Influence of Salt on the Solution Dynamics of a Phosphorylcholine-Based Polyzwitterion

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ABSTRACT

The diffusion of a polyzwitterion, poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), in aqueous solution containing different alkali halides was studied by fluorescence correlation spectroscopy at single molecule level. It was found that the halide anion has a greater effect on the radius of zwitterionic PMPC molecules than alkali cations, which is due to the mechanism by which PMPC molecules interact with the surrounding hydrogen bond network of water molecules and adsorbed ions. With the addition of salt, the size of PMPC remains constant while its diffusion coefficient is reduced slightly, although larger cations (e.g. K^+) result in slightly increased diffusion coefficient for 1 M potassium chloride-based solutions. This enhanced diffusion coefficient is attributed to the decrease in the viscosity of the aqueous solution on the addition of salt. When the counter-ion was varied in potassium-based salts, different effects were observed for different anions, resulting a reduction in the diffusion coefficient as a function of salt concentration. This reduction was modest for KBr, but significant for KI. Overall, no discernible changes were observed as the size of the PMPC coil was varied, except in case of KI for which a significant increase was observed at higher ionic strength. Divalent cations (Ca^{2+} and Mg^{2+}), produced similar effects to those found for monovalent cations. These effects are explained by the interaction of PMPC with the hydrogen bond network of water molecules and with the adsorbed ions.

INTRODUCTION

Understanding the dynamics of polyelectrolytes in aqueous solution is challenging because long-range electrostatic interactions between charged groups on the chains play an important role alongside their interactions with mobile counter-ions and solvent molecules.[1-2] The solution properties of polyzwitterions (a type of polymer that contains both positive and negative charged groups in each repeat monomer unit) have been found to exhibit the so-called antipolyelectrolyte effect.[3] In contrast to polyelectrolytes and proteins that tend to precipitate upon the addition of salt, most polyzwitterions become increasingly soluble as salt concentration increases, as attractive dipole-dipole interactions between the polyzwitterions are screened by electrostatic interactions and counter-ion adsorption. The antipolyelectrolyte effect is anticipated to be dependent upon the chemical structure and composition of the polymer as well as the solution conditions.

The phosphorylcholine (PC) group in phospholipids has inspired the development of a number of materials for the preparation of biomimetic, antifouling materials for bioscience and bioengineering applications. Of these, poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC)

is perhaps the most promising because it possesses characteristics that make for ideal biomaterial coatings, such as biocompatibility,[4-5] colloidal stabilisation,[6-7] anti-fouling,[8-9] and lubrication properties.[10-11] Since biological fluids are complex solutions containing biomolecules and salts, it is crucial to understand the effect of ions on PMPC to enhance its performance. Such knowledge will also facilitate the understanding of the effect of ions on other PC-based materials, e.g. phospholipid bilayers that serve multiple functional roles in signal transduction, since these are often exposed to physiological environments.[12-15]

Different views have been reported in the literature regarding the effect of ions on PMPC in aqueous solution. Both frictional properties and the swelling structure of surface-grown PMPC brushes exposed to NaCl solutions were examined as a function of salt concentration in the region of 0 – 5 M. It was concluded that the effect of salt on PMPC brushes was negligible.[16-18] Furthermore, the size of free PMPC molecules in aqueous solution was measured using static and dynamic light scattering techniques in concentrations from 0 to 0.5 M, and it was concluded that both the hydrodynamic radius and second virial coefficient of PMPC were independent of salt concentration.[19-22] However, in another study,[23] the hydrodynamic volumes of PMPC molecules were analysed using size exclusion chromatography (SEC) in various salt solutions at concentrations from 0 to 0.3 M. It was reported that the concentration of ions has a significant effect on the size of PMPC, and a qualitative correlation between the type and concentration of salts and the solution dynamics of PMPC was established. In recent studies, the friction coefficient between two PC-functionalized surfaces was found to increase with the addition of sodium nitrate ($NaNO_3$), which was attributed to the salting-out of some PC groups at higher salt concentration.[10, 24]

Fluorescence correlation spectroscopy (FCS) was employed in the present work to investigate the solution dynamics of PMPC in aqueous solutions at the single molecule level to enhance the understanding of the effect of salts on the PC group. Based on a statistical analysis of the fluctuation of the fluorescence signal in a confined volume, FCS is able to observe the dynamics of molecular events, such as diffusion and conformational fluctuations of molecules. Unlike techniques such as light scattering that require relatively high concentration of solute, extremely low concentrations (a few nM) are used in FCS measurements. This eliminates interference due to intermolecular interactions that are dominant at higher concentrations. Consequently only the self-diffusion of single molecules is measured.[25-27] Previous studies have successfully demonstrated the capability and advantage of FCS in examining the solution dynamics of several polyelectrolytes in different aqueous solutions or neutral polymers in polyelectrolyte environments.[28-31]

In particular, FCS is used to measure the diffusive motion of individual PMPC chains quantitatively at the single molecule level. Because the viscosities of the corresponding aqueous media in which PMPC molecules were dissolved are known, it is possible to evaluate to what extent the interaction between ions and PMPC contributes to the diffusion and consequently the size of PMPC molecules. The ion-specific effect on the PC group was then assessed resulting in a systematic knowledge of the effect of ions on the solution dynamics of PMPC. This work demonstrates that FCS is a powerful and ultrasensitive single molecule tool that can study the solution dynamics of polyzwitterions under a wide range of experimental conditions.

