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Synthesis of polyelectrolytes bearing phosphorylcholine moieties

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Abstract

We report on the modification of the polyelectrolytes poly(L-glutamic acid) (PGA), poly(acrylic acid) (PAA) and poly(L-lysine) (PLL) with side-groups bearing phosphorylcholine (PC) groups. Two different side-chains were synthesized from monoamino tri or tetraethylene glycol, both with PC group linked to the OH end: the tert-butyloxycarbonyl protected compounds BOCNH(EO)_nPC (n = 2,3) and the succinylated compound SucNH(EO)₃PC. BOCNH(EO)_nPC was deprotected and the resulting amine was condensed with PGA or PAA and SucNH(EO)₃PC was directly coupled with PLL. Degrees of substitution as high as 80 % were obtained with the described method. These polyelectrolytes are potential candidates to fabricate multilayers with protein repellent properties.

Introduction

The phosphorylcholine group (PC) is a major component of biological membranes and well known for its excellent resistance to protein adsorption and its hemocompatibility. First interest in the chemical base of hemocompatibility arose when Zwaal et al.^[1] were able to show that the phospholipids that are predominantly found in the outer leaflet of the

erythrocyte membrane are non-thrombogenic. Many results show that mainly the electrically neutral, zwitterionic phosphorylcholine head group is responsible for these non-thrombogenic properties and the resistance to protein adsorption.^[1-3] This observation has triggered much work to impart protein repellent properties to biomaterials by mimicking the outer layer of erythrocytes through the incorporation of PC.^[4-6] Different strategies were followed depending upon the nature of the substrate. Self-assembled monolayers were used on gold^[7] and silicon substrates.^[8-10] Direct grafting of PC containing polymers on various substrates either by conventional radical polymerization^[11] or by surface-initiated ATRP^[12, 13] were carried out. Carbon-chain polymers bearing PC side chains, the best known being the polymer based on 2-methacryloyloxyethyl phosphorylcholine (MPC) monomer, were synthesized to create nonfouling coatings for biomaterials.^[5, 14]

Most of these surface modifications are substrate or geometry specific. In contrast, polyelectrolyte multilayers^[15] can be fabricated on many different surfaces almost independently of the chemical nature, the size and the shape of the underlying substrate. Polyelectrolyte multilayers are obtained by bringing a substrate alternately in contact with a polyanion and a polycation solution and constitute a very versatile tool to coat and render surfaces biologically active^[16, 17] or tunable.^[18] This can be achieved by incorporation through simple adsorption of active compounds such as anti-inflammatory drugs^[19], active peptides^[20] or proteins,^[21] DNA^[22] or through inclusion of active vesicles.^[23] We have recently reviewed the fabrication of biofunctional surfaces using layer-by-layer assembly.^[24] Schlenoff et al.^[25] already showed that in polyelectrolyte multilayers, when the outer layers bear sulfobetain zwitterionic groups, cellular adhesion is greatly reduced. Polyelectrolytes bearing zwitterionic groups and in particular PC groups thus could constitute a very interesting class of macromolecules to create nonfouling surfaces employing layer-by-layer deposition. This technique would, in addition, allow conferring additional properties to the coatings and thus

render them multifunctional. A thorough investigation of the development of such films requires however to have access to a whole library of polyelectrolytes.

PC-bearing polymers are usually obtained by polymerization of PC-based monomers.^[26, 27]

Such an approach can, however, hardly be generalized to a large class of polyelectrolytes, each case necessitating a different reaction scheme. Following the pioneering work of Winnik^[28, 29] et al., we propose a route based on the modification of existing polymers with side groups bearing PC moieties. Winnik et al. reported on the modification of amino polyelectrolytes like chitosan by reductive amination of PC-CHO. This approach, even if it proceeds in high yield, can only be applied to polyamines. For other polymers, the amine groups have to be introduced either by copolymerization with an amino-monomer or by a modification of the respective polyelectrolyte. In this article we propose an alternative route that is based on the synthesis of the heterobifunctional oligo(ethylene oxide) $\text{H}_2\text{N}(\text{EO})_n\text{PC}$, (EO : ethylene oxide). These compounds can be directly coupled to the carboxylic groups of a polyelectrolyte backbone via a carbodiimide coupling or coupled to the amine groups of polyamines via an additional transformation into a succinylated derivative $\text{SucHN}(\text{EO})_n\text{PC}$. The synthesized polyelectrolytes are represented on scheme 1. Our synthetic strategy is thus applicable to a very broad range of polyelectrolytes and can be generalized to other spacers than $(\text{EO})_n$ oligomers. However, we chose the oligoethyleneoxide spacers because of their known hydrophilicity and biocompatibility.^[30] Moreover, it was also shown that the introduction of two EO units between the methacrylate and the PC groups in MPC butylmethacrylate copolymers increased only slightly the fibrinogen adsorption, compared to the parent MPC-BMA copolymer without spacer and that platelet adhesion is effectively prevented for about 30% of PC units in both cases.^[31, 32]

Experimental Part

Materials

Organic solvents were purchased in analytical grade quality from Acros, Aldrich, Fluka, Merck, Riedel-de-Haën and VWR. They were used as received for chromatography. Ethyl acetate, chloroform and acetonitrile were distilled under inert atmosphere with the usual drying agents prior to their use as reaction solvents. Millipore water was used for all the experiments.

