Synthesis of Phosphorylcholine-Based Hydrophobically Modified Polybetaines

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Introduction. Polyzwitterions are charged polymers carrying a positive and a negative charge on the same monomer unit or on two different units. In solution, they may be anionic, cationic, or without net electric charge, depending on the solution pH. The first synthetic polyzwitterions, polycarboxybetaines based on poly(4-vinylpyridine), were prepared by Ladenheim and Morawetz.[1] Since then, a number of zwitterionic groups have been linked to polymer chains, including sulfobetaines[2−8] and phosphorylcholines.[9−14] Hydrophobically modified (HM) polyzwitterions have been described as well. They include adamantane-substituted polysulfobetaines,[15] copolymers of styrene/*N*,*N*-(dimethylmaleimido)propylammonium propanesulfonate,[16] acrylamido-based copolymers of [2-(methacryloyloxy)ethyl]trimethylammonium chloride, sodium 2-acrylamido-2-methylpropanesulfonate and *N*,*N*-dihexylacrylamide,[17] conjugates of phospholipids to poly(isopropylacylamide),[18] and acrylamide-based hydrophobically modified polymers containing low levels of either sulfobetaines or carboxybetaines.[19]

In this communication, we report the preparation of water-soluble HM phosphorylcholine (PC) polymers which belong to the general class of "phosphocholine polymers"[20] originally designed by Nakaya.[21] Many biomedical devices have been developed based on PC polymers, including high-performance surgical stents and contact lenses,[22] as these polymers impart remarkable biocompatibility and hemocompatibility to surfaces onto which they are coated.[9,10,23] The nonthrombogenic nature of PC-coated surfaces has been attributed to their ability to resist the adsorption of proteins,[24] possibly by triggering the formation of structured water layers.[25−27]

Phosphorylcholine polymers are traditionally obtained by polymerization of PC-based monomers, either alone or in the presence of comonomers.[20,28,29] For the preparation of hydrophobically modified PC polymers reported here, we took a different approach which involved the attachment of phosphorylcholine groups to a preformed hydrophobically modified polymer. This route avoids the tedious preparation and purification of PC monomers. It should also favor random distribution of the hydrophobic groups and of the water-soluble PC groups along the chain, as the precursor copolymers can be obtained by solution copolymerization of *N*-alkyl acrylamides.[30] After exploring several approaches, we conceived a simple, efficient, and versatile synthesis of a family of HM PC polymers, where phosphatidylglyceraldehyde is coupled via reductive amination to polymer-linked primary amines. The method was applied to the preparation of random copolymers of *N*-isopropylacrylamide (NIPAM) and *N*-(phosphorylcholine)-*N*′-(ethylenedioxybis(ethyl))acrylamide (NPCAM) and the hydrophobic monomers *N*-(*n*-octadecyl)acrylamide, *N*-(1*H*,1*H*-perfluoro-*n*-octyl)acrylamide, or *N*-[(1-pyrenyl)-4-butyl]-*N*-(*n*-octadecyl)acrylamide. The conformation of the polymers in water, methanol, and chloroform is described on the basis of NMR experiments.

Table 1. Composition (in mol %) of the Polymers Prepared

polymer	NIPAM[a]	PC[b] (residual NH_2)[c]	$C_{18}H_{37}$[a]	Py[d]	$C_8F_{15}H_2$[e]
PNIPAM-PC	49.6	50.4 (0)			
PNIPAM-PC-C_{18}(5)	52.6	42.6 (1)	4.8		
PNIPAM-PC-C_{18}(10)	42.5	48.8 (2)	8.7		
PNIPAM-PC-C_{18}(15)	44.8	41.4 (0)	13.8		
PNIPAM-PC-C_{18}/Py	45.1	52.0 (6)	2.9	2.9	
PNIPAM-PC-F	47.6	47.6 (3)			4.8

[a] From ^{1}H NMR measurements. [b] From phosphate colorimetric test. [c] From $-NH_2$ colorimetric test. [d] From UV absorbance measurement. [e] From quantitative ^{19}F NMR measurement using CF_3COOH as internal standard and known amounts of 1*H*,1*H*-perfluoro-*n*-octylamine. The integrations of the CF_3 signals of the amine (84.2 ppm) and of the perfluoro substituent of the polymer (78.67 ppm) were compared.

