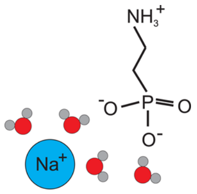
Observation of Water Separated Ion-Pairs between Cations and Phospholipid Headgroups

Sietse T. van der Post,[†] Johannes Hunger,[‡] Mischa Bonn,[‡] and Huib J. Bakker*,[†]

[†]FOM Institute AMOLF, Science Park 104, Amsterdam, The Netherlands
[‡]Max-Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany



ABSTRACT: In this work, we present evidence for ion pair formation of cations with a high surface charge density (Na^+ and Ca^{2+}) and phosphate groups of phospholipids. We used femto-second infrared pump−probe and dielectric spectroscopy to probe the dynamics of water molecules in solutions of phosphorylethanolamine and different types of cations. We find that sodium and calcium cooperatively retard the dynamics of water in solutions of phosphorylethanolamine, implying the formation of solvent separated ion pairs. This ion-specific interaction is absent for potassium, cesium and ammonium. We compare our results to dielectric spectroscopy experiments, which probes the rotation of all dipolar molecules and ions in solution. The rotation of the dipolar phosphorylethanolamine ion shows that long-lived ion-pairs are only formed with calcium and not with ammonium, cesium, potassium, and sodium. This finding implies that the association between calcium and the phosphate is strong with lifetimes exceeding 200 ps, while the interaction with sodium is relatively short-lived (∼20−100 ps).

■ INTRODUCTION

Phospholipids are the main building blocks of lipid membranes that are ubiquitous in all living organisms. Because of their hydrophilic headgroups and hydrophobic tails, phospholipids form stacked bilayers in the presence of water. The molecular character of the hydrophilic headgroup governs its interaction with proteins adsorbed at the membrane surface. Lipid membranes thereby influence the properties of these proteins and catalyze biochemical processes.[1,2] As has become clear in the last decades, water does not play a passive role as just the medium, but is actively involved in membrane−protein interaction.[3,4] In addition, water has an influence on the physical properties of the membrane. The dynamics of the phospholipid headgroups is for example found to be more collective when less water is present at the lipid surface.[5]

Ions have been found to change some of the properties of a lipid membrane such as thickness, headgroup conformation and lateral diffusion.[1,6−12] In a number of studies it was found that the tendency of anions to adsorb at the membrane surface follows the Hofmeister series: weaker hydrated anions (with a low surface charge density) such as iodide are attracted, while fluoride is repelled.[8,12] For cations the effect is rather opposite: strongly hydrated ions (with a large surface charge density) such as Ca^{2+} and Na^+ can penetrate into the interfacial region of the membrane and tend to form clusters with lipids by binding to their carbonyl and phosphate groups.[6,9,10,12−14] These results suggest a delicate balance of the interactions between the hydrophilic groups of the phospholipid headgroups, water and the cosolvated ions.

In recent work it was found that cations can influence the dynamics of water in the solvation shell of their counterions in a subtle way.[15,16] Water that hydrogen-bonds to an anion is restricted in its reorientation depending on the surface charge density of nearby cations. In this work, we study the combined effect of different types of cations and phosphorylethanolamine on the dynamics of water. We study the solutions with femtosecond infrared spectroscopy and GHz dielectric relaxation spectroscopy, which have both proven to be powerful techniques to study aqueous salt solutions.[15,17−25]

■ EXPERIMENT

fs-IR Pump−Probe. We use polarization-resolved femtosecond infrared pump−probe spectroscopy to measure the reorientation of OD groups in isotopically diluted water (8% HDO in H_2O), for various concentrations of phosphorylethanolamine salts (M^+PE^-, see Figure 1, M^+ being different types of cations). In this experiment we excite the OD-stretch vibration with an intense, linearly polarized pump pulse, after a delay time t followed by a much weaker probe pulse with its polarization either parallel or perpendicular to the pump polarization. Preferably the OD groups that have their transition dipole parallel to the pump-polarization are excited, resulting in an anisotropically excited subensemble of OD groups. The excitation thus leads to a polarization dependent change in absorption, which is recorded by two probe pulses

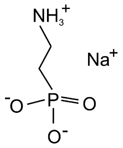


Figure 1. Molecular structure formula of NaPE in water. PE^- is a zwitterion at neutral pH.

with mutual perpendicular polarizations. By increasing the delay time between the pump and the probe pulses, the dynamics of the absorption changes are monitored. Vibrational relaxation causes the oscillators to relax to the ground state, resulting in a decrease of the absorption changes. In addition, with increasing delay the excited OD groups reorient and thus become more isotropically distributed, causing the parallel and perpendicular signals to become increasingly similar. The dynamics of the vibrational relaxation can be extracted by taking the weighted sum of the parallel and perpendicular signal,

$$\Delta\alpha_{\mathrm{iso}}(\omega, t) = \frac{\Delta\alpha_{\parallel}(\omega, t) + 2\Delta\alpha_{\perp}(\omega, t)}{3} \tag{1}$$

where $\Delta\alpha_{\parallel}(\omega,t)$ and $\Delta\alpha_{\perp}(\omega,t)$ are the transient absorption changes probed by the probe parallel and perpendicular to the polarization of the pump. The typical relaxation time of the OD-stretch vibration in isotopically diluted water is 1.8 ± 0.1 ps.[26] The reorientation dynamics, free from the effect of the vibrational relaxation, is expressed in the anisotropy parameter $R(t)$, defined by,

$$R(\omega, t) = \frac{\Delta\alpha_{\parallel}(\omega, t) - \Delta\alpha_{\perp}(\omega, t)}{\Delta\alpha_{\parallel}(\omega, t) + 2\Delta\alpha_{\perp}(\omega, t)} \tag{2}$$

