Novel Nonionic Oligosaccharide Surfactant Polymers Derived from Poly(vinylamine) with Pendant Dextran and Hexanoyl Groups

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Received May 27, 1997; Revised Manuscript Received October 8, 1997[⊗]

ABSTRACT: A series of novel surfactant polymers: poly(*N*-vinyldextran aldonamide-*co*-*N*-vinylhexanamide) (PNVDA-*co*-PNVH), in which hydrophilic dextran oligosaccharides and hydrophobic hexanoyl groups were incorporated simultaneously on to poly(vinylamine) (PVAm), were synthesized, and characterized by FTIR and ^{1}H NMR spectroscopy. Structurally well-defined poly(vinylamine) was prepared by hydrolysis of poly(*N*-vinylformamide), which was obtained by free radical polymerization of *N*-vinylformamide. Dextran molecules ($M_w = 1600$) were attached to poly(vinylamine) by reacting amine groups with dextran lactone, while hexanoyl groups were attached by reacting amine groups with *N*-(hexanoyloxy)succinimide. By adjustment of the feed ratio, surfactant polymers with different hydrophilic/hydrophobic balances were prepared. Surface activity of the surfactants at an air/water interface was demonstrated by significant reductions in water surface tension. Surface activity at a solid surface/water interface was demonstrated by atomic force microscopy of surfactant molecules adsorbed onto a highly oriented pyrolytic graphite. The synthetic approach described in this report provides a route to prepare a variety of oligosaccharide surfactant polymers with well-defined structures and hydrophilic/hydrophobic balances, by selecting different combinations of oligosaccharide and alkanoyl groups or by adjusting feed ratio of the oligosaccharide to alkanoyl groups.

Introduction

Research interest in oligosaccharide surfactants has increased substantially over the past decade,[1−15] primarily because they offer natural alternatives to existing surfactants for many industrial applications. However, oligosaccharide surfactants and glycoconjugates also offer a wide range of unexplored possibilities for applications in the biomedical field, including drug delivery systems and biomaterials.[16−18] Many of the current problems with implanted biomaterials or drug delivery devices are related to the spontaneous adsorption of proteins that initiate characteristic failure mechanisms such as thrombus formation on blood-contacting implants.[19] We approach the problem of inhibiting protein adsorption from the perspective of how the body controls adhesive and nonadhesive interactions. Cell and molecular interactions are strongly influenced by pendant oligosaccharides that are clustered on many plasma proteins and cell surface glycomolecules that constitute a cell's glycocalyx.[20] The glycocalyx is the oligosaccharide-rich and structurally complex surface of a cell. The densely-packed, highly hydrated oligosaccharides provide a physical basis for maximizing entropic repulsion and preventing undesirable molecular and cellular adhesions. Desirable adhesive interactions and biological specificity are often achieved through electrostatic interactions with cell-surface glycoprotein receptor molecules that possess complex three-dimensional charge distributions that create sufficient attractive force to overcome entropic repulsion.

In following this biological perspective, we have explored physical adsorption/assembly of oligosaccharide surfactants onto hydrophobic biomaterials in an attempt to mimic the nonadhesive properties of a glycocalyx.[21] That is, using the hydrophobic effect between surfactant and solid surface to create a new biomaterial oligosaccharide-rich interface capable of minimizing protein adsorption. Our reports on novel oligosaccharide surfactants have laid emphasis on manipulating surfactant structure to achieve desired interfacial behavior.[22−24] We reported on the synthesis and surface active properties of structurally well-defined linear oligosaccharide surfactants with different molecular geometries (AB diblock and ABA bolaform-type surfactants, where "A" represents the sugar headgroup and "B" represents the hydrophobic alkyl tail), and different hydrophobic and hydrophilic chain lengths.[22−24]

In this report, we describe the synthesis, characterization, and surface activity at air/water and solid surface/water interfaces of a novel series of well-defined, comblike, surfactant polymers derived from low molecular weight poly(vinylamine) (PVAm). Dextran molecules were attached to PVAm by reacting amine groups with dextran lactone. Hydrophobic hexanoyl groups are attached to PVAm by reacting the amine groups with *N*-(hexanoyloxy)succinimide, the *N*-hydroxysuccinimide active ester of hexanoic acid. The macromolecular design of surfactants presented here provide a glycocalyx-like surface when adsorbed under aqueous conditions on a hydrophobic highly oriented pyrolytic graphite (HOPG) surface. The surfactant polymers derived from PVAm with multiple hexanoyl and dextran pendant groups facilitate both increased surface contact and increased oligosaccharide density. The stability on the hydrophobic surface is provided by the hexanoyl seg-

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[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1997.

S0024-9297(97)00740-7 CCC: $15.00
Published on Web 01/13/1998

ments, while high sugar density is provided by attached dextran molecules. The synthetic approach described in this report offers numerous possibilities for quantitative manipulation of composition and molecular architecture for achieving desired interfacial properties, and ultimately to structures that may realistically mimic a glycocalyx.

Experimental Section

(1) Materials. Acetaldehyde, formamide, formic acid, hexanoic acid, *N*-hydroxysuccinimide and dicyclohexylcarbodiimide (DCCI) were purchased from Aldrich Chemical Co. and used as received. Dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were purchased from Aldrich Chemical Co. and freshly distilled before use. Dextran lactone ($M_w = 1600$, $M_w/M_n = 1.16$, DP = 9) was prepared according to the method reported previously.[22] Strong anionic exchange resin Amberlite IR-400 (Sigma Chemical Co.) was rinsed with distilled water before use. All other reagents and solvents were used as received unless otherwise specified.