Triethylene glycol mono-amine (Molecular Biosciences, 99.5%) and tetraethylene glycol mono-amine (Molecular Biosciences, 99%) were transformed into the BOC derivative by known procedures.^[33, 34] Choline tosylate (ABCR, > 97%), Di-*tert*-butyl dicarbonate (Boc₂O, Fluka, ≥ 98%), N'-(3-dimethylaminopropyl)-N-ethyl carbodiimide hydrochloride (EDCI, Fluka, ≥ 98%), POCl₃ (Acros, 99%), pyridine (Fluka, absolute over molar sieve), succinic anhydride (Acros, ≥ 99%), trimethylamine (Fluka, > 99%), 1-hydroxybenzotriazole hydrate (HOBT, Fluka, ≥ 99%), poly-L-glutamic acid sodium salt (PGA, Sigma, MW 50-100,000), poly-L-lysine hydro bromide (PLL, Sigma, MW 25,500), poly(acrylic acid) (PAA, Aldrich, 35 wt.% in water, Mw 100,000), 1,2-dibutyroyl-*sn*-glycero-3-phosphocholine (04:0 PC, Avanti Polar Lipids) were used as received.

NMR measurements

NMR spectra were recorded on a Bruker Avance 400 operating at 400 MHz for ¹H and 100 MHz for ¹³C. ¹H-¹H and ¹H-¹³C shift correlated spectra were recorded using the standard pulse sequences provided by Bruker. The degree of substitution (DS) was determined from the ratios of integrals of the ¹H-signals of the protons belonging to the spacers and PC groups to the integrals of the signals of the repeat unit. In the case of PGA(EO)₃PC for example, the calculation was based on the signals at 4.05 (d), 3.78-3.67 (e, f, g, b), 3.63 (h), 3.40 (i) or 3.24

(a) ppm (the letters correspond to the labelling of figure 3B). The values calculated from each of these signals were found equal within an error of 3%.

FTIR measurements

The FTIR spectra were recorded on a Bruker Vertex 70 spectrometer. The ATR-mode was used for the small molecules. For the determination of DS, the transmission spectra of the polymers were measured in D₂O buffered with Trizma Base (0.12 M, pH adjusted to 9.4 with DCl) using Specac™ cells purchased from Eurolabo, with ZnSe windows and an optical path length of 0.1 mm. The bands at 1564 and 1405 cm⁻¹ (ν_{as} and ν_s COO⁻) were used to determine the conc. [COO⁻] from a calibration curve established with PGA. Both bands gave the same results. The bands at 1087 and 971 cm⁻¹ (ν_s PO₂⁻ and ν_{as} (CH₃)₃N⁺) were used to determine [PC], the phosphorylcholine conc. from a calibration curve established with 04:0 PC. The degree of substitution DS was calculated according to : $DS = [PC]/([PC]+[COO^-])$.

SEC measurements

The SEC experiments have been performed with an Agilent 1100 pumping unit and three sequential columns Shodex OH (1803 HQ, 1804 HQ, 1806 HQ), a differential refractometer Optilab rEX (from Wyatt Techn.) and a Dawn Heleos multiangle light scattering detector (from Wyatt Techn.). The solvent was a solution of NaNO₃ (0.1 M) and NaN₃ (5 mM) in tridistilled water. Volumes of 100 μ L of solution at 5 mg/mL were injected and eluted at 0.5 mL/min.