Experimental Section. NMR spectra were recorded on a Bruker ARX-400 400 MHz spectrometer. UV spectra were measured on a Hewlett-Packard 8452A photodiode array spectrometer and IR spectra (KBr pellets) on a Bomem IR spectrometer. GPC measurements were carried out on a GPC system equipped with a Waters binary HPLC pump 1525 and a Waters 2410 refractive index detector using HR3, HR4, and HR6 styrogel columns (Waters) eluted with tetrahydrofuran (1 mL min^{-1}) at 35 °C. The molecular weights were calibrated against polystyrene standards. All chemicals were purchased from Sigma-Aldrich Chemicals. The concentrations of free amine groups[31] and phosphorylcholine groups[32] were determined by colorimetric tests. Copolymers with different compositions (see Table 1) were prepared by the procedure exemplified here for the preparation of PNIPAM-PC-C_{18} (Figure 1).

Copolymerization of N-Isopropylacrylamide (NIPAM), N-(n-Octadecyl)acrylamide, and N-BOC-N′-[(ethylenedioxybis(ethyl))acrylamide] (PNIPAM-NH-BOC-C_{18}). A solution of NIPAM, *N*-(*n*-octadecyl)acrylamide,[33] and *N*-BOC-*N*′-[(ethylenedioxybis(ethyl))acrylamide][34] (total monomer concentration: 0.3 mol L^{-1}; initiator concentration: 8.0×10^{-4} mol L^{-1}) in dioxane was degassed for 30 min by vigorously bubbling nitrogen through the solution. The mixture was heated to 60 °C. Azobis(isobutyronitrile) (AIBN) was added at once. The polymerization mixture was stirred at 60 °C for 16 h. It was cooled to room temperature. The solvent was removed by evaporation. The polymer, PNIPAM-NH-BOC-C_{18}, was purified by two precipitations from THF into hexane and dried in vacuo (yield: 65−88%); M_n 20 400, M_w 35 500, M_w/M_n 1.71.

Preparation of PNIPAM-NH_2-C_{18}. Trifluoroacetic acid (20 equiv/BOC equiv) was added to a solution of the copolymer in dichloromethane cooled in an ice/water bath. The reaction mixture was stirred for 24 h. As the