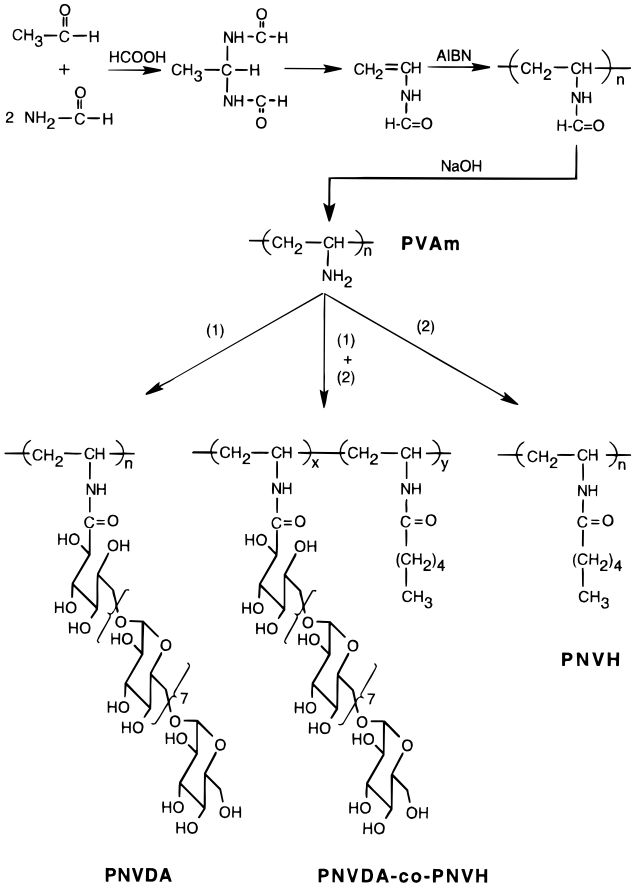
(2) Methods. ^{1}H NMR spectra were recorded at ambient temperature, using a 200 MHz Varian Gemini-200 or a 400 MHz Bruker MSL-400 spectrometer, in parts per million downfield from tetramethylsilane (TMS) as internal standard and DMSO-d_6 or D_2O as solvent. Transmission IR spectra in the range of 400−4000 cm^{-1} were recorded using a Digilab FTS-40 FTIR spectrometer. The materials were ground with KBr and pressed into pellets under reduced pressure. For each sample, 256 scans were collected with a resolution of 8 cm^{-1}. Gel permeation chromatography (GPC) was carried out using a HEMA-BIO100 GPC column equipped with a Rainin HPXL solvent delivery system and a DYNAMAX refractive index detector. The column was calibrated with dextrans with narrow molecular weight distribution. The flow rate was 1.0 mL/min with distilled water as eluent.

The surface activity of the comblike surfactant polymers in solution at an air/water interface was demonstrated by water surface tension measurement. Surface tensions of aqueous surfactant solutions were measured at 25 °C and ambient pressure, using a Du Nouy ring tensiometer. The tensiometer was calibrated with ultrapure water before use. The platinum ring was cleaned by flaming, while the glassware was cleaned with a strong basic solution and rinsed with tap water and ultrapure water. A 5 wt % surfactant solution was freshly prepared as a starting solution and then diluted to the desired concentration for each measurement. Surface tension was measured three times at each concentration.

The surface activity of surfactant polymers at a solid surface/water interface was studied by atomic force microscopy (AFM). AFM tapping mode imaging of surfactants on highly oriented pyrolytic graphite (HOPG) under aqueous medium was performed using a Nanoscope III Multimode AFM (Digital Instruments, Santa Barbara, CA) equipped with a glass fluid cell attachment. Silicon nitride (Si_3N_4) cantilevers with integrated Si_3N_4 tips (Nanoprobes, Digital Instruments) were used. A freshly cleaved HOPG substrate was first imaged in water as a reference. Surfactant solution (0.5 mg/mL) was injected into the fluid cell without withdrawing the AFM probe, so that the same surface region of HOPG, before and after adsorption of surfactant molecules, could be imaged. Imaging was carried out over a 16 h period.

(3) Synthesis of Poly(vinylamine) (PVAm). To obtain well-defined, defect-free, PVAm, we used poly(*N*-vinylformamide) as the precursor. The synthesis of PVAm involved 4 steps as shown in Scheme 1: (i) synthesis of ethylidenebis(formamide); (ii) pyrolysis of ethylidene bis(formamide) to *N*-vinylformamide and formamide; (iii) polymerization of *N*-vinylformamide to poly(*N*-vinylformamide); (iv) hydrolysis of poly(*N*-vinylformamide) to PVAm, which is precipitated as a PVAm·HCl salt and then desalted by ion exchange chromatography. The product of each step was confirmed by spectroscopic analysis to ensure a well-defined final product of PVAm.

Scheme 1. Synthetic Route to Poly(vinylamine) and Derivatization of Poly(vinylamine) with (1) Dextran Lactone and (2) *N*-(Hexanoyloxy)succinimide



Ethylidenebis(formamide) ($CH_3CH(NHCHO)_2$). To a solution of 45 g (1.0 mol) formamide and 50 g of formic acid heated to 40 °C with an oil bath was added acetaldehyde (8.8 g, 0.2 mol) dropwise. The reaction mixture was stirred at 85 °C for 4 h. Excess formamide and formic acid were evaporated by vacuum distillation with the bath temperature below 120 °C. The yellow-brown residue was refluxed in acetone (300 mL) and then cooled to −70 °C to precipitate white crystals. The crystals were filtered and dried under vacuum at 70 °C to give 15.0 g (65%) of ethylidenebis(formamide), which was further purified by recrystallization from a mixture of 2-propanol and hexane. IR (KBr): 3211 cm^{-1} (ν(N−H)), 2900 cm^{-1}, 3042 cm^{-1} (ν(C−H) of CH_3 and CH), 1689 cm^{-1} (amide I), 1545 cm^{-1} (amide II). ^{1}H NMR (DMSO-d_6, ppm): 1.24−1.31 (3H, $-CH_3$), 5.56−5.59 (1H, CH_3CH-), 7.92−8.15 (2H, from two −CHO), 8.40−8.64 (2H, from two −CONH−).