Synthesis of the side-chains

[2-(2-N-tert-Butoxycarbonylamino-ethoxy)-ethoxy]-ethylphosphorylcholine

(BOCNH(EO)₃PC): To a solution of POCl₃ (3.35 g, 21.8 mmol, 1.3 equiv.) in CHCl₃ (20 mL), a solution of BOCNH(EO)₃H (4.16 g, 16.7 mmol, 1 equiv.) and NEt₃ (12.6 mL, 90.4 mmol, 5.4 equiv.) in CHCl₃ (100 mL) was added dropwise at 0 °C and the mixture was stirred for 2h at 25°C. The mixture was cooled at 0°C and choline tosylate (10.39 g, 37.7 mmol, 2.26

equiv.) and pyridine (15.4 mL) were added. The reaction mixture was stirred for an additional 12 h at room temperature and water (6.2 mL) was added. After 45 min the solvents were evaporated under reduced pressure and a red solid residue was obtained. Chromatography (SiO₂, 25-35 % water-acetone gradient elution) afforded BOCNH(EO)₃PC as a white deliquescent solid (3.05 g, 50 % yield): ¹H NMR (D₂O, 400 MHz) : δ = 4.34 (br m, 2 H, POCH₂CH₂N), 4.05 (br m, 2 H, POCH₂CH₂O), 3.76-3.68 (m, 8 H, CH₂OCH₂CH₂O, CH₂N(CH₃)₃), 3.61 (t, 2 H, *J* = 5.36 Hz, OCH₂CH₂N), 3.28 (t, 2H, *J* = 5.36 Hz, CH₂N), 3.24 (s, 9H, N(CH₃)₃), 1.44 (s, 9 H, C(CH₃)₃); ¹³C-NMR (D₂O, 100 MHz) : δ = 158.9 (CO), 81.7 (C(CH₃)₃), 70.8 (d, *J* = 7.7 Hz, OCH₂CH₂OP), 70.4, 70.1 (OCH₂CH₂) , 66.7 (m, CH₂N(CH₃)₃) , 65.5 (d, *J* = 5.8 Hz, POCH₂CH₂O) , 60.1 (d, *J* = 4.8 Hz, POCH₂CH₂N) , 54.6 (t, *J* = 3.7 Hz, N(CH₃)₃) , 40.3 (CH₂N), 28.4 (C(CH₃)₃); IR (ATR) ν_{max} : 3355 (sh) and 3223 (νNH), 2975 (ν_{as} CH₃), 2937 (ν_{as} CH₂), 2878 (ν_s CH₂), 1696 (amide I), 1525 (amide II), 1484 (δ_{as} (CH₃)₃N⁺), 1458; 1391 and 1365 (δ CH₃), 1247, 1172, 1089 (ν_s PO₂⁻), 1058, 968 (sh) and 954 (ν_{as} CH₃)₃N⁺), 920, 865, 777 cm⁻¹. HRMS (ESI⁺) : *m/z* 415.2216 (MH⁺, C₁₆H₃₅N₂O₈P requires 415.2203), 437.2036 (MNa⁺, C₁₆H₃₅N₂O₈P requires 437.2029).

(BOCNH(EO)₄PC) was synthesized according to the same protocol (see supp. Info for characterization)

2-[2-(2-N-succinylamino-ethoxy)-ethoxy]-ethylphosphorylcholine (SucNH(EO)₃PC) :

BOCNH(EO)₃PC (2.53 g, 6.10 mmol, 1 equiv.) was mixed with aq. HCl (2.5 M, 12 mL) and the mixture stirred at 25 °C during 90 min. The solution was evaporated under vacuum and dissolved in water (4.5 mL). The pH was adjusted to 9 by addition of 2 M aq. NaOH and a solution of succinic anhydride (1.16 g, 11.60 mmol, 1.9 equiv.) in DMSO (3 mL) was added at once and the pH readjusted to 9.5. The reaction mixture was stirred for 24 h at 25 °C, a second batch of succinic anhydride (2.25 g, 22.50 mmol, 3.7 equiv.) in DMSO (6 mL) was added and the pH was readjusted to 9.5. A third batch of succinic anhydride (1.03 g, 10.30

mmol, 1.7 equiv.) in DMSO (3 mL) was added after 24h. The reaction mixture was stirred for an additional 24h at 25 °C. It was precipitated in EtOH (400 mL). The precipitate was filtered off and the filtrate was evaporated under vacuum. The obtained solid was redissolved in MeOH (10 mL) and the minimal amount of water to solubilize it (ca 1 mL) and mixed with diethyl ether (100 mL). The organic phase was removed by decantation to yield a paste containing water and the crude product. Chromatography of this residue (SiO₂, 25-33 % water-acetone gradient eluent) afforded SucNH(EO)₃PC as a white solid (1.59 g, yield 63%) :