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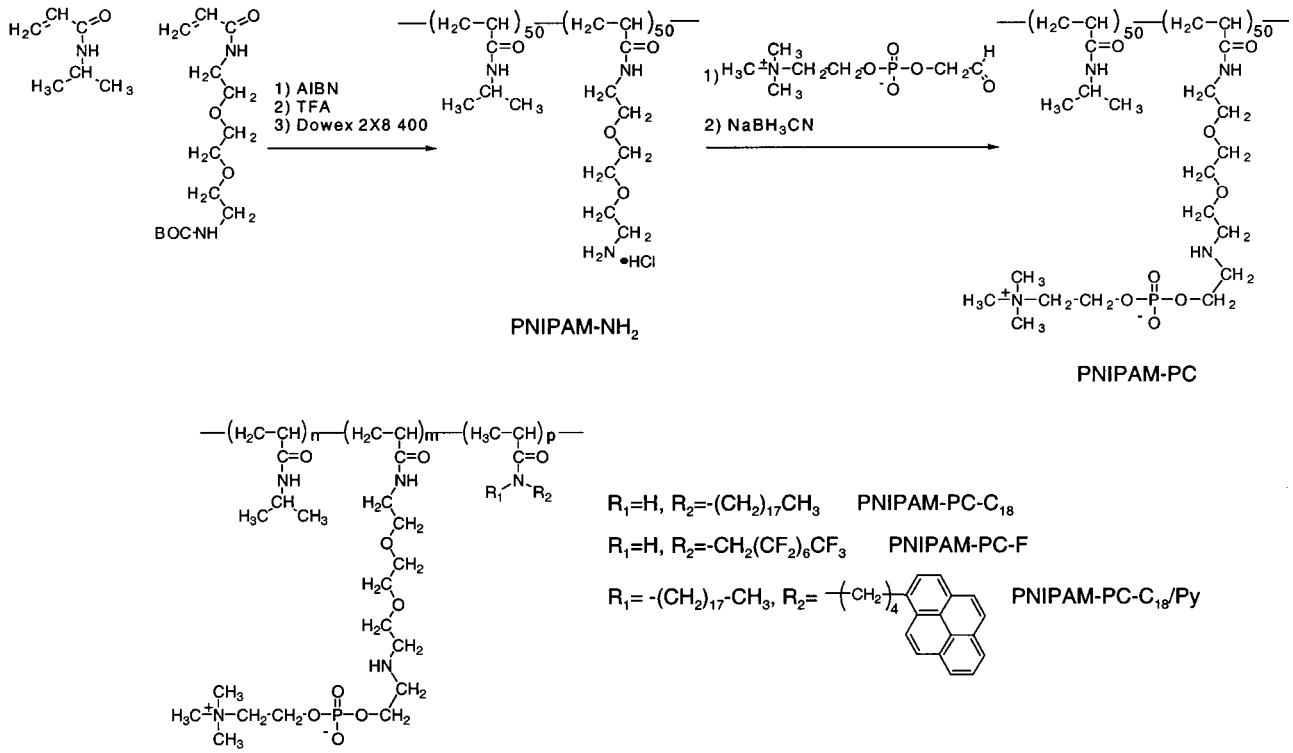

Figure 1. Synthetic sequence for the preparation of phosphorylcholine-based polybetaines exemplified for the preparation of a copolymer of *N*-(isopropylacrylamide) and *N*-(phosphorylcholine)-*N′*-[ethylenedioxybis(ethyl)]acrylamide (PNIPAM-PC) and structure of the polymers prepared. BOC = *N*-(*tert*-butoxycarbonyl); AIBN = azobis(isobutyronitrile); TFA = trifluoroacetic acid.

reaction proceeded, several aliquots of methanol were added to keep the reaction mixture homogeneous. The solvent was evaporated. The polymer PNIPAM-NH_2-C_{18}·CF_3COOH was purified by two precipitations from THF into diethyl ether/hexane (1/1 v/v) and dried in vacuo (yield: ~100%). To convert the ammonium trifluoroacetate groups to ammonium chlorides, a Dowex 2X8-400 resin (4.0 g) was added to a solution of PNIPAM-NH_2-C_{18}·CF_3COOH (0.5−2.0 g) in water/ethanol (2/1 v/v). The mixture was stirred at room temperature for 1 h. The resin was separated by filtration, and the filtrate was concentrated in vacuo to yield PNIPAM-NH_2-C_{18}·HCl.

Preparation of PNIPAM-PC-C_{18}. A solution of phosphatidylglyceraldehyde[35] (2.5 equiv/NH_2 equiv) in methanol was added to a solution of PNIPAM-NH_2-C_{18}·HCl in methanol. The mixture was stirred at room temperature for 3.5 h. A solution of sodium cyanoborohydride (1 equiv/phosphatidyl glyceraldehyde) in methanol was added dropwise to the reaction mixture cooled to 0 °C. At the end of the addition the reaction mixture was warmed to room temperature and stirred for 19 h. The polymer was purified by dialysis against distilled water for 3 days and was isolated by lyophilization. The same procedure, but starting with *N*-[(1-pyrenyl)-4-butyl]-*N*-*n*-(octadecyl)acrylamide[33] or *N*-(1*H*,1*H*-perfluoro-*n*-octyl)acrylamide,[36] led to PNIPAM-PC-C_{18}/Py and PNIPAM-PC-F, respectively. All polymers were characterized by NMR, IR, UV spectroscopy, and GPC.