***N*-Vinylformamide.** A mixture of 6.96 g (60 mmol) ethylidenebis(formamide) and 2.0 g calcium carbonate was heated to 210 °C under vacuum with a sodium nitrite bath. The pyrolyzed product was vacuum distilled to give 5.80 g (91%) of a slightly yellow liquid consisting of a 1:1 molar ratio of *N*-vinylformamide and formamide. ^{1}H NMR (DMSO-d_6, ppm): 4.19−4.76 (2H, CH_2=CH−), 6.60−6.94 (1H, CH_2=C*H*−), 7.23−7.47 (2H, $-NH_2$), 7.84−8.09 (2H, HCO−), 10.02 (1H, −CONH−).

Poly(*N*-vinylformamide). To 2.32 g of the 1:1 molar ratio mixture of *N*-vinylformamide (20 mmol) and formamide (20 mmol) was added 5 mL of 2-propanol. The reaction mixture was purged with argon and freeze−thawed three times using liquid nitrogen to eliminate oxygen. 2, 2′-Azobisisobutyronitrile (AIBN, 32 mg, 0.12 mmol) was added to the reaction solution, which was then refluxed for 4 h under argon. 2-Propanol was removed by rotary vacuum evaporation. The residue was dissolved in a small amount of water and precipitated in acetone. The precipitate was filtered and dried

under vacuum at 70 °C overnight to give 1.3 g (89%) of poly(*N*-vinylformamide). GPC measurement showed that the number average molecular weight was ~10 000. IR (KBr): 1677 cm^{-1} (amide I), 1538 cm^{-1} (amide II). ^1H NMR (D_2O, ppm): 1.57 (2H, $-CH_2-$), 3.79 (1H, $-CH-$), 7.90 (1H, HCO−).

Poly(vinylamine) Hydrochloride (PVAm·HCl). To a 4 mL aqueous solution of 0.85 g (12 mmol) of poly(*N*-vinylformamide) was added 4 mL of aqueous NaOH (0.72 g, 18 mmol) solution. The mixture was stirred at 80 °C for 6 h under nitrogen. After cooling to ambient temperature, the solution was acidified with concentrated HCl, to precipitate the PVAm·HCl salt. The precipitate was washed with methanol to neutral pH and dried under vacuum to give 0.62 g (65%) PVAm·HCl. IR (KBr) ~3420 cm^{-1} (υ(N−H) of NH_2), ~3000 cm^{-1} (wide and strong overlapping peak from υ(N−H) of $-NH_3^+$ and υ(C−H) of CH_2 and CH), 1606 and 1512 cm^{-1} ((δ_{as}(N−H) and δ_s(N−H) of $-NH_3^+$). No amide peaks were observed. ^1H NMR (D_2O, ppm): 2.16 (2H, $-CH_2-$), 3.74 (1H, $-CH-$).

Poly(vinylamine) (PVAm). PVAm was obtained by passing the aqueous solution of PVAm·HCl through a strong anionic exchange column (Amberlite IR-400), followed by lyophilizing the eluate. IR (KBr): ~3300 cm^{-1} (υ(N−H) of $-NH_2$), 2870 and 2930 cm^{-1} (υ(C−H) of CH_2 and CH), 1591 cm^{-1} (δ(N−H) of $-NH_2$). ^1H NMR (D_2O, ppm): 1.22 (2H, $-CH_2-$), 2.85 (1H, $-CH-$). ^1H NMR (DMSO-d_6, ppm): 1.18 ($-CH_2-$), 2.92 ($-CH-$), 2.6 ($-NH_2$) (a small amount of PVAm can be dissolved in DMSO-d_6 at 60 °C), ^1H NMR (DMSO-d_6 with a drop of D_2O, ppm): 1.20 (2H, $-CH_2-$), 2.88(1H, $-CH-$).

(4) *N*-(Hexanoyloxy)succinimide. To a solution of 4.64 g (0.04 mol) of hexanoic acid and 5.75 g (0.05 mol) of *N*-hydroxysuccinimide in 100 mL distilled DMF was added 10.3 g (0.05 mol) of DCCI. The mixture was cooled with an ice−water bath and stirred for 5 h. The dicyclohexyl urea (DCU) precipitate was removed by filtration, and the DMF solvent was removed by vacuum rotary evaporation. The yellow oil residue was washed with water and hexane to yield a white solid, which was vacuum-dried at 78 °C to give 6.34 g (74%) of *N*-(hexanoyloxy)succinimide. IR (KBr): 1816 cm^{-1} (υ(C═O) of ester), 1745−1786 cm^{-1} (υ(C═O) of imide). ^1H NMR (DMSO-d_6, ppm): 0.86 (3 H, $-CH_3$), 1.31 (4H, $-(CH_2)_2-CH_3$), 1.62 (2H, $-CH_2CH_2COO-$), 2.64 (2H, $-CH_2COO-$), 2.80 (4H, $-COCH_2CH_2CO-$).

(5) Synthesis of Poly(vinylamine) Derivatives. The synthetic route to PVAm derivatives, poly(*N*-vinylhexanamide) (PNVH), poly(*N*-vinyldextran aldonamide) (PNVDA), and poly(*N*-vinyldextran aldonamide-*co*-*N*-vinylhexanamide) (PNVDA-*co*-PNVH), is shown in Scheme 1.

Poly(*N*-vinylhexanamide) (PNVH). PNVH was prepared by reacting the amino groups of PVAm with *N*-(hexanoyloxy)succinimide. To a 2 mL methanol solution of 107 mg (0.5 mmol) *N*-(hexanoyloxy)succinimide was added 21.5 mg of PVAm (0.5 mmol amino groups). The reaction mixture was stirred at ambient temperature overnight and then precipitated from 50 mL aqueous solution of 1% sodium bicarbonate. The precipitate was washed several times with distilled water, filtered, and vacuum-dried at 78 °C overnight to give 40 mg (56%) of PNVH. IR (KBr): 2957−2856 cm^{-1} (υ(C−H) of CH_2 and CH), 1633 cm^{-1} (υ(C═O) of amide), 1548 cm^{-1} (υ(N−H) of amide).