EXPERIMENTAL

Materials

Rhodamine 6G (99 %) was obtained from Acros Organics (Geel, Belgium) and used as received. HPLC grade acetonitrile, diethyl ether, dichloromethane, methanol, tetrahydrofuran, isopropanol, *n*-heptane and regenerated cellulose dialysis membrane (1,000 MWCO) were

obtained from Fisher Scientific (Loughborough, UK) and used as received. 2-Methacryloyloxyethyl phosphorylcholine (MPC, 99.9%) was donated by Biocompatibles UK Ltd (Farnham, UK) and was used as received. *N*-hydroxyethyl piperazine (98 %), 2-bromoisobutyryl bromide (98 %), 2-bromoisobutyric acid (98 %), anhydrous methanol (MeOH, 99.8 %), copper bromide (CuBr, 99.999%), 2,2'-bipyridine (bpy, 99%), lithium chloride (LiCl, ACS reagent grade), sodium chloride (NaCl, ACS reagent grade), potassium chloride (KCl, ACS reagent grade), potassium bromide (KBr, ACS reagent grade), potassium iodide (KI, ACS reagent grade), calcium chloride ($CaCl_2$, ACS reagent grade), and magnesium chloride ($MgCl_2$, ACS reagent grade) were purchased from Sigma-Aldrich (Dorset, UK) and used as received. HPLC grade water (CHROMASOLV, 2.5L) purchased from Sigma-Aldrich was used to prepare polymer solutions.

Synthesis of rhodamine 6G-labelled PMPC

Rhodamine 6G was derivatized in its 2' position by direct reaction with *N*-hydroxyethyl piperazine. Subsequent esterification of the hydroxyl-group with 2-bromoisobutyryl anhydride gave a fluorescent 2-bromoisobutyryl ester which was used as a (fluorescent) atom transfer radical polymerization (ATRP) initiator for the controlled polymerization of 2-methacryloyloxyethyl phosphorylcholine (MPC). This method of synthesis ensures that each PMPC contained precisely one rhodamine 6G molecule at the terminus of the chain. Details of this procedure can be found in a previous report.[32]

A typical protocol for the controlled polymerization of MPC by (ATRP) was as follows. The ATRP initiator (derivatized rhodamine 6G, 0.0229 g, 0.0339 mmol, 1.0 equiv.) and MPC (2.00 g, 6.77 mmol, 200 equiv.) were dissolved in 3.0 mL anhydrous methanol. After purging with nitrogen for 20 min, Cu(I)Br catalyst (4.9 mg, 0.034 mmol, 1.0 equiv.) and bpy ligand (10.6 mg, 0.0679 mmol, 2.0 equiv.) were added to the stirred solution under nitrogen. After leaving the reaction for 15 h, protons from methacrylic groups were no longer detected by ^{1}H NMR. At this point, the reaction mixture was diluted with methanol and exposed to air. The solution was passed through a column of silica using methanol as the eluent to remove copper salts. The solution was then transferred to a dialysis bag (MWCO 1,000) and dialysed first against methanol with daily changes of solvent until the dialysate was colourless. Then the solvent was changed to water by 3 consecutive solvent changes. Finally the polymer was freeze-dried overnight, followed by drying in a vacuum oven at 80°C for two days. The polymer was isolated in a 75% yield as a dark red powder.

Fluorescence Correlation Spectroscopy

Rhodamine 6G-labelled PMPC was dissolved in HPLC grade water to prepare a solution with a concentration of 3.15×10^5 nM, and subsequently stored in a fridge. Diluted PMPC solutions (10 nM) with the desired ionic strength were prepared immediately before each measurement, and subsequently placed in a chambered cover glass (8-well Lab-Tek, Thermo Fisher Scientific, UK). To reduce experimental errors that could be caused by variations in laser output, control experiments (10 nM PMPC in pure water) were performed before each measurement. FCS measurements were made using a ConfoCor2 FCS module fitted to a LSM510 inverted confocal microscope (Zeiss). Rhodamine 6G-labelled PMPC was excited using the 514 nm line of an argon laser. Fluorescence emission was collected through a 530-600 nm band-pass filter and recorded with an avalanche photodiode. Photobleaching was inhibited by attenuation of the laser using an embedded neutral density filter. Fluctuations in the fluorescence signal from rhodamine 6G-labelled PMPC molecules were quantified by temporal autocorrelation of the fluorescence intensity signal. A solution of rhodamine 6G (10 μM) was used to calibrate the alignment of the confocal optics. Based on Equation 2, the width of the confocal volume was

calibrated by carrying out diffusion measurements on a 10 nM rhodamine 6G solution, whose diffusion rate in water is well documented following characterisation by other techniques.[33]

Data Analysis

The autocorrelation functions ($G(\tau)$) acquired were fitted using[34]

$$G(t) = 1 + \frac{1}{N}\left[\frac{1}{\left(1+\frac{t}{t_D}\right)}.\frac{1}{\left(1+\frac{t}{S^2 t_D}\right)^{1/2}}\right].\left[1+\left(\frac{P_t}{1-P_t}\right).\exp\left(-\frac{t}{t_t}\right)\right], \qquad (1)$$

where N is the number of fluorophores (rhodamine 6G or rhodamine 6G-labelled PMPC in this case) within the detection volume, τ_D is the diffusion time of the fluorophore (the averaged time taken to cross the confocal volume), S is the structural parameter of the confocal volume, P_t denotes the fraction of fluorophores excited to a triplet state, and τ_t is the corresponding triplet lifetime. Equation (1) was then fitted to the data using pro Fit v6.1.8 (QuantumSoft, Switzerland). A Monte Carlo algorithm was used initially to determine the starting parameters, and a Levenberg-Marquardt routine was then used to find best-fit parameters.

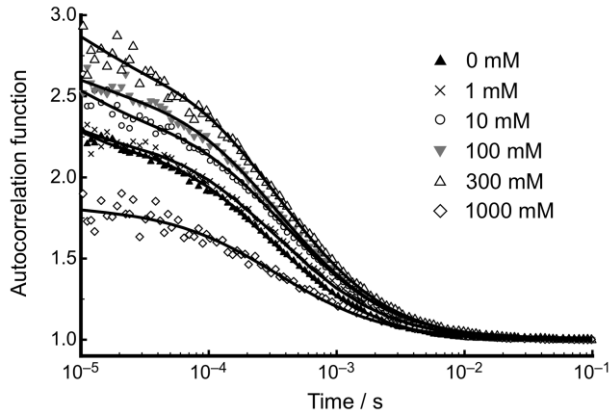

Figure 1. Representative autocorrelation plots for rhodamine 6G-labelled PMPC in a series of KBr solutions of different concentrations. Solid lines show best fits to Equation (1).