Results and Discussion. The synthetic strategy selected, which involves a one-step linkage of a phosphorylcholine moiety to a polymer-bound functional group, is presented (Figure 1) for the preparation of the copolymer PNIPAM-PC. Free radical copolymerization of NIPAM and *N*-BOC-*N′*-[(ethylenedioxybis(ethyl))-acrylamide] followed by deprotection of the terminal amine groups yielded a copolymer of *N*-isopropylacrylamide (NIPAM) and amino-*N′*-[(ethylenedioxybis-(ethyl))acrylamide]. The composition of the BOC-protected copolymer was determined by ^{1}H NMR spectroscopy, using the singlet at δ 1.14 ppm, assigned to the resonance of the *tert*-butyl protons of the BOC units,

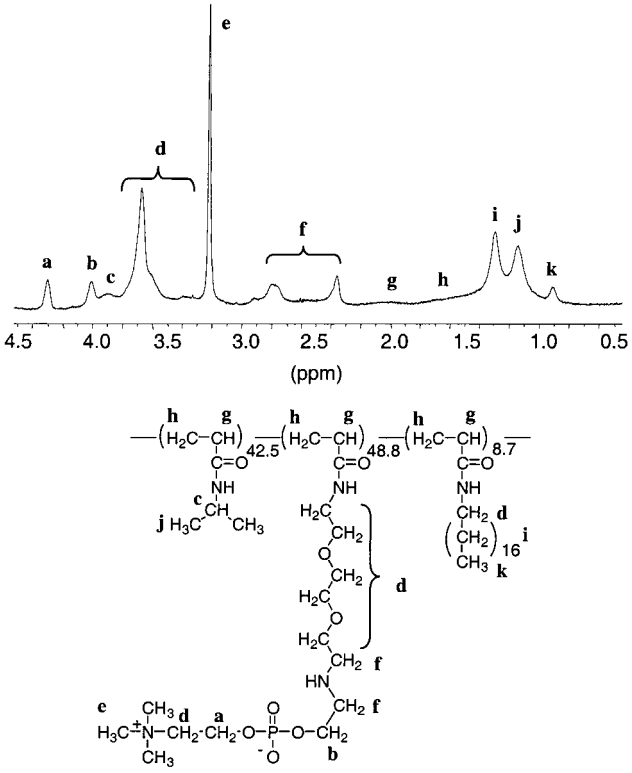

Figure 2. ^{1}H NMR spectrum of a solution of PNIPAM-PC−C_{18}(10) in D_2O indicating the structural assignments of the signals; polymer concentration = 5.0 g L^{-1}.