¹H NMR (D₂O, 400 MHz) : δ = 4.32 (br m, 2H, POCH₂CH₂N), 4.04 (br m, 2H, POCH₂CH₂O), 3.76-3.67 (m, 8H, CH₂OCH₂CH₂O, CH₂N(CH₃)₃), 3.64 (t, 2H, J = 5.49 Hz, OCH₂CH₂NHCO), 3.39 (t, 2H, J = 5.42 Hz, CH₂NHCO), 3.23 (s, 9H, N(CH₃)₃), 2.47 (m, 4H, COCH₂CH₂COO). ¹³C-NMR (D₂O, 100 MHz) : δ = 181.5 (CO), 176.6 (CO), 70.8 (d, J = 5.4 Hz, OCH₂CH₂OP), 70.4, 70.1, 69.5, (OCH₂CH₂O, OCH₂CH₂N), 66.7 (m, CH₂N(CH₃)₃), 65.6 (d, J = 5.4 Hz, POCH₂CH₂O), 60.1 (d, J = 4.9 Hz, POCH₂CH₂N), 54.7 (t, J = 3.7 Hz, N(CH₃)₃), 39.6 (CH₂N), 33.6 (CH₂CO), 33.0 (CH₂CO). IR (ATR) ν_{max} : 3385 (ν COOH), 3297 (ν NH), 3031, 2928 (ν_{as} CH₂), 2883 (ν_{as} CH₂), 2479; 2402, 1639 (amide I); 1573, 1470, 1398, 1356, 1236, 1133, 1088 (ν_{s} PO₂⁻), 1057, 954 (ν_{as} (CH₃)₃N⁺), 924, 877, 787, 748, 638 cm⁻¹.

HRMS (ESI+) : 421.1890 m/z (MLi⁺, requires 421.1922).

Coupling reactions.

The polymers were modified according to the procedure exemplified here by the synthesis of PGA(EO)₃PC 35 and PLLSuc(EO)₃PC 35.

PGA(EO)₃PC 35 : BOCNH(EO)₃H (220 mg, 0.53 mmol, 0.40 equiv.) was mixed with aq. HCl (6 mL, 2.5M) and stirred at 25 °C for 90 min. The mixture was dried under vacuum. The residue was mixed with PGA (200 mg, 1.32 mmol of glutamyl units) and water (1.5 mL).

HOBt (180 mg, 1.32 mmol, 1equiv.) was dissolved in water (1.5 mL) and the minimal amount of NEt₃ and added to the reaction mixture. The pH was adjusted with HCl and NEt₃ between 4.7 and 5.0 and EDCI (190 mg, 1.22 mmol, 0.93 equiv.) was added at 0°C. The reaction mixture was stirred for 24 h at 25 °C and dialyzed with a cellulose ester membrane (MWCO 5000) against 1M aq. NaCl, 0.5 M aq NaCl and finally Millipore water during 3 days. Lyophilization of the solution afforded PGA(EO)₃PC 35 as a white solid (250 mg , yield 81%). See Figure 3A, 3B and 4 for characteristic peaks in ¹H NMR and IR. Full spectroscopic data is given in suppl. info.

PLLSuc(EO)₃PC 35: PLL (61 mg, 0.29 mmol of lysine units) and sucNH(EO)₃PC (48 mg, 0.12 mmol, 0.41 equiv.) were dissolved in water (1.5 mL). HOBt (0.040 g, 0.30 mmol, 1.03 equiv.) dissolved in slightly basic water (0.7 mL) was added. The pH was adjusted between 4.7 and 5.0 by the addition of HCl and NaOH aq. solutions (1M). EDCI (43 mg, 0.28 mmol, 0.97 equiv.) was added to the reaction mixture at 0°C. The reaction mixture was stirred for 24 h at 25 °C. The pH was maintained with the HCl and NaOH solutions. The reaction mixture was dialyzed with a cellulose ester membrane (MWCO 5000) against 1M aq. NaCl, 0.5 M aq. NaCl and finally Millipore water for 3 days. One obtains 78 mg of a white powder after lyophilization (yield 74%). See Figure 3C, 3D for characteristic peaks in ¹H NMR. Full spectroscopic data is given in suppl. info.

Results and Discussion

The synthesized polymers are described on figure 1. The overall synthetic scheme (figure 2) consists in synthesizing first the side-groups bearing PC at one end and an amine or acid group at the other end, then coupling them to the polymer. The lateral substituents were synthesized (scheme 2) by the method introduced by Brockerhof,^[35, 36] which consists in the

sequential reaction of POCl_3 with two different alcohols. First POCl_3 is reacted with $\text{BOCNH(EO)}_n\text{H}$ in the presence of NEt_3 . Then, reaction in situ of the resulting dichlorophosphate with choline tosylate in the presence of pyridine, followed by hydrolysis affords the desired compound in good yield (50 %). The nature of the base used for the second step is very important : when NEt_3 is used instead of pyridine, the yield is less than 5 %, probably because pyridine increases the solubility of the choline salt in the reaction mixture.