Poly(*N*-vinyldextran aldonamide) (PNVDA). PNVDA was prepared by reacting the amino groups of PVAm with dextran lactone. Dextran lactone was prepared according to the method described previously.[22] To a 1 mL methanol solution of 8.6 mg of PVAm (0.2 mmol amino groups) was added 0.27 g (0.2 mmol) of dextran lactone in 3 mL of DMSO. The reaction mixture was stirred at 70 °C for 2 days. The solution was concentrated by vacuum distillation and the product precipitated by the addition of acetone. The precipitate was filtered and dried under vacuum at 78 °C overnight to give 0.25 g (92%) of PNVDA. The raw product was further purified by extensive dialysis against water to remove unreacted dextran lactone, using Spectra/Por 3 regenerated cellulose membrane with a 3500 molecular weight cutoff. IR: 3310 cm^{-1} (υ(O−H)), 2920 cm^{-1} (υ(C−H) of CH_2 and CH), 1643 cm^{-1} (υ(C═O) of amide), 1152−1014 cm^{-1} (υ(C−O)). The υ(C═O) peak of unreacted lactone at 1780 cm^{-1} was not observed in the IR spectrum of the purified product.

(6) Poly(*N*-vinyldextran aldonamide-*co*-*N*-vinylhexanamide) (PNVDA-*co*-PNVH). Surfactant polymers PNVDA-*co*-PNVH were prepared by reacting amino groups of PVAm with dextran lactone and *N*-(hexanoyloxy)succinimide simultaneously. PNVDA-*co*-PNVH with 3 different molar feed ratios of dextran lactone to *N*-(hexanoyloxy)succinimide (1:1, 1:2 and 1:4) were prepared. To prepare PNVDA-*co*-PNVH (1:1 feed ratio), 0.54 g (0.4 mmol) of dextran lactone in 6 mL of DMSO was added to a 2 mL methanol solution containing 34.2 mg of PVAm (0.8 mmol amino groups) and 85.2 mg (0.4 mmol) of *N*-(hexanoyloxy)succinimide. After being stirred for 4 h at room temperature, the solution was heated to 70 °C with an oil bath and stirred for 2 days. The product solution was concentrated by vacuum distillation and precipitated by the addition of acetone. The precipitate was filtered and dried under vacuum at 78 °C overnight to give 0.53 g (81% yield) raw product. This was purified by extensive dialysis against water as described previously. PNVDA-*co*-PNVH (1:2 and 1:4 feed ratios) were prepared using similar procedures. IR: 3310 cm^{-1} (υ(O−H)), 2930−2874 cm^{-1} (υ(C−H) of CH_2 and CH), 1643 cm^{-1} (amide I), 1547 cm^{-1} (amide II), 1149−1032 cm^{-1} (υ(C−O)). ^1H NMR (DMSO-d_6, ppm): 0.85 ($-CH_3$ of hexanoyl groups, $CH_3(CH_2)_3CH_2CO-$), 1.1−1.6 ((CH_2)$_3$ of $CH_3(CH_2)_3CH_2-CO-$ and $-CH_2-$ from PVAm backbone), 2.1 (CH_2 of $CH_3(CH_2)_3CH_2CO-$), 3.0−4.1 ($-CH-$ of PVAm backbone and all dextran CH's and CH_2's except the ones at the glycosidic linkages), 4.1−5.3 (all dextran OH's and CH at the glycosidic linkages), 7−8 ($-NH-$ of amide linkages).

Results and Discussion

Synthesis of Poly(vinylamine). The synthesis of well-defined PVAm required 4 reaction steps (Scheme 1). In the first step, acetaldehyde was condensed with formamide under acidic conditions to generate ethylidenebis(formamide), which was purified by recrystallization. In the second step, ethylidenebis(formamide) was pyrolyzed to *N*-vinylformamide and formamide. These two compounds are both liquids at ambient temperature, with very similar boiling points, but separation is not necessary. Thus, without any further purification, the *N*-vinylformamide in the mixture was polymerized in 2-propanol solution using AIBN as initiator. Poly(*N*-vinylformamide) is water soluble with average molecular weight ~10 000 as determined by GPC using dextrans as standards. In the last step, poly(*N*-vinylformamide) was hydrolyzed to PVAm under basic conditions and then precipitated in concentrated HCl solution as PVAm·HCl. The hydrolysis was carried out under basic, rather than acidic, conditions in order to achieve complete conversion. Under acidic conditions, positive charges build up along polymer chain during hydrolysis, which then limits further hydrolysis of the remaining formamide. Under basic conditions, no charges build up during hydrolysis, and complete conversion can be realized. No amide peaks were observed in the IR spectrum of PVAm·HCl; instead, very strong $-NH_3^+$ peaks occur, indicating complete conversion of poly(*N*-vinylformamide) to PVAm·HCl.