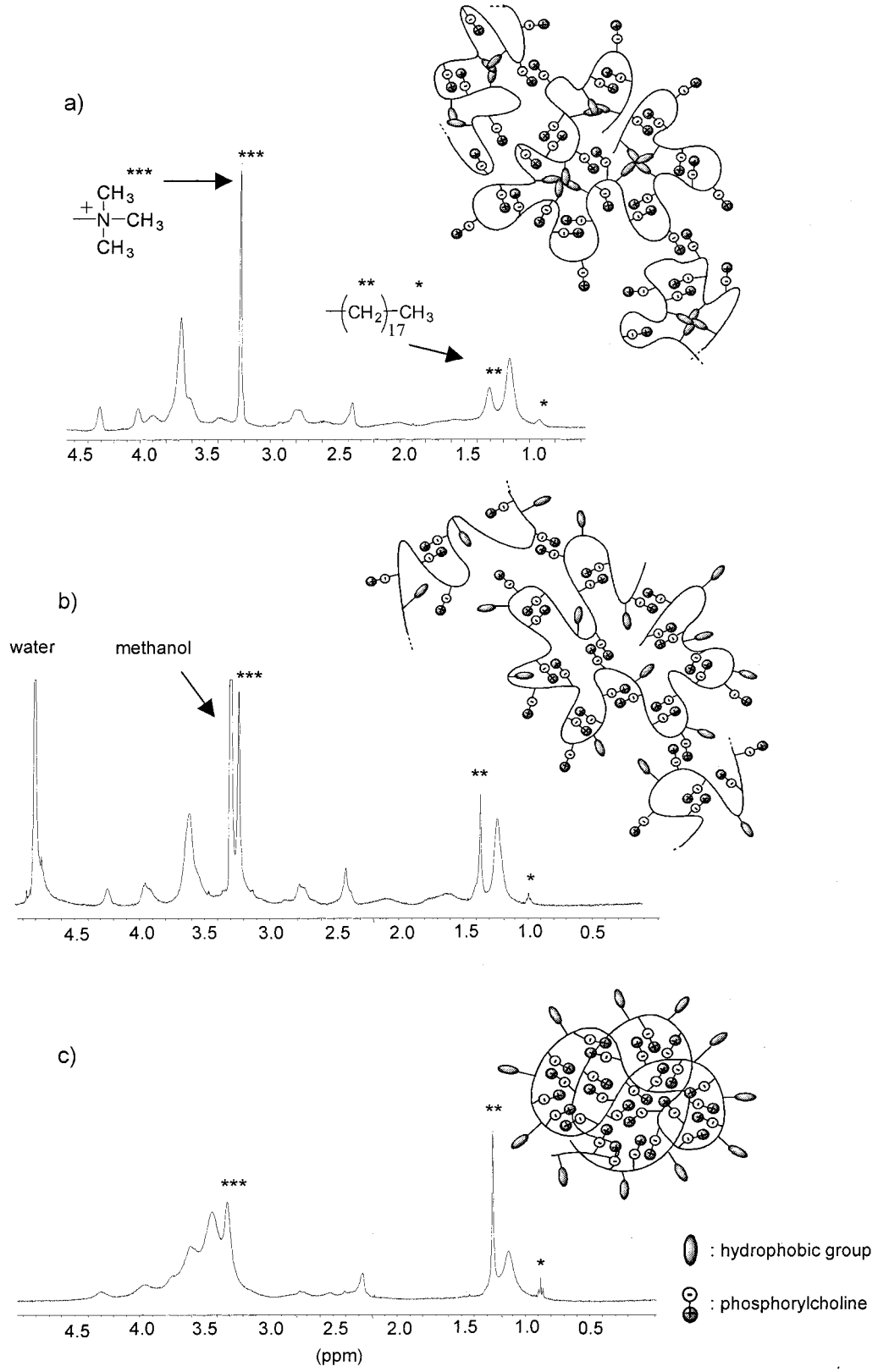

Figure 3. ^{1}H NMR spectra of PNIPAM-PC-C_{18}(5) in deuterated water (a), methanol (b), and chloroform (c). Idealized conformations of the polymer in each solvent highlight the formation of hydrophobic microdomains in water (a) and of PC clusters in chloroform (c); polymer concentration = 5.0 g L^{-1}.

and the broad singlet at δ 3.90 ppm, assigned to the resonance of the methine NIPAM protons, to calculate the molar ratio of *N*-BOC-*N*′-[(ethylenedioxybis(ethyl))-acrylamide] and NIPAM units. A primary-amine specific colorimetric assay[31] was performed on PNIPAM-NH_2 to ascertain the composition of the deprotected copolymer.