Figure 1 presents representative autocorrelation data of PMPC molecules in pure water and KBr solutions of different concentrations, obtained by FCS. Curves fitted using equation (1) are also shown, from which the diffusion time τ_D and number of molecules N within the confocal volume can be obtained.

The diffusion coefficient (D) of a particle can be extracted from its diffusion time if the characteristic size of the detection volume is known,[35] giving

$$D = \frac{a^2}{4t_D}, \qquad (2)$$

where a is the calibrated width of the confocal volume.

The Stokes-Einstein equation is the fundamental model used to describe the diffusive motion of a solid spherical particle of radius R through a medium of viscosity η. The Stokes-Einstein diffusion coefficient (D_{SE}) is defined by

$$D_{SE} = \frac{k_B T}{6\rho Rh}, \qquad (3)$$

where k_B is the Boltzmann's constant, R is the radius of the polymer, and T is the absolute temperature.

RESULTS

Influence of ions on fluorescence probe

To elucidate the influence of ions on the fluorescent probe, diffusivity of free rhodamine 6G was examined as a function of salt concentration (in pure water and KCl solutions up to 1000 mM) using FCS. It was found that the diffusion coefficients of rhodamine 6G were independent of salt concentration, which ensures that any change observed by FCS can be attributed to the solution dynamics of the derivatized PMPC molecule. Raw autocorrelation curves of rhodamine 6G, collected from solutions with different quantities of salt, were found to superimpose each other (Figure S1 in Supporting Information). This confirms that both diffusion coefficients and the number of rhodamine 6G molecules within the confocal volume were constant over the range of salt concentrations (up to 1000 mM) used in the present work. Consequently the fluorescence properties of rhodamine 6G was considered to be unaffected by variations of the concentration of KCl solutions. This allowed the absence of free rhodamine 6G to be confirmed in polymer diffusion measurements as well as confirming that the dye label was not itself affected by salt.

As the effect of varying the concentration of KI was also examined, it should be noted that KI has been reported to exhibit quenching of rhodamine 6G at high concentrations due to an antioxidant property of iodide.[37] This quenching caused noticeable noise in the FCS measurements of rhodamine 6G labelled PMPC molecules at high KI (1000 mM) concentration, resulting in poor autocorrelation functions. As such, data collected from this specific concentration were not included.

Effect of Monovalent Salts on PMPC

Solution dynamics of PMPC (with a target degree of polymerization of 200, a number average molar mass, M_n measured by aqueous gel permeation chromatography of 59.7 kDa, and a dispersity, PDI of 1.75) in aqueous solutions were investigated using FCS. The averaged diffusion coefficients of PMPC in pure water is 32.0 ± 1.5 $\mu m^2 s^{-1}$, with R = 2.7 nm obtained from Equation 3. It should be noted that the diffusion coefficients of PMPC in the presence of salts as presented in subsequent sections are normalized to the same PMPC samples measured in pure water immediately before each experiment to account for variations in laser output and laboratory conditions.

Diffusion coefficients of PMPC molecules were measured in KCl solutions with concentration ranges from 1 to 1000 mM and are shown in Figure 2a. Even though changes were observed as the salt concentration increased, the variation in diffusion coefficient is not significant. The ion-specific effect on the solution dynamics of PMPC was evaluated by changing the anionic or cationic components of the salt; KBr and KI were chosen for the former, while NaCl and LiCl were used to evaluate the role of different cations. The normalized diffusion coefficients and radii of PMPC chains, as a function of ionic strength, can be found in Figure 2.

In all salt solutions examined in the present work, the diffusion coefficient of PMPC was found to change slightly with the addition of salt, compared with the control value acquired in pure

water (shown in Figure 2a and 2b). However, no significant change was observed with all variations within 15% of the control value.

In Figure 2c and 2d, the normalized radii of PMPC chains, based on Equation 3, are presented as a function of salt concentration after being corrected for changes in the solution viscosity due to the presence of salt ions. By taking into account the viscosity of the solution, the effect of ions on the size of the solvated PMPC can be investigated. Figure 2c presents the normalized radii of PMPC as a function of ionic strength in the presence of three different cations. Even though the diffusion coefficient of PMPC chains decreases slightly as a function of the concentration of the alkali salts in Figure 2a, only subtle changes in their radius were found in Figure 2b. This observation is in agreement with previous reports stating that the size of PMPC is independent of the NaCl concentration[19-22] and that alkali cations have no effect on the hydrodynamic radius of PMPC.[23] The comparison between the diffusion coefficient and the size of PMPC chains suggests that the size remains constant in the alkali salt solutions, and the changes in diffusion coefficients at high concentration regime is due to the variations in solution viscosity.

As shown in Figure 2d, the effect of changing the halide anion on the hydrodynamic radius of PMPC is more pronounced. The normalized radius varied by ~10% in KBr solutions over the salt concentration range examined, but increased significantly in KI solutions. After correction by taking into account of the changing viscosity of the solution, the size of PMPC molecules were found to swell by ~50% in a 300 mM KI solution compared to that in pure water. The changes in radius of PMPC chains further confirm that the PC functional group responds to anions. The effect of anions on PMPC is in agreement with the study by Mahon and Zhu[23] as well as reports on other PC-based materials, such as phospholipid membranes.[38] It is also worth noting that iodide may quench the fluorescence as reported elsewhere[37] and confirmed in the present work, and hence it might have an influence on the quality of the FCS measurements, especially at higher concentration. It is also possible that, because the rhodamine interacts with iodide through fluorescence quenching, it might also affect the PMPC chain conformation. Because the PMPC is end-labelled, this latter possibility is rather unlikely.