PVAm was obtained by passing aqueous PVAm·HCl solution through a strong anionic exchange column. The PVAm is readily soluble in water and methanol. From the ^1H NMR of PVAm in D_2O (Figure 1A), the peak integrations of $-CH_2-$ and $-CH-$ agree with the predicted molecular structure. As reported by others,[25] the proton resonance peak of $-NH_2$ was not observed for PVAm in D_2O, because of rapid proton exchange between $-NH_2$ and any H_2O in D_2O. The proton

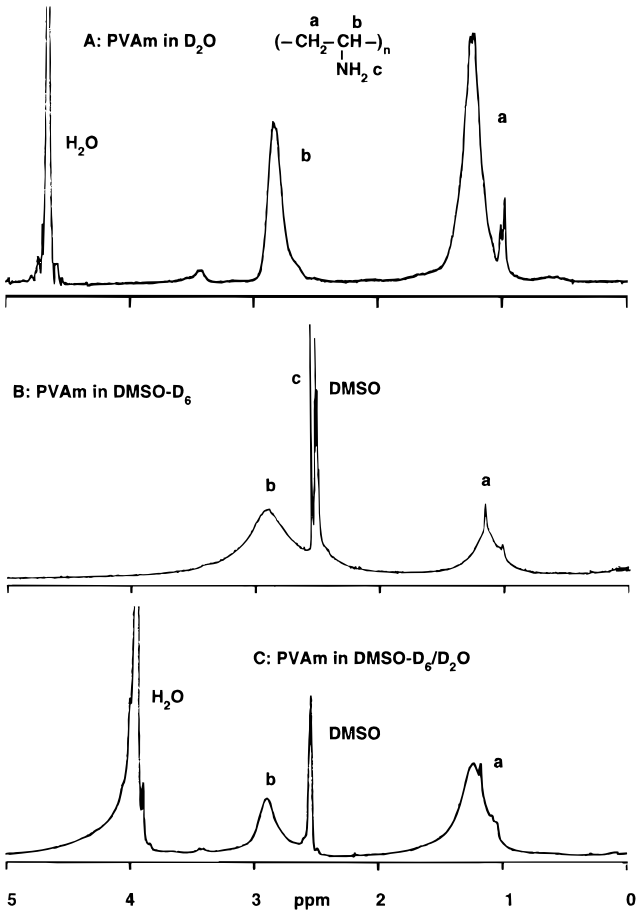


Figure 1. ^{1}H NMR spectra of poly(vinylamine) obtained on a 200 MHz Varian Gemini 200 spectrometer. (A) in D_2O, proton peaks of $-CH_2-$ and $-CH-$ with 2:1 peak integration ratio; (B) in DMSO-d_6, proton peak of $-NH_2$ together with broad peaks of $-CH_2-$ and $-CH-$; (C) in DMSO-d_6 with a drop of D_2O, proton peak of $-NH_2$ disappears.

resonance peak of $-NH_2$ (2.6 ppm) was observed when using vigorously dried DMSO-d_6 as solvent (Figure 1B). However, after adding a drop of D_2O into the PVAm–DMSO–d_6 solution, the peak disappears (Figure 1C). The proton peaks of $-CH_2-$ and $-CH-$ are broad because of the poor solubility of PVAm in DMSO. In addition, the proton peak of the trace H_2O is shifted, probably due to the interaction between H_2O and DMSO.

Derivatization of PVAm. To attach functional moieties to a PVAm backbone, PVAm has been derivatized with active derivatives of carboxylic compounds.[25–30] However, in each case, a homopolymer with only one type of functional moiety attached to PVAm was reported. To achieve surface-active properties, both hydrophilic and hydrophobic pendant groups need to be attached to the PVAm. This was accomplished by reacting the amino groups of PVAm with oligosaccharide lactone and *N*-(hexanoyloxy)succinimide simultaneously. Before we attempted to make the comblike surfactant, two homopolymers containing either alkanoyl or oligosaccharide pendant groups were prepared.

PNVH was prepared by reacting the amine groups of PVAm with *N*-(hexanoyloxy)succinimide. *N*-(Hexanoyloxy)succinimide is selective toward amino groups.[29] Selectivity is important in the preparation of the comblike surfactants, because both amino and hydroxyl

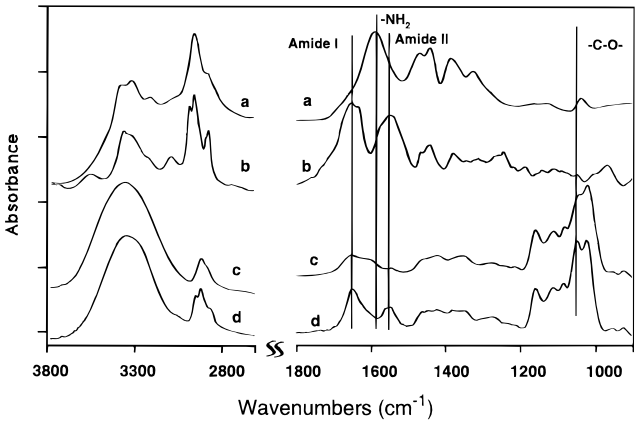


Figure 2. IR spectra (a) PVAm; (b) PNVH; (c) PNVDA; (d) PNVDA-*co*-PNVH.

groups are present in the reaction system. PNVH was soluble in the methanol reaction solution but was hardly soluble in any solvent after precipitation, possibly due to the aggregation of pendant hexanoyl groups. Instead, it swells in most solvents. The IR spectrum of PNVH (Figure 2b) indicates the presence of both amide and alkyl groups; a weak shoulder at 1590 cm^{-1} indicates some unreacted amino groups.

PNVDA was prepared by reacting amino groups of PVAm with dextran lactone (1:1 ratio). Initially, PVAm was insoluble in DMSO, but gradually dissolved as the reaction proceeded. The aminolysis of dextran lactone is selective, and thus protection of dextran hydroxyl groups is not necessary.[22] The IR spectrum of crude PNVDA (spectrum not shown) exhibits the presence of both amide and hydroxyl groups, indicating the successful derivatization of PVAm with dextran lactone. However, a peak at 1780 cm^{-1} due to unreacted lactone also was observed, which meant that not all the amino groups along the PVAm chain were consumed by dextran lactone. The unreacted dextran lactone which precipitated along with the surfactant from the reaction mixture was removed by extensive dialysis. The lactone peak at 1780 cm^{-1}, which is present in the crude PNVDA, was not observed in purified PNVDA (Figure 2c). The resultant PNVDA is soluble in water and in aprotic solvents such as DMF and DMSO.