Coupling of phosphorylcholine residues to PNIPAM-PC was achieved via reductive amination of phosphatidylglyceraldehyde (Figure 1). The success of the linkage was confirmed by the presence of a singlet at 3.22 ppm in the ^{1}H NMR spectrum of PNIPAM-PC, attributed to the resonance of the trimethylammonium protons. A

signal at δ 1.75 ppm due to the resonance of the PC phosphorus was observed in the ^{31}P NMR of a solution of PNIPAM-PC. Further evidence for the successful incorporation of PC moieties on the polymer chain was gathered from the IR spectrum of PNIPAM-PC which displays bands at ν 1231 cm^{-1} (ν_{as} PO_2^-) and at ν 970 cm^{-1} (ν_{as} N−CH_3).[37]

The HM-PNIPAM-PC samples were obtained via an analogous sequence, starting with terpolymers of NIPAM, amino-*N*′-[(ethylenedioxybis(ethyl))acrylamide], and *N*-(*n*-octadecyl)acrylamide, *N*-(1*H*,1*H*-perfluoro-*n*-octyl)-acrylamide, or *N*-[(1-pyrenyl)-4-butyl]-*N*-(*n*-octadecyl)-acrylamide, yielding PNIPAM-PC-C_{18}, PNIPAM-PC-F, and PNIPAM-PC-C_{18}/Py, respectively (Figure 1). The composition of the polymers was determined by ^{1}H and ^{19}F NMR spectroscopy, UV absorbance (PNIPAM-C_{18}/Py), and colorimetric tests. A representative ^{1}H NMR spectrum of PNIPAM-PC-C_{18}(10) in water is presented in Figure 2, together with the peak assignments. In all cases, the polymer composition was identical, within experimental error, to the monomer feed ratio and conversion of the aminated polymer to the PC derivative was nearly quantitative.

^{1}H NMR spectroscopy was used to gain information on the conformation adopted by each polymer in D_2O (a), CD_3OD (b), and $CDCl_3$ (c) (Figure 3). In the spectrum of the polymers in D_2O (Figure 3a), the signal due to the resonance of the PC trimethylammonium protons (δ 3.22 ppm) is sharp, whereas the signals attributed to the octadecyl protons (δ 0.91 ppm, methyl) and (δ 1.30 ppm, methylene) appear as unresolved broad signals. Line broadening of ^{1}H NMR signals indicates that the motion of the corresponding protons is restricted, providing evidence for the occurrence of hydrophobic microdomains via association of the octadecyl chains. Hydrophobic interactions all but disappear when the polymer is dissolved in methanol: the spectrum of PNIPAM-PC-C_{18} in CD_3OD (Figure 3b) presents sharp signals, not only for the trimethylammonium protons but also for the octadecyl protons. However, in the spectrum of PNIPAM-PC-C_{18} in $CDCl_3$ (Figure 3c), while the signals attributed to the octadecyl protons remain sharp, the signal due to the PC trimethylammonium protons experiences significant broadening together with a downfield shift. Thus, in $CDCl_3$, the motion of the $(NMe_3)^+$ group is highly restricted. This indicates that the PC moieties are confined in a restricted environment, while the alkyl chains rotate freely and do not associate. These observations were corroborated by T_1 measurements made with solutions of PNIPAM-PC-C_{18} in the three solvents. The T_1 values for the trimethylammonium protons decreased from 700 ms in D_2O to 420 ms in $CDCl_3$, while the T_1 values for the octadecyl methyl protons increased from 880 ms in D_2O to 1630 ms in $CDCl_3$. Similar trends were reported previously for other PC polymers.[29]

Studies of the change in conformation experienced by HM PC polymers from inter/intrapolymeric micellar structures with a hydrophobic core in water to inverse polymeric micelles with cores made up of agglomerated PC units in a nonpolar solvent are in progress using other techniques, to gain further insight into the solution properties of this new class of biocompatible and hemocompatible polymers.

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(30) In the case of the copolymers prepared here, it has been demonstrated that the monomers employed have similar reactive ratios and that further polymer modifications can be performed at random along the polymer chain (Principi,

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