In neat 8% $HDO{:}H_2O$ $R(t)$ of the pure OD-stretch excitation (i.e., the transient spectra corrected for the accompanied heating of the sample) decays monoexponentially for a delay $t > 0.4$ ps with a time constant of 2.5 ± 0.1 ps.[26]

GHz Dielectric Relaxation. We performed GHz dielectric relaxation (GHz-DR) measurements on the same salt solutions as studied with fs-IR, but now dissolved in pure water instead of $HDO{:}H_2O$. In this experiment the frequency dependent complex dielectric function of the sample is obtained in the range of 200 MHz to 70 GHz by the phase-sensitive measurement of reflected microwaves. A detailed description of the experiment can be found in ref 27.

GHz-DR measures the correlation function of the macroscopic polarization of the sample as a response to an externally applied electric field. In the presence of an electric field the partial alignment of molecular dipoles against thermal fluctuations results in a polarization effect. This polarization can be expressed in terms of the complex permittivity $\hat{\varepsilon}(\nu) = \varepsilon'(\omega) - i\varepsilon''(\omega)$. For electrolyte solutions the translation of the ions, as determined by the macroscopic conductivity, σ, gives rise to an additional contribution to the imaginary part ε'' of the permittivity. This contribution of $((-i\sigma)/(2\pi\nu\varepsilon_0))$ is subtracted from the data in line with the analysis done in refs 27 and 28.

For single-component molecular liquids, for which the polarization decays with a single-exponential functional shape, the measured permittivity can often be described by a Debye relaxation model

$$\hat{\varepsilon}(\nu) - \varepsilon_\infty = \frac{S}{1 + 2\pi i\nu\tau} \tag{3}$$

where ε_∞ is the permittivity at infinite frequency, τ is the characteristic relaxation time and S the amplitude of the Debye mode. For a mixture of different dipolar species that show distinct time constants, each species can be described with a separate Debye mode. The amplitude S of each relaxation mode is proportional to the corresponding concentration c of dipoles and their effective dipole strength μ_{eff} through the Cavell equation,[29]

$$S = \frac{\varepsilon_s}{3\left(\varepsilon_s + \frac{1}{3}(1 - \varepsilon_s)\right)} \frac{N_{\mathrm{A}}}{k_{\mathrm{B}} T \varepsilon_0} {\mu_{eff}}^2 c \tag{4}$$

where ε_s is the static permittivity.

Samples. We studied the molecular anion phosphorylethanolamine (PE) in combination with different cations $M = NH_4^+$, Cs^+, K^+, Na^+, Ca^{2+} (see the structure formula of NaPE in Figure 1). PE forms the polar headgroup of a very common class of phospholipids, the phosphatidylethanolamines. Phosphorylethanolamine (98% purity) was obtained in pure form from Sigma-Aldrich. After dissolving PE in water (isotopically diluted or pure H_2O), a stoichiometric amount of hydroxide with the appropriate cation was added to obtain the salt solution of interest. The solutions were brought to pH = 8 by adding a small amount (a few percent maximum) of a solution with the same PE concentration. At pH = 8, the phosphate fragment is fully deprotonated and the ammonium group is positively charged,[30] as illustrated in Figure 1. A small amount of D_2O was added to the samples used in the fs-IR experiments, such that the solvent consisted of 8% $HDO{:}H_2O$. Samples for the dielectric experiments were prepared in volumetric flasks, in order to obtain well-defined molar concentrations of all components, required for the analysis of the dielectric data. Throughout this manuscript all concentrations mentioned will refer to the concentration of PE^-, meaning that the amount of bivalent Ca^{2+} cations in a solution of $Ca_{0.5}PE$ is only half the amount of Na^+ cations in a solution of NaPE of the same (PE) concentration.

■ FS-IR RESULTS

Figure 2 shows transient differential absorption spectra for ten pump−probe delay times for a 1 mol/kg solution of KPE. The negative absorption change (bleach) for short delay times results from the depletion of the ground state and stimulated emission of the probe from the $v = 1$ to the $v = 0$ state for the excited fraction (a few percent) of HDO molecules. The excited state decays in a few picoseconds to a differential absorption spectrum that remains constant on the time scale of the experiment ($10 < t/\mathrm{ps} < 100$). This differential absorption signal results from the increase of temperature after the excitation has completely equilibrated over all the bath modes, and hence will be referred to as the thermal difference