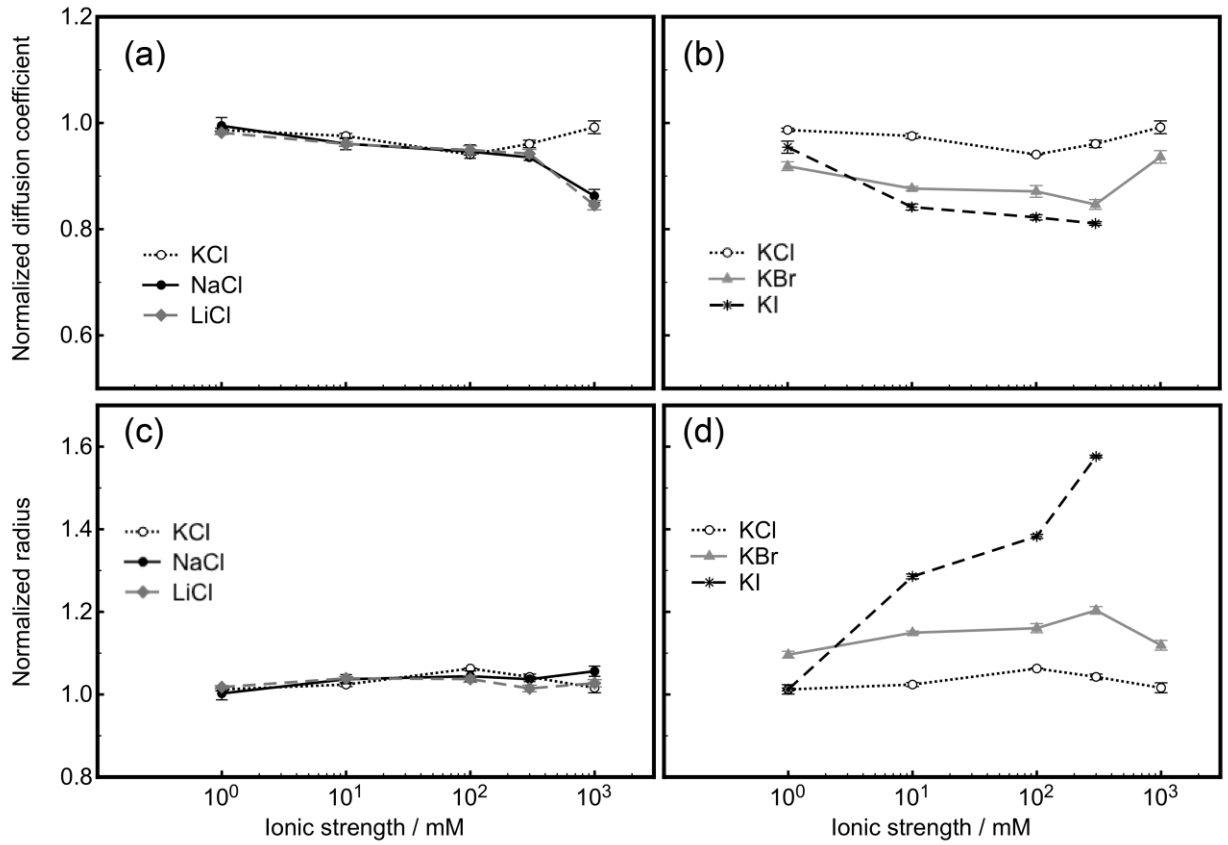

Figure 2: (a, b) Diffusion coefficients and (c, d) radii of PMPC chains, normalized to those in pure water (diffusion coefficient 32.0 $\mu m^2\ s^{-1}$ with corresponding radius 2.7 nm), as a function of ionic strength, where either the cations (a, c) or the anions (b, d) is varied.

Divalent Salts

Previous work on phospholipids demonstrates that divalent cations have a greater affinity to PC functional groups than monovalent cations, For instance, a binding constant of 40 M^{-1} for Ca^{2+} to egg phosphatidylcholine compared to 0.15 M^{-1} for Na^+ has been reported previously.[39] The effects of two types of divalent salt, $CaCl_2$ and $MgCl_2$ on PMPC were examined in the present work (Figure 3). The diffusion coefficient of PMPC chains in both divalent salt solutions was found to decrease with increased salt concentration, which is similar to what was found with LiCl and NaCl (see Figure 2). It is worth noting that Mg^{2+} has a greater effect than Ca^{2+} on the diffusion coefficient of PMPC, as shown in Figure 3a.

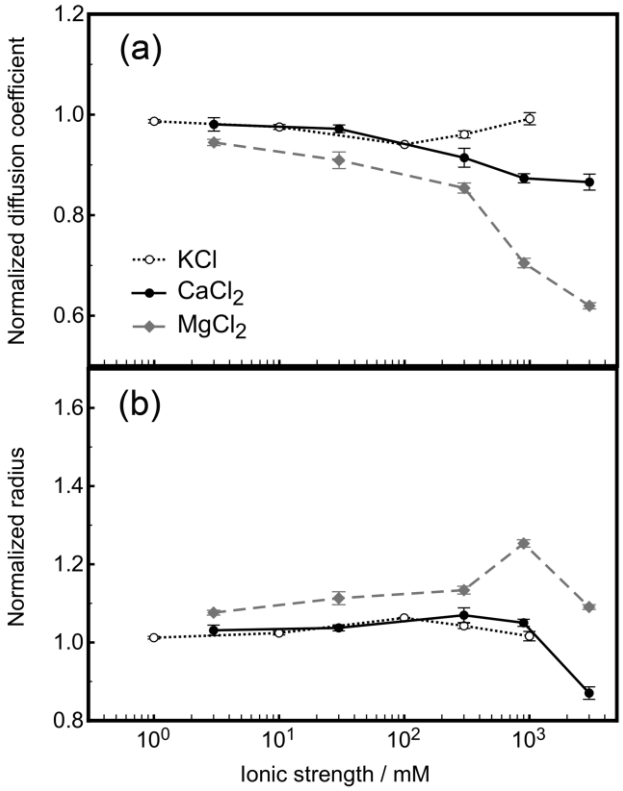

Figure 3: (a) Diffusion coefficients and (b) radii of PMPC chains as a function of ionic strength (the data are normalized to diffusion coefficient 32.0 μm^2 s^{-1} with corresponding radius 2.7 nm in pure water), where the valency and type of the cation is varied. Data acquired in KCl solutions are included for comparison.

The effect of ionic strength on the size of PMPC is less significant, as shown in Figure 3b. Once the variations in the solution viscosity have been accounted for, the normalized radius (against that in pure water) remains constant except for the highest salt concentrations. This is consistent with observations for monovalent alkaline salts (KCl and KBr, see Figure 2). In $CaCl_2$ solution, the hydrodynamic radius of PMPC chains does not change in solutions up to 1000 mM ionic strength. This confirms that the viscosity of salt solution contributes significantly to the diffusion of PMPC molecules when divalent salts are used.