To minimize pendant group interactions along the chain, derivatized PVAm could presumably adopt either an atactic, syndiotactic, or isotactic conformation. There are two extreme conformational possibilities: extended isotactic and syndiotactic conformation of PVAm. The theoretical distance between adjacent amino groups is estimated to be about 0.25 nm. According to computer simulations, using Biosym software, dextran lactone exhibits a highly compact helical conformation with a diameter of about 1.3 nm for a dextran M_w of 1600. It was quite obvious from the molecular modeling that it was not possible to derivatize all of the amino groups with dextran lactone because of the steric hindrance between dextran groups. Theoretically, we estimate that only 16.7 or 33.3 mol % of amino groups can be derivatized with dextran lactone when PVAm is in either a fully extended isotactic or syndiotactic conformation, respectively.

Synthesis of Oligosaccharide Surfactant Polymer: Our initial results demonstrated the feasibility of derivatizing PVAm with either hydrophilic dextran or hydrophobic alkanoyl groups. The comblike

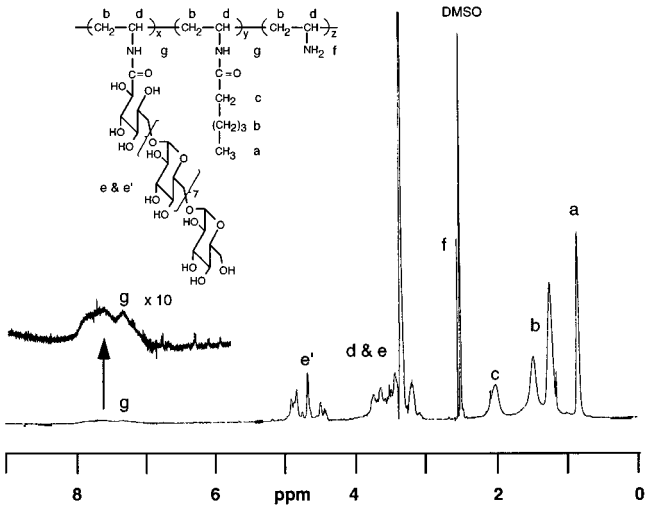


Figure 3. ^{1}H NMR spectrum of PNVDA-*co*-PNVH in DMSO-d_6, where x, y, and z is the mole percentage of dextran, hexanoyl and unreacted amino groups, respectively. The values of x, y, and z were estimated by solving three equations based on the ^{1}H NMR integration, where $x + y + z = 1$, $z/x = Z/X$, and $y/x = Y/X$. X = (integral of peaks d, e, and e′)/91 (i.e., 90 protons from dextran and 1 proton from −CH− of PVAm); Y = (integral of peaks a, b, and c)/13 (i.e., 11 protons from hexanoyl and 2 from $-CH_2-$ of PVAm); Z = integral of peak f/2 (proton number for unreacted amino groups in PVAm).

surfactant polymer, poly(*N*-vinyldextran aldonamide-*co*-*N*-vinylhexanamide) (PNVDA-*co*-PNVH) was then prepared by reacting the amino groups with dextran lactone and *N*-(hexanoyloxy)succinimide simultaneously. Three different molar feed ratios of dextran lactone to *N*-(hexanoyloxy)succinimide (1:1, 1:2 and 1:4) were used to prepare PNVDA-*co*-PNVH with different compositions.

The IR spectrum of PNVDA-*co*-PNVH (Figure 2d) is an overlay of those of PNVH (Figure 2b) and PNVDA (Figure 2c). The spectrum shows absorption peaks indicative of amide, hydroxyl, and alkyl groups. Any unreacted dextran lactone was removed by extensive dialysis. The IR results demonstrated qualitatively the expected comblike structure of PNVDA-*co*-PNVH. The ^{1}H NMR spectra of the purified surfactants (Figure 3) show proton peaks derived from PVAm, dextran, and hexanoyl groups, confirming the expected composition for the surfactants.

The compositions for PNVDA-*co*-PNVHs prepared from the three different molar feed ratios were estimated, based on proton integration of ^{1}H NMR spectra. Both theoretical and experimentally determined compositions are listed in Table 1. The measured compositions are, as expected, lower than the extreme theoretical values calculated by assuming syndiotactic PVAm conformation. The dextran molar content in the surfactants decreases from about 22% to 19% to 15%, which correspond to dextran weight percentages of about 85% to 72% to 66%, or about 30 to 26 to 21 dextrans per surfactant molecule, respectively. Correspondingly, the hexanoyl molar content in the surfactants increases from about 23% to 69% to 76%, which correspond to weight percentages of about 9% to 27% to 33%, or about 32 to 97 to 107 hexanoyl groups per surfactant molecule. Accordingly, the ratios of dextran to hexanoyl for the three surfactants prepared from feed ratios 1:1, 1:2, and 1:4 were determined to be 1:1, 1:3.7, and 1:5, respectively. These quantitative data demonstrate successful synthesis of the comblike dextran surfactants with adjustable multiple hydrophilic dextran molecules and hydrophobic hexanoyl groups. The surfactant polymers with varied hydrophilic/hydrophobic balances were designated as PNVDA-*co*-PNVH (1:1), PNVDA-*co*-PNVH (1:3.7), and PNVDA-*co*-PNVH (1:5). PNVDA-*co*-PNVH (1:1) and PNVDA-*co*-PNVH (1:3.7) are soluble in water, DMF, and DMSO, while PNVDA-*co*-PNVH (1:5) is not readily soluble in water but is soluble in DMSO. However, PNVDA-*co*-PNVH (1:5) becomes partially soluble in water with vigorous sonication (≥1.5 h). An opaque solution can be prepared at low concentration (∼1 mg/mL).

