and 2.6 mmol (447 mg) 1,10-diaminodecane was added and the reaction mixture shaken at 50 rpm at 25 °C for 6 h. Reduction of the Schiff bases was accomplished as described above, using 3.2 mmol (200 mg) $NaBH_3CN$. Solution-state ^{13}C NMR (D_2O δ ppm) 19 (C5′, C6′ alkyl chain), 29 (multiple, C3′, C4′, C7′, C8′), 38 (C2′, C9′), 42 (C1′, C10′), 51 (C1, C6, sugar moiety), 66 (C2), 68 (C5), 75 (C3), 77 (C4). Solid-state ^{13}C NMR (δ ppm) 29 (broad, alkyl chain carbons), 74 (broad, sugar carbons).

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