DISCUSSION

Effect of ionic strength

A key characteristic of PMPC is its high level of hydration with 15 or more water molecules associated with each monomer unit. Techniques including DSC,[41-42] Raman Spectroscopy,[43] and ATR-FTIR have been used to investigate the structure of water in the vicinity of PMPC chains. These studies indicate that, unlike polyelectrolytes, PMPC has a very small effect on the structure of the hydrogen bonding network of surrounding water both in bulk solution and in thin films.[44]

To understand the aqueous solution dynamics of PMPC, information related to the interaction between the PC groups in PMPC and surrounding ions is key. As depicted in Figure 4, although each PC functional group has both a positive and a negative charge, the quaternary ammonium group is expected to be more accessible to ions in the salt solution because the phosphate

group is partially screened due to its position between the ammonium group and the hydrophobic backbone. Previous studies have demonstrated that ions have significant effects on both local and global properties of model PC-based phospholipid membranes.[45] It has also been reported that lipid lamellar phases swell when immersed in monovalent salt solutions where the swelling of the lipid multilayers increases monotonically with increasing salt concentration.[46] Moreover, electrostatic repulsion of membranes was observed and attributed to the binding of anions to the PC group, where the magnitude of the repulsion is ion-specific. It was rationalized that the electrostatic repulsion between PC groups is progressively screened by increasing salt concentration, which also screens the van der Waals attraction. However, the effect of ions on the conformation of PMPC chains was rather limited except for potassium iodide. Even though the dominating moiety in PMPC molecule is the PC group that has been proved responding to ions, these are tethered to the backbone of the polymer via a strong covalent bond, which limits the flexibility of PC side groups. Furthermore, it is probable that the negatively charged phosphate has a role in preventing salting out, which lowers the affinity of the quaternary ion to other anions. As a consequence, PMPC, in contrast to the behaviour expected for polyelectrolytes, did not exhibit a significant response to ionic strength in the experiments described here.

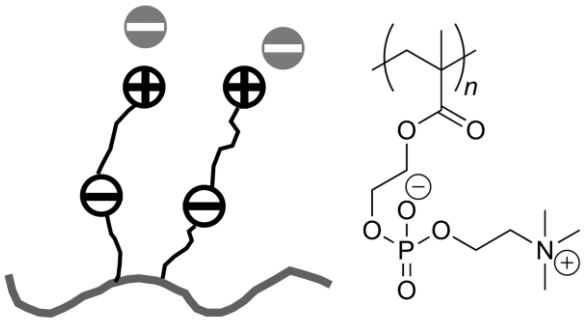

Figure 4. (left) A schematic diagram shows that anions have greater effect on PMPC. (right) Chemical structure of PMPC.

The observed solution dynamics of PMPC do not agree with the general characteristics of other polybetaines (polyzwitterions in which both positive, which must be an onium bearing no hydrogen, and negative charged groups are present but not adjacent in the same monomer unit) that are poorly soluble in pure water. The formation of intra- and interchain ionic contacts result in an ionically crosslinked network structure, and consequently render them insoluble in pure water. With the addition of salt, the ions could penetrate such an ionic network, screen the electrostatic interactions and change the Debye length, which would alter the conformation of polybetaines and expand the size of the polymeric chain. However, such a 'salting-in' process was not observed in the present study. It is highly likely that the PMPC is fully expanded in pure water, due to the hydrogen bonding network formed by surrounding water molecules, and the conformation of PMPC remains relatively constant over the broad range of salt concentrations explored. In the competition between the 'swelling' effect by water and the ionic interactions between PC groups (including the electrostatic screening), it is clear that the former factor plays the determining role.

In previous light scattering experiments, the conformation of free PMPC molecules and those immobilized on silica nanoparticles in aqueous solutions was found to be independent of salt concentration in agreement with the results reported here.[19-20] Although there was no explanation for the constant size of PMPC in aqueous solution, it was suggested that the mechanism by which PMPC dissolves in water without perturbing the hydrogen network

between water molecules is responsible for its particularly stable characteristics. Furthermore, in studies of surface grafted PMPC chains immersed in salt solutions using neutron reflectometry, it was found that the height and swollen structure of PMPC brushes were independent of salt concentration.[18]

Ion-specific effect

It has to be noted that only NaCl was used in the aforementioned studies where PMPC showed no response to the presence of salt. However, in the present work, noticeable changes in the diffusion coefficient and size of PMPC were observed when concentrated salt solutions containing large halide anions (KBr and KI) and divalent salts ($CaCl_2$ and $MgCl_2$) were used. This could be due to the unique mechanism by which PMPC interact with surrounding water molecules; the electrostatic interaction exists locally between ions and the individual PC groups of each repeat unit. Both experimental and molecular dynamics simulation studies on phospholipid membranes have revealed detailed information about the location of ions with respect to the polar head groups and hydrocarbon chains.[12, 38, 47-50] These works show that the binding of ions modifies the area per lipid, lipid ordering, orientation of the lipid head dipole, and the charge distribution along the system, among other effects. It has been found that the binding of potassium ions to PC bilayers is almost fully absent: most K^+ ions are located in the aqueous phase and not associated to the membrane. In addition, studies have shown that halide anions have a stronger interaction with PC lipids than those of cations. The strength of these interactions increases with the size of the anion: $Cl^- < Br^- < I^-$. Calculations show that iodide exhibits a genuine affinity for the PC lipids, which is due to its pairing with the choline group and its propensity for the nonpolar region of the lipid chains.[38] The attractive interaction between iodide and the PC group could potentially cause the PMPC chain to expand. Similar results have also been reported with zwitterionic micelles; the affinity for different anions follows the Hofmeister series: less hydrophilic anions, such as ClO_4^-, are adsorbed more strongly than more hydrophilic anions, such as Cl^-.[51]

Because of its location, the negatively charged phosphate has to overcome shielding caused by the positively charged ammonium group before interacting with cations in the salt solution. Secondly, the short distance between the phosphate and the backbone results in a reduced flexibility, which may be required for it to interact with cations. It is possible for a cation to be trapped between nearby phosphates, which for normal polyelectrolytes would cause the chain to change conformation because the repulsion is shielded. However, the presence of the ammonium leads to repulsion, which counters the shielding effect. As a consequence PMPC shows a more noticeable response to halide anions.