The compounds $\text{BOCNH(EO)}_n\text{PC}$ obtained by this method can be purified by flash chromatography on silica gel with water-acetone eluent, which allows obtaining the pure compounds at the scale of the gram (NMR spectra given in figure 3A). For the coupling step, $\text{BOCNH(EO)}_n\text{PC}$ is deprotected quantitatively by acidic treatment (HCl 2.5M). The resulting amine $\text{H}_2\text{N(EO)}_n\text{PC}$ is obtained in the form of its ammonium chloride. It is not isolated but coupled in situ with PGA or PAA in water, at pH between 4.7 and 5.0, with an excess of EDCI and HOBt as auxiliary agent. The resulting polyelectrolyte is purified by dialysis against NaCl first and water then.

The same coupling conditions could be implemented to the coupling of PLL. For this purpose, $\text{H}_2\text{N(EO)}_3\text{PC}$ was transformed in the succinylated derivative $\text{SucHN(EO)}_3\text{PC}$. This was achieved simply by the deprotection of the amino group followed by the reaction with succinyle anhydride in large excess. As expected the competing hydrolysis of the succinyle anhydride occurs, but the formed byproducts do not hamper the purification steps, and $\text{SucNH(EO)}_3\text{PC}$ could be obtained in good purity. Its coupling to PLL and the following purification steps proceeded the same way as with the PGA.

All the polymers obtained were very hygroscopic and deliquescent at air. However, except water no other impurities were observed. All final polymers gave very clean and well resolved NMR spectra (figure 3A and 3C), The spectra were interpreted by a combined

analysis of ^1H , ^{13}C , ^1H - ^1H shift correlated (COSY) and ^1H - ^{13}C shift correlated spectra (Suppl. info., figure S2a, S2b). The assignments of the signals are given on figure 3. They are coherent with the findings in the literature^[29, 37, 38] and with the structure of the polymers. For PGA(EO)₃PC, the signals of the PC and spacer groups are found at 4.34 (c), 4.05 (d), 3.78-3.67 (e-g, b), 3.63 (h) and 3.23 ppm (a) (the letters refer to the indexation given in figure 3B). In the non-coupled BOCNH(EO)₃PC, the same protons give signals at similar shifts. But the triplet at 3.40 corresponding to the protons (i) is shifted downfield compared to the same protons in the starting BOC derivative (at 3.29); the new shift is coherent with the formation of an amide. This proves the effective coupling of the PC containing lateral groups. Similar conclusions were obtained for the other polymers. The IR spectra (figure 4) of the polymers also exhibit the characteristic bands of PC groups: The band at 1087 cm^{-1} can clearly be assigned to the PO_2^- symmetric stretching ($\nu_s(\text{PO}_2^-)$), the one at 967 cm^{-1} to the $\text{N}^+-(\text{CH}_3)_3$ antisymmetric stretching, ($\nu_{as}(\text{N}-\text{CH}_3)$), the 930 cm^{-1} band to the $\text{C}-\text{N}^+-(\text{CH}_3)_3$ symmetric stretching, ($\nu_s(\text{N}-\text{CH}_3)$), and the one at 802 cm^{-1} to the $\text{P}-(\text{OC})_2$ antisymmetric stretching ($\nu_{as}(\text{P}-(\text{OC})_2)$) vibrations.^[29, 39] No chloride was detected by ICP-MS in the final compounds.

The degree of substitution (DS) can be calculated from the NMR spectra with a satisfactory accuracy. IR spectroscopy was used to confirm these DS. The NCH_3 and the COO^- stretching bands were measured simultaneously for each sample and used to calculate the choline and the COO^- concentrations respectively. This double measurement enables the calculation of the DS independently from the water content of the polymers and avoids the accurate determination of the concentration in these hygroscopic polymers. The DS calculated from NMR and IR (Table 1) are in good agreement. The efficiency of the coupling reaction is above 85 % for $\text{H}_2\text{N}(\text{EO})_3\text{PC}$ and $\text{SucNH}(\text{EO})_3\text{PC}$ and 45 % for $\text{H}_2\text{N}(\text{EO})_4\text{PC}$. It is similar for PLL and PGA, but surprisingly, PAA and $\text{H}_2\text{N}(\text{EO})_3\text{PC}$ couple with nearly quantitative yield.

A DS greater than 80 % could be obtained with a sufficient excess of amine with a good efficiency (60 %).