A three-dimensional molecular model of the surfactant with a 1:5 molar ratio of dextran to alkanoyl is shown in Figure 4. Steric hindrance between the bulky dextran molecules resulted in some unreacted amino groups in the PVAm. However, as shown in Table 1, the number of unreacted amino groups in PNVDA-*co*-PNVH (1:3.7) and PNVDA-*co*-PNVH (1:5) decreases significantly compared with PNVDA-*co*-PNVH (1:1), due to a decreased amount of dextran relative to *N*-(hexanoyloxy)succinimide used in the reaction. All the amino groups in PVAm can be fully derivatized by introducing a smaller oligosaccharide than dextran, such as gluconolactone. To confirm this hypothesis, a surfactant, poly(*N*-vinylglucose aldonamide-*co*-*N*-vinylhexanamide) was prepared from a 1:1 ratio of gluconolactone to *N*-(hexanoyloxy)succinimide (PNVGA-*co*-PNVH, (1:1)). The measured composition from ^{1}H NMR is 49.8 mol % glucose and 50.2 mol % hexanoyl.

Surface Active Properties. Air/Water Interface. Surface active properties of the surfactant polymers at an air/water interface were determined from surface tension measurements. Surface tension plotted against the logarithm of surfactant concentration is shown in Figure 5 for PNVDA and the PNVDA-*co*-PNVH surfactants. The surface tension of PNVDA-*co*-PNVH (1:5) was not measured because of its poor solubility in water. As expected, PNVDA, a homopolymer composed of PVAm with pendant dextran molecules, showed virtually no surface activity, as indicated by the small decrease (∼2 dyn/cm) in water surface tension with increasing surfactant concentration (Figure 5a). In contrast, the surfactants PNVDA-*co*-PNVH (1:1) and PNVDA-*co*-PNVH (1:3.7) exhibit significant surface active behavior, as indicated by the substantial decrease in surface tension with increasing surfactant concentration (Figure 5b,c). The lowest surface tension achieved is about 41 dyn/cm, a decrease of 31 dyn compared with pure water. In both cases, no critical micelle phenomenon was observed within the measured concentration range.

A surfactant's surface activity is characterized by its efficiency and effectiveness. The efficiency is defined by the negative logarithm of the bulk concentration (mol/L) necessary to reduce the surface tension by 20 dyn/cm, designated as pC 20. The pC 20 of PNVDA-*co*-PNVH (1:1) and PNVDA-*co*-PNVH (1:3.7) are estimated to be 4.9 and 5.2 respectively, based on an average molecular weight of 50 000 for the polymer surfactants, estimated from the molecular weight of PVAm and the surfactant compositions. Effectiveness is defined by the extent of surface tension reduction attained at the critical micelle concentration, π_{CMC}. No CMC was observed for either PNVDA-*co*-PNVH (1:1) or PNVDA-*co*-PNVH (1:3.7). However, the maximum surface tension reduction of 31 dyn/cm was approximately

Table 1. Theoretical and Measured Compositions for PNVDA-*co*-PNVH[a]

Dextran/Hexanoyl ratio	molar feed ratio	1:1	1:2	1:4
	measured molar ratio	1:1.06	1:3.7	1:5.05
*Theoretical maximum composition (mol %)[b]	dextran	33.3	33.3	0.2
	hexanoyl groups	50.0	66.7	0.8
	amino groups	16.7	0.0	0.0
Measured composition (mol %)	dextran	21.6	18.9	15.1
	hexanoyl groups	22.9	69.1	76.2
	amino groups	55.5	12.0	8.7
Measured composition (wt %)	dextran	84.5	72.0	65.7
	hexanoyl groups	8.9	26.6	33.2
	amino groups	6.6	1.4	1.1
Estimated number of groups per surfactant molecule[c]	dextran	30	26	21
	hexanoyl groups	32	97	107
	amino groups	78	17	12

[a] Measured compositions of PNVDA-*co*-PNVH determined by ^{1}H NMR. [b] Calculated from hypothetical syndiotactic PVAm conformation. [c] Calculated based on the measured molar composition and 140 amino groups per PVAm molecule, estimated from molecular weight data of PNVF.

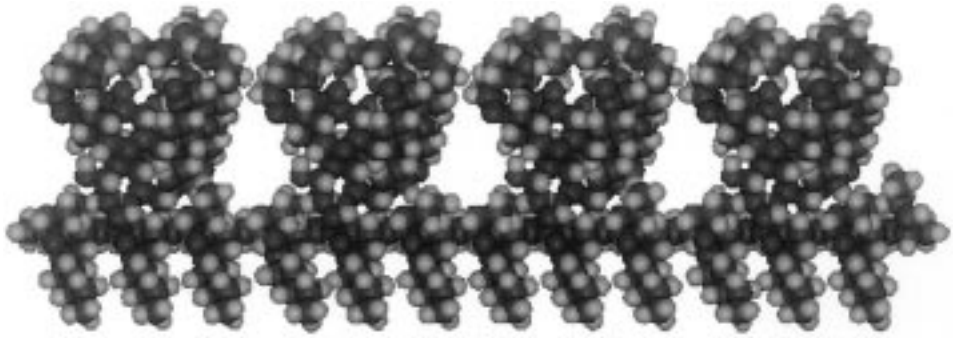


Figure 4. Molecular model of oligosaccharide surfactant polymer with a 1:5 molar ratio dextran to hexanoyl. Here 4 dextran molecules (upper) and 20 hexanoyl groups (lower) bonded to a PVAm backbone (middle) are depicted in the model

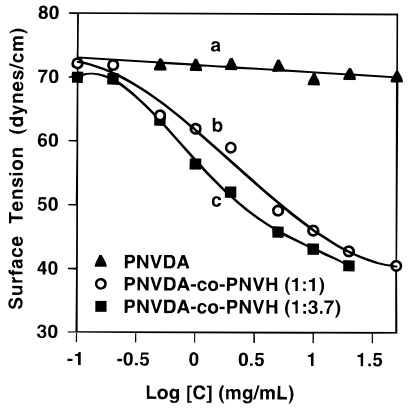


Figure 5. Plots of surface tension vs logarithm of the concentration of aqueous surfactant solutions. (a) PNVDA; (b and c) PNVDA-*co*-PNVH surfactant polymers.

the same for both surfactants.