Studies have shown that divalent cations are capable of charging a phospholipid membrane. The degree of saturation of the hydrocarbon chains was found to have a significant effect on the binding of multivalent cations, which predominantly bind to membranes containing lipids with saturated tails.[45, 52] For a PC-based bilayer in the gel phase in the presence of three different cations (sodium, potassium, and calcium), it was found that the area per unit cell did not change significantly under the influence of these three ions. However, the lipid molecules re-order non-isotropically under the influence of the salts. This re-ordering was attributed to the change in the highly directional intermolecular interactions caused by a variation in the dipole-dipole bonding arising from a tilt of the head group out of the bilayer plane. Furthermore it has been shown that calcium ions interact more strongly with the PC lipids and increase the area per unit cell more than potassium ions.[53] There are other studies that reported a similar effect with PMPC and related phosphorylcholine-containing polymers.[23, 54-55] For example, it has been reported[55] that changes in ionic strength lead to minor changes in the expansion of PNIPAM-PC if the added salt is monovalent, but they also trigger a noticeable change in polymer conformation and aggregation if the added salt is divalent. Such observation is consistent with results of the

present work where divalent ions have greater influence on PMPC than monovalent ions do at high ionic strength.

CONCLUSION

A single molecule technique, fluorescence correlation spectroscopy, was employed to examine the solution dynamics of PMPC which was found to have a limited response to both the ionic strength of the aqueous solution and the nature of the ions in the solution.

It was found that both the viscosity of the aqueous solutions and the interaction between ions and PC groups contribute to changes in the diffusion coefficient. With known values of the viscosity of the salt solution, the ion-PC group interaction was evaluated. The result confirms that cations have very little influence on the hydrodynamic radius of the PMPC chains due to the reduced opportunity to bind to the PC group relative to that of anions, and that PMPC remains in a highly swollen state even at relatively high salt concentration. The unique mechanism by which PMPC interacts with the hydrogen bonding network of the surrounding water molecules is believed to be the main cause for its stable size in salt solutions, even though the ion-PC group interaction does exist locally.

The knowledge of the interaction between ions and PC lipids has been applied to rationalize that the effect of halide anions is much more substantial than that of alkali cations, which is in agreement with the ionic-specific effect observed on PC-based lipids. When it comes to divalent ions, although the diffusion coefficient of PMPC changed substantially, it appears that viscosity of salt solutions contributes more than the ion-PC interaction.

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REFERENCE

1. Dobrynin, A. V.; Rubinstein, M. Theory of Polyelectrolytes in Solutions and at Surfaces. *Prog. Polym. Sci.,* **2005,** *30*, 1049-1118.
2. Manning, G. S. The Molecular Theory of Polyelectrolyte Solutions with Applications to the Electrostatic Properties of Polynucleotides. *Quart. Rev. Biophys.,* **1978,** *11*, 179-246.
3. Lowe, A. B.; McCormick, C. L. Synthesis and Solution Properties of Zwitterionic Polymers. *Chem. Rev.,* **2002,** *102*, 4177-4189.
4. Ma, I. Y.; Lobb, E. J.; Billingham, N. C.; Armes, S. P.; Lewis, A. L.; Lloyd, A. W.; Salvage, J. Synthesis of Biocompatible Polymers. 1. Homopolymerization of 2-Methacryloyloxyethyl Phosphorylcholine via ATRP in Protic Solvents: An Optimization Study. *Macromolecules,* **2002,** *35*, 9306-9314.
5. Ishihara, K.; Nomura, H.; Mihara, T.; Kurita, K.; Iwasaki, Y.; Nakabayashi, N. Why Do Phospholipid Polymers Reduce Protein Adsorption? *J. Biomed. Mater. Res.,* **1998,** *39*, 323-330.
6. Sugihara, S.; Sugihara, K.; Armes, S. P.; Ahmad, H.; and Lewis, A. L. Synthesis of Biomimetic Poly(2-(methacryloyloxy)ethyl phosphorylcholine) Nanolatexes via Atom Transfer Radical Dispersion Polymerization in Alcohol/Water Mixtures. *Macromolecules,* **2010,** *43*, 6321-6329.
7. Thompson, K. L.; Bannister, I.; Armes, S. P.; and Lewis, A. L. Preparation of Biocompatible Sterically Stabilized Latexes Using Well-Defined Poly(2-(methacryloyloxy)ethyl phosphorylcholine) Macromonomers. *Langmuir,* **2010,** *26*, 4693-4702.