The masses of the modified PGA were determined by SEC coupled with MALLS. The measured masses M_w for the starting PGA, PGA(EO)₄PC 35 and PGA(EO)₄PC 16 are 65, 60 and 55 kDa respectively, which shows that a limited mass diminution occurs during the coupling reaction. The resulting mass is still acceptable for layer-by-layer deposition. Two groups have shown^[40, 41] that carbodiimides may cleave the main chain of poly(- α -glutamic acid) or poly(- γ -glutamic acid) resulting in 10 to 50 times shorter chain fragments. The chain cleavage was attributed to side reactions that occur with the O-acylurea intermediates formed from the addition of the acid side group to the carbodiimide. One of these groups^[40] had not used HOBt. This auxiliary nucleophile reduces the concentration of the O-acylurea and the side reactions due to this species. However, the other group^[41] had used a 20 fold excess of HOBt and still observed cleavage in a large extent. The reaction conditions described herein are different since we have used a 100 times larger concentration in acid and a 9 times larger concentration in HOBt compared with the referenced work. These higher concentrations induce a lower ratio of the concentration in O-acylurea and amines and explain why the chain cleavage is limited. This high concentration also proved beneficial to reach high substitution levels.

Conclusion

Weak polyelectrolytes were functionalized with phosphorylcholine connected through oligo(ethylenoxide) to the main chain. First, side-chains with either acid or amine end groups were synthesized in a good purity level and at the scale of the gram, from the same pool of intermediates. Then these side groups were coupled by standard methods with the polymers. The method allows the functionalization of a wide range of polyacids or polyamines. The

coupling reactions proceed with a high efficiency and allow degrees of substitution up to 80 %.

Both the scale of the preparations and the purity levels attained make these polymers suitable to explore their physicochemical behavior in solution and serve as a library to investigate new types of polyelectrolyte multilayers.

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Supplementary information

Full characterization of BOCHN(EO)₃PC. NMR datas of PGA(EO)₄PC 16, PAA(EO)₃PC 25 .

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Table 1

Polymer	PC/monomer molar	PC/monomer molar ratio
	ratio in the feed	in polymer (%) NMR
	(%)	(IR)
PGA(EO) ₃ PC 25	30	22 ± 3
PGA(EO) ₃ PC 35	40	35 ± 3 (34 ± 5)
PGA(EO) ₃ PC 80	120	83 ± 5 (85 ± 3)
PGA(EO) ₄ PC	38	15 ± 2 (18 ± 5)
PAA(EO) ₃ PC	26	25 ± 3
PLL(EO) ₃ PC	41	36 ± 3

Figure captions

Figure 1. Target polymers and nomenclature

Figure 2. Synthetic scheme. Top: synthesis of BOCNH(EO)₃PC and Suc(EO)₃PC and bottom: coupling to the polyelectrolytes

Table 1. Preparation conditions and composition of the PC substituted polyelectrolytes.

Figure 3. 400 MHz ¹H-NMR spectra of the unmodified polymers, the side chains and the polymers bearing PC moieties and assignment of signals for the latter. a) Spectra of (from top to bottom): PGA(EO)₃PC with DS of 80 and 25 %, BOCNH(EO)₃PC, PGA and assignment of the signals according to b. b) Atom labelling for PGA(EO)₃PC. The same indexation from a to i holds also for BOCNH(EO)₃PC. c) Spectra of PLL(EO)₃PC, SucNH(EO)₃PC and PLL and the corresponding atom labeling (d).

Figure 4. IR spectra of PGA(EO)_nPC and BOCNH(EO)_nPC in D₂O. The spectrum of 4:0 PC (1,2-dibutyroyl-*sn*-glycero-3-phosphocholine) is given as reference.

n = 3, PGA(EO)₃PC

n = 4, PGA(EO)₄PC

PLLSuc(EO)₃PC

PAA(EO)₃PC

Figure 1

Figure 2

Figure 3

Figure 4

Table of Content

Polyelectrolytes were modified with side-groups bearing zwitterionic phosphorylcholine groups (PC), known to impart protein repellent properties to biomaterials. Side groups containing PC and terminated either by an acid or an amine function can be synthesized at the scale of the gram. They can be readily condensed with polycations such as PLL or polyanions such as PGA or PAA. Degrees of substitution as high as 80 % are obtained.

Supplementary Information

2-{2-[2-(2-N-tertButoxycarbonylamino-ethoxy)-ethoxy]-ethoxy}-ethylphosphorylcholine (BOCNH(EO)₄PC): the method described for BOC(EO)₃PC was applied to BOCNH(EO)₄OH (0.67 g, 2.29 mmol) as the starting material and afforded BOCNH(EO)₄PC as a white powder (0.59 g, 55 % yield).