The results indicated no significant difference between the surface active properties of PNVDA-*co*-PNVH (1:1) and PNVDA-*co*-PNVH (1:3.7). Both surfactants gave similar curves for surface tension vs concentration. Since PNVDA-*co*-PNVH (1:1) and PNVDA-*co*-PNVH (1:3.7) exhibit similar effects on water surface tension reduction, we may assume that residual free amine groups do not contribute significantly to the surface active properties and that packings of hexanoyl groups at the air/water interface, which is responsible for reducing the surface tension, are similar in both cases. The results suggests the surfactants have considerable conformational freedom in order to facilitate comparable hexanoyl packing at the air/water interface. Thus, to achieve further reductions in surface tension suggests increasing the length of the alkanoyl ligand in the molecular design, rather than further increasing the density of hexanoyl ligand groups. This is a useful finding, because it should also permit increased oligosaccharide-to-alkanoyl ratios in the polymer surfactants, an important consideration for maximizing oligosaccharide density while maintaining surface activity.

Solid Surface/Water Interface. The observed surface active properties of the surfactants at an air/water interface should lead to similar properties at a solid/water interface. To examine this hypothesis, we carried out preliminary studies on the adsorption of PNVDA-*co*-PNVH (1:1) on a hydrophobic solid surface under aqueous conditions. The solid surface activity was confirmed directly by AFM imaging of PNVDA-*co*-PNVH (1:1) molecules adsorbed on HOPG from dilute aqueous surfactant solutions. HOPG cleaves across a single atomic plane and is therefore extremely smooth, except for occasional step edges. The ultra-smooth topography of HOPG substrate in water is shown as an insert in Figure 6. This allowed topographical features of adsorbed surfactant molecules to be easily distinguished from the underlying HOPG surface. The geometry of the surfactant polymers inhibits micelle formation, rendering adsorption onto the solid surface as the lowest energy state for the surfactant. This should give rise to a maximally coated surface under at very dilute bulk surfactant concentrations. Accordingly, assembly of the surfactant adsorbed on HOPG was observed in AFM images. An example demonstrating the solid surface activity of PNVDA-*co*-PNVH (1:1) is shown in Figure 6. The adsorption of PNVDA-*co*-PNVH (1:1) on HOPG after 4 h was visualized as molecular aggregates by AFM. The surface coverage increases over a period of about 16 h adsorption time until the surface appears to be fully covered by surfac-

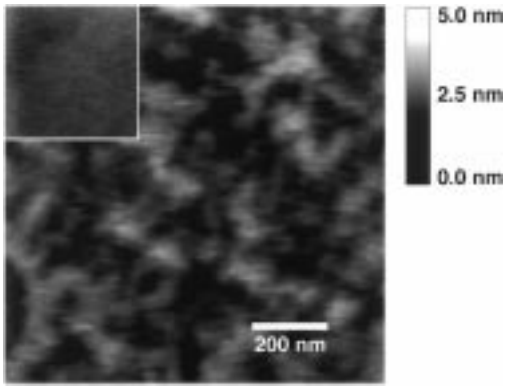


Figure 6. Tapping mode AFM images of PNVDA-*co*-PNVH (1:1) surfactant polymer adsorbed on HOPG from aqueous surfactant solution (0.5 mg/mL). The image was obtained after 4 h adsorption time. The AFM tapping mode image of HOPG before surfactant adsorption is shown as an insert.

tant molecules. Multiple hydrophobic interactions between the hexanoyl groups of the surfactant and the HOPG surface provides the driving force for the attachment of the surfactant polymer. The hydrophilic dextrans orient away from the surface and interact with the aqueous phase to maximize hydration. The adsorbed surfactants create a high surface density of exposed oligosaccharide—a result that provides preliminary evidence toward a surface modification that mimics a glycocalyx.

The promising results obtained concerning the solid surface activity of PNVDA-*co*-PNVH (1:1) surfactant are stimulating further experiments that explore structure–property relations of these novel surfactant polymers on hydrophobic solid surfaces. Our preliminary results, studied by AFM, are suggesting interesting surface induced molecular ordering and assembly of the surfactants. Details will be described in a separate report that focuses on the solid surface activity of these surfactants.[31] In addition, the PNVDA-*co*-PNVH surfactants are being examined as potential surface-modifying agents for polymers used as implantable biomaterials.[32] In this regard, we have obtained promising results on the surface modification of low-density polyethylene and for the inhibition of nonspecific protein adsorption on PNVDA-*co*-PNVH surfactant modified surfaces.[32]

Conclusions. A novel series of nonionic oligosaccharide surfactant polymers with well-defined structure and adjustable hydrophilic/hydrophobic balances was synthesized by incorporating dextran oligosaccharide molecules and hydrophobic hexanoyl groups simultaneously into poly(vinylamine). The surfactants demonstrate surface activity at an air/water interface, as determined by water surface tension measurements, and at a solid surface/water interface, as visualized by AFM imaging of adsorbed surfactants on HOPG. The synthetic approach described in this report offers numerous possibilities and opportunities for manipulating the surfactant polymer design, ligand composition, and hydrophilic/hydrophobic ratios, to accommodate a wide range of potential applications that require surface modification with well-defined molecular structures.

Acknowledgment. We gratefully acknowledge the financial support of this work provided by NIH Grant HL-40047 and the Whitaker Foundation and use of facilities at the Center for Cardiovascular Biomaterials.

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MA9707401