8. Feng, W.; Brash, J. L.; Zhu, S. Non-biofouling Materials Prepared by Atom Transfer Radical Polymerization Grafting of 2-methacryloyloxyethyl Phosphorylcholine: Separate Effects of Graft Density and Chain Length on Protein Repulsion. *Biomaterials,* **2006,** *27*, 847-855.
9. Kobayashi, M.; Terayama, Y.; Yamaguchi, H.; Terada, M.; Murakami, D.; Ishihara, K.; Takahara, A. Wettability and Antifouling Behavior on the Surfaces of Superhydrophilic Polymer Brushes. *Langmuir,* **2012,** *28*, 7212-7222.
10. Chen, M.; Briscoe, W. H.; Armes, S. P.; Klein, J. Lubrication at Physiological Pressures by Polyzwitterionic Brushes. *Science,* **2009,** *323*, 1698-1701.
11. Zhang, Z.; Morse, A. J.; Armes, S. P.; Lewis, A. L.; Geoghegan, M.; Leggett, G. J. Effect of Brush Thickness and Solvent Composition on the Friction Force Response of Poly(2-(methacryloyloxy)ethylphosphorylcholine) Brushes. *Langmuir,* **2011**, 2514-2521.
12. Böckmann, R. A.; Hac, A.; Heimburg, T.; Grubmüller, H. Effect of Sodium Chloride on a Lipid Bilayer. *Biophys. J.,* **2003,** *85*, 1647-1655.
13. Vácha, R.; Siu, S. W. I.; Petrov, M.; Böckmann, R. A.; Barucha-Kraszewska, J.; Jurkiewicz, P.; Hof, M.; Berkowitz, M. L.; Jungwirth, P. Effects of Alkali Cations and Halide Anions on the DOPC Lipid Membrane. *J. Phys. Chem. A,* **2009,** *113*, 7235-7243.
14. Weiß, K.; Enderlein, J. Lipid Diffusion within Black Lipid Membranes Measured with Dual-Focus Fluorescence Correlation Spectroscopy. *ChemPhysChem,* **2012,** *13*, 990-1000.
15. Filippov, A.; Orädd, G.; Lindblom, G. Effect of NaCl and CaCl2 on the Lateral Diffusion of Zwitterionic and Anionic Lipids in Bilayers. *Chem. Phys. Lipids,* **2009,** *159*, 81-87.
16. Kobayashi, M.; Yamaguchi, H.; Terayama, Y.; Wang, Z.; Ishihara, K.; Hino, M.; Takahara, A. Structure and Surface Properties of High-density Polyelectrolyte Brushes at the Interface of Aqueous Solution. *Macromol. Symp.,* **2009,** *279*, 79-87.
17. Kobayashi, M.; Takahara, A. Tribological Properties of Hydrophilic Polymer Brushes Under Wet Conditions. *Chem. Rec.,* **2010,** *10*, 208-216.
18. Kobayashi, M.; Mitamura, K.; Terada, M.; Yamada, N. L.; Takahara, A. Characterization of Swollen States of Polyelectrolyte Brushes in Salt Solution by Neutron Reflectivity. *J. Phys.: Conf. Ser.,* **2011,** *272*, 012019.
19. Matsuda, Y.; Kobayashi, M.; Annaka, M.; Ishihara, K.; Takahara, A. Dimension of Poly(2-methacryloyloxyethyl phosphorylcholine) in Aqueous Solutions with Various Ionic Strength. *Chem. Lett.,* **2006,** *35*, 1310-1311.
20. Matsuda, Y.; Kobayashi, M.; Annaka, M.; Ishihara, K.; Takahara, A. Dimensions of a Free Linear Polymer and Polymer Immobilized on Silica Nanoparticles of a Zwitterionic Polymer in Aqueous Solutions with Various Ionic Strengths. *Langmuir,* **2008,** *24*, 8772-8778.
21. Kikuchi, M.; Terayama, Y.; Ishikawa, T.; Hoshino, T.; Kobayashi, M.; Ogawa, H.; Masunaga, H.; Koike, J.; Horigome, M.; Ishihara, K.; Takahara, A. Chain Dimension of Polyampholytes in Solution and Immobilized Brush States. *Polym. J.,* **2012,** *44*, 121-130.
22. Kobayashi, M.; Terayama, Y.; Kikuchi, M.; Takahara, A. Chain Dimensions and Surface Characterization of Superhydrophilic Polymer Brushes with Zwitterion Side Groups. *Soft Matter,* **2013,** *9*, 5138-5148.
23. Mahon, J.; Zhu, S. Interactions of Poly(2-methacryloyloxyethyl phosphorylcholine) with Various Salts Studied by Size Exclusion Chromatography. *Colloid Polym. Sci.,* **2008,** *286*, 1443-1454.
24. Goldberg, R.; Schroeder, A.; Barenholz, Y.; Klein, J. Interactions between Adsorbed Hydrogenated Soy Phosphatidylcholine (HSPC) Vesicles at Physiologically High Pressures and Salt Concentrations. *Biophys. J.,* **2011,** *100*, 2403-2411.

25. Jia, P.; Gong, Y.; Wang, S.; Zhao, J. Advantage of Fluorescence Correlation Spectroscopy for the Study of Polyelectrolytes. *Chin. J. Chem.,* **2012,** *30*, 2237-2240.
26. Pristinski, D.; Kozlovskaya, V.; Sukhishvili, S. A. Fluorescence Correlation Spectroscopy Studies of Diffusion of a Weak Polyelectrolyte in Aqueous Solutions. *J. Chem. Phys.,* **2005,** *122*, 014907.
27. Koynov, K.; Butt, H.-J. Fluorescence Correlation Spectroscopy in Colloid and Interface Science. *Curr. Opin. Colloid Interface Sci.,* **2012,** *17*, 377-387.
28. AL-Baradi, A. M.; Mears, M.; Jones, R. A. L.; Geoghegan, M. Diffusion of Dextran Within Poly(methacrylic acid) Hydrogels. *J. Polym. Sci., Part B: Polym. Phys.,* **2012,** *50*, 1286-1292.
29. Bosco, S. J.; Zettl, H.; Crassous, J. J.; Ballauff, M.; Krausch, G. Interactions between Methyl Cellulose and Sodium Dodecyl Sulfate in Aqueous Solution Studied by Single Molecule Fluorescence Correlation Spectroscopy. *Macromolecules,* **2006,** *39*, 8793-8798.
30. Zustiak, S. P.; Nossal, R.; Sackett, D. L. Hindered Diffusion in Polymeric Solutions Studied by Fluorescence Correlation Spectroscopy. *Biophys. J.,* **2011,** *101*, 255-264.
31. Jia, P.; Zhao, J. Single Chain Contraction and Re-expansion of Polystyrene Sulfonate: a Study on Its Re-entrant Condensation at Single Molecular Level. *J. Chem. Phys.,* **2009,** *131*, 2311103.
32. Madsen, J.; Warren, N. J.; Armes, S. P.; Lewis, A. L. Synthesis of Rhodamine 6G-Based Compounds for the ATRP Synthesis of Fluorescently Labeled Biocompatible Polymers. *Biomacromolecules,* **2011,** *12*, 2225-2234.
33. Petrášek, Z.; Schwille, P. Precise Measurement of Diffusion Coefficients using Scanning Fluorescence Correlation Spectroscopy. *Biophys. J.,* **2008,** *94*, 1437-1448.
34. Widengren, J.; Mets, Ü.; Rigler, R. Fluorescence Correlation Spectroscopy of Triplet States in Solution: A Theoretical and Experimental Study. *J. Phys. Chem.,* **1995,** *99*, 13368-13379.
35. Varma, B. K.; Fujita, Y.; Takahashi, M.; Nose, T. Hydrodynamic Radius and Intrinsic Viscosity of Polystyrene in the Crossover Region from θ to Good-solvent Conditions. *J. Polym. Sci., Part B: Polym. Phys.,* **1984,** *22*, 1781-1797.
36. Rubinstein, M.; Colby, R. H., *Polymer Physics*. Oxford University Press: New York, 2003.
37. Chmyrov, A.; Sandén, T.; Widengren, J. Iodide as a Fluorescence Quencher and Promoter—Mechanisms and Possible Implications. *J. Phys. Chem. B,* **2010,** *114*, 11282-11291.
38. Vácha, R.; Jurkiewicz, P.; Petrov, M.; Berkowitz, M. L.; Böckmann, R. A.; Barucha-Kraszewska, J.; Hof, M.; Jungwirth, P. Mechanism of Interaction of Monovalent Ions with Phosphatidylcholine Lipid Membranes. *J. Phys. Chem. B,* **2010,** *114*, 9504-9509.
39. Tatulian, S. A. Binding of Alkaline-Earth Metal Cations and Some Anions to Phosphatidylcholine Liposomes. *Eur. J. Biochem.,* **1987,** *170*, 413-420.
40. Zettl, H.; Portnoy, Y.; Gottlieb, M.; Krausch, G. Investigation of Micelle Formation by Fluorescence Correlation Spectroscopy. *J. Phys. Chem. B,* **2005,** *109*, 13397-13401.
41. Hatakeyama, T.; Tanaka, M.; Hatakeyama, H. Studies on Bound Water Restrained by Poly(2-methacryloyloxyethyl phosphorylcholine): Comparison with Polysaccharide-water Systems. *Acta Biomater.,* **2010,** *6*, 2077-2082.
42. Morisaku, T.; Watanabe, J.; Konno, T.; Takai, M.; Ishihara, K. Hydration of Phosphorylcholine Groups Attached to Highly Swollen Polymer Hydrogels Studied by Thermal Analysis. *Polymer,* **2008,** *49*, 4652-4657.