¹H-NMR (D₂O, 400 Mhz) δ = 4.34 (br m, 2H, POCH₂CH₂N), 4.06 (br m, 2 H, POCH₂CH₂O), 3.81-3.68 (m, 8 H, CH₂OCH₂CH₂O, CH₂N(CH₃)₃), 3.62 (t, 2 H, *J* = 5.4 Hz, OCH₂CH₂N), 3.29 (t, 2 H, *J* = 5.4 Hz, CH₂N), 3.26 (s, 9H, N(CH₃)₃), 1.47 (s, 9H, C(CH₃)₃). ¹³C-NMR (D₂O, 100 Mhz) : δ = 158.8 (CO), 81.6 (C(CH₃)₃), 70.8 (d, *J* = 7.7 Hz, POCH₂CH₂O), 70.4, 70.3, 70.3, 70.1, (OCH₂CH₂), 66.7 (m, CH₂N(CH₃)₃), 65.5 (d, *J* = 5.9 Hz, POCH₂CH₂O), 60.1 (d, *J* = 5.1 Hz, POCH₂CH₂N), 54.7 (t, *J* = 3.9 Hz, N(CH₃)₃), 40.3 (CH₂N), 28.4 (C(CH₃)₃). IR (ATR) ν_{max} : 3365 , 3240 (ν NH), 2977 (ν CH₃), 2935 (ν_{as} CH₂), 2874 (ν_{as} CH₂), 1689 (amide I), 1522 (amide II), 1482 (δ_{as} (CH₃)₃N⁺), 1455, 1419, 1366 (δ CH₃), 1238, 1137, 1086 (ν_{s} PO₂⁻), 1058, 970, 953 (ν_{as} (CH₃)₃N⁺), 925, 875, 781 cm⁻¹.

PGA(EO)₃PC 35 :

¹H-NMR (D₂O, 400 MHz) : δ = 4.34 (broad s, POCH₂CH₂N, NHCHCO), 4.05 (broad m, POCH₂CH₂O), 3.78-3.67 (m, CH₂OCH₂CH₂O, CH₂N(CH₃)₃), 3.63 (t, *J* = 5.6 Hz, OCH₂CH₂N), 3.40 (t, *J* = 5.6 Hz, CH₂N), 3.24 (s, N(CH₃)₃), 2.33 (broad s, CH₂CO), 2.07 (broad s, CHCH₂CH₂), 1.97 (broad s, CHCH₂CH₂). ¹³C-NMR (D₂O, 100 MHz) : δ = 180.6 (CO), 175.5 (CO), 174.2 (CO), 173.8 (CO), 70.7 (d, *J* = 7.7 Hz, POCH₂CH₂), 70.3, 70.1, 69.4, (OCH₂CH₂), 66.6 (m, CH₂N(CH₃)₃), 65.5 (d, *J* = 5.5, POCH₂CH₂O), 60.1 (d, *J* = 4.5 Hz, POCH₂CH₂N), 54.6 (t, *J* = 3.7 Hz, N(CH₃)₃), 54.1 (CH), 53.7 (CH), 39.5 (CH₂N), 33.2

(CH₂CO), 32.4 (CH₂CO), 28.05 (CHCH₂CH₂), 27.8 (CHCH₂CH₂). IR (sol. D₂O) $\nu_{\max}$: 3406, 1697 (ν COO), 1645 (amide I), 1566 (amide II), 1458, 1404, 1366, 1086 (ν_s PO₂⁻), 1064, 973, 953 (ν_{as} (CH₃)₃N⁺), 799 cm⁻¹.

PGA(EO)₄PC 16 : this compound is synthesized as described above for PGA(EO)₃PC with PGA (153 mg, 1.01 mmol of monomer units) and BOCNH(EO)₄PC (179 mg, 0.39 mmol, 0.38 equiv.) . PGA(EO)₄PC 16 is obtained as a very hygroscopic white powder (150 mg, yield 71%): ¹H NMR (D₂O) : δ = 4.33 (broad s, POCH₂CH₂N, NHCHCO), 4.03 (broad m, POCH₂CH₂O), 3.80 – 3.66 (m, CH₂OCH₂CH₂O, CH₂N(CH₃)₃), 3.61 (t, J = 5.58 Hz, OCH₂CH₂N), 3.39 (broad s, CH₂N), 3.22 (s, N(CH₃)₃), 2.37 (broad s, CH₂CO), 2.07 (broad s, CHCH₂CH₂), 1.97 (broad s, CHCH₂CH₂). ¹³C NMR (D₂O) : δ = 180.6 (CO), 175.5 (CO), 174.2 (CO), 173.8 (CO), 70.7 (d, J =7.63 Hz, POCH₂CH₂O), 70.3, 70.2, 70.1, 69.5 (OCH₂CH₂), 66.7 (m, CH₂N(CH₃)₃), 65.6 (d, J =5.41 Hz, POCH₂CH₂O), 60.1 (d, J = 5.11 Hz, POCH₂CH₂N), 56.4 (broad s, CH), 54.7 (t, J =3.63 Hz, N(CH₃)₃), 39.6 (CH₂N), 32.4 (CH₂CO), 32.0 (CH₂CO), 26.2 (broad s, CHCH₂CH₂). IR (sol. D₂O) $\nu_{\max}$: 3406, 1697 (ν COO), 1644 (amide I), 1564 (amide II), 1482 (δ_{as} (CH₃)₃N⁺), 1463, 1447, 1405, 1086 (ν_s PO₂⁻), 1066, 972, 956 (ν_{as} (CH₃)₃N⁺), 798 cm⁻¹.

PAA(EO)₃PC 25 : this compound is synthesized as described above for PGA(EO)₃PC 35 with PAA (77 mg, 1.06 mmol of monomer units) and BOCNH(EO)₃PC (110 mg, 0.27 mmol, 0.25 equiv.) as the starting materials. Pure PAA(EO)₃PC 25 is obtained as a white powder (126 mg, 82% yield). ¹H NMR (D₂O, 400 Mhz): δ = 4.32 (broad s, POCH₂CH₂N, CH₂CH), 4.03 (broad m, POCH₂CH₂O), 3.79–3.65 (m, CH₂OCH₂CH₂O, CH₂N(CH₃)₃), 3.60 (broad s), 3.37 (broad s, CH₂N), 3.23 (s, N(CH₃)₃), 2.22 (broad s), 2.08 (broad s), 1.74 (broad s), 1.57 (broad s), (CH₂CH). ¹³C NMR (D₂O, 100 Mhz): δ = 182.0 (CO), 181.8 (CO), 177.3 (CO), 70.1 (d, J = 7.7 Hz, POCH₂CH₂O), 69.7, 69.5, 68.8, (OCH₂CH₂), 65.9 (m, CH₂N(CH₃)₃), 64.9

(d, $J = 5.1$ Hz, $\text{POCH}_2\text{CH}_2\text{O}$), 59.4 (d, $J = 4.8$ Hz, $\text{POCH}_2\text{CH}_2\text{N}$), 54.0 (t, $J = 3.3$ Hz, $\text{N}(\text{CH}_3)_3$), 44.0 (broad s), 42.6 (broad s), 38.8 (broad s), 36.1 (broad s) (CH_2CH).

PLLSuc(EO)₃PC 35:

^1H -NMR (D_2O , 400 MHz) : $\delta = 4.33$ (broad s, $\text{POCH}_2\text{CH}_2\text{N}$, CH_2CH), 4.05 (broad m, $\text{POCH}_2\text{CH}_2\text{O}$), 3.78 -3.67 (broad m, $\text{CH}_2\text{OCH}_2\text{CH}_2\text{O}$, $\text{CH}_2\text{N}(\text{CH}_3)_3$), 3.64 (t, $J = 5.3$ Hz, $\text{OCH}_2\text{CH}_2\text{N}$), 3.25 (s, $\text{N}(\text{CH}_3)_3$), 3.40 (t, $J = 5.3$ Hz, $\text{OCH}_2\text{CH}_2\text{NHCO}$), 3.17 (m, CH_2NHCO), 3.02 (t, $J = 7.11$ Hz, CH_2NH_2), 2.54 (broad s, $\text{COCH}_2\text{CH}_2\text{CO}$), 1.72 (broad m), ($\text{CHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$), 1.49 (broad m). ^{13}C -NMR (D_2O , 100MHz) : $\delta = 174.5$ (CO), 173.7 (CO), 173.5 (CO), 70.1 (d, $J = 7.8$ Hz, $\text{OCH}_2\text{CH}_2\text{OP}$), 69.7, 69.4, 68.8, (OCH_2CH_2), 66.0 (m, $\text{CH}_2\text{N}(\text{CH}_3)_3$), 64.9 (d, $J = 5.9$ Hz, $\text{POCH}_2\text{CH}_2\text{O}$), 59.4 (d, $J = 4.78$ Hz, $\text{POCH}_2\text{CH}_2\text{N}$), 54.0 (t, $J = 3.81$ Hz, $\text{N}(\text{CH}_3)_3$), 53.4 (CH), 39.2 (CH_2NH_2), 39.1 (CH_2NHCO), 39.0 ($\text{OCH}_2\text{CH}_2\text{NHCO}$), 31.2, 31.2, ($\text{COCH}_2\text{CH}_2\text{CO}$), 30.6, 27.9, 26.5, 22.4, 22.2, ($\text{CHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$).

Figure S1. Spectrum of crude PC(EO)₃NH₃Cl.

Figure S2. section of the COSY spectrum of BOCNH(EO)₃PC.

Figure S3a. COSY spectrum of PGA(EO)₃PC 80.

Figure S3b. ¹H-¹³C correlation spectrum of PGA(EO)₃PC 80

Figure S4. SEC measurements. Green: PGA; blue PGA(EO)₄PC 16; red: PGA(EO)₃PC 35.