43. Kitano, H.; Sudo, K.; Ichikawa, K.; Ide, M.; Ishihara, K. Raman Spectroscopic Study on the Structure of Water in Aqueous Polyelectrolyte Solutions. *J. Phys. Chem. B,* **2000,** *104*, 11425-11429.
44. Kitano, H.; Imai, M.; Mori, T.; Gemmei-Ide, M.; Yokoyama, Y.; Ishihara, K. Structure of Water in the Vicinity of Phospholipid Analogue Copolymers as Studied by Vibrational Spectroscopy. *Langmuir,* **2003,** *19*, 10260-10266.
45. Pabst, G.; Hodzic, A.; Štrancar, J.; Danner, S.; Rappolt, M.; Laggner, P. Rigidification of Neutral Lipid Bilayers in the Presence of Salts. *Biophys. J.,* **2007,** *93*, 2688-2696.
46. Petrache, H. I.; Zemb, T.; Belloni, L.; Parsegian, V. A. Salt Screening and Specific Ion Adsorption Determine Neutral-lipid Membrane Interactions. *Proc. Natl. Acad. Sci. U.S.A.,* **2006,** *103*, 7982-7987.
47. Klasczyk, B.; Knecht, V.; Lipowsky, R.; Dimova, R. Interactions of Alkali Metal Chlorides with Phosphatidylcholine Vesicles. *Langmuir,* **2010,** *26*, 18951-18958.
48. Lee, S.-J.; Song, Y.; Baker, N. A. Molecular Dynamics Simulations of Asymmetric NaCl and KCl Solutions Separated by Phosphatidylcholine Bilayers: Potential Drops and Structural Changes Induced by Strong Na+-Lipid Interactions and Finite Size Effects. *Biophys. J.,* **2008,** *94*, 3565-3576.
49. Gurtovenko, A. A.; Vattulainen, I. Effect of NaCl and KCl on Phosphatidylcholine and Phosphatidylethanolamine Lipid Membranes: Insight from Atomic-Scale Simulations for Understanding Salt-Induced Effects in the Plasma Membrane. *J. Phys. Chem. B,* **2008,** *112*, 1953-1962.
50. Aroti, A.; Leontidis, E.; Dubois, M.; Zemb, T. Effects of Monovalent Anions of the Hofmeister Series on DPPC Lipid Bilayers Part I: Swelling and In-Plane Equations of State. *Biophys. J.,* **2007,** *93*, 1580-1590.
51. Drinkel, E.; Souza, F. D.; Fiedler, H. D.; Nome, F. The Chameleon Effect in Zwitterionic Micelles: Binding of Anions and Cations and Use as Nanoparticle Stabilizing Agents. *Curr. Opin. Colloid Interf. Sci.,* **2013,** *18*, 26-34.
52. Szekely, O.; Steiner, A.; Szekely, P.; Amit, E.; Asor, R.; Tamburu, C.; Raviv, U. The Structure of Ions and Zwitterionic Lipids Regulates the Charge of Dipolar Membranes. *Langmuir,* **2011,** *27*, 7419-7438.
53. Ferber, U. M.; Kaggwa, G.; Jarvis, S. P. Direct Imaging of Salt Effects on Lipid Bilayer Ordering at Sub-molecular Resolution. *Eur. Biophys. J.,* **2011,** *40*, 329-338.
54. Wang, H.; Miyamoto, A.; Hirano, T.; Seno, M.; Sato, T. Radical Polymerization of 2-methacryloyloxyethyl phosphorylcholine in Water: Kinetics and Salt Effects. *Euro. Polym. J.,* **2004,** *40*, 2287-2290.
55. Miyazawa, K.; Winnik, F. M. Solution Properties of Hydrophobically-Modified Phosphorylcholine-Based Polymers in Water and in the Presence of Surfactants. *J. Phys. Chem. B,* **2003,** *107*, 10677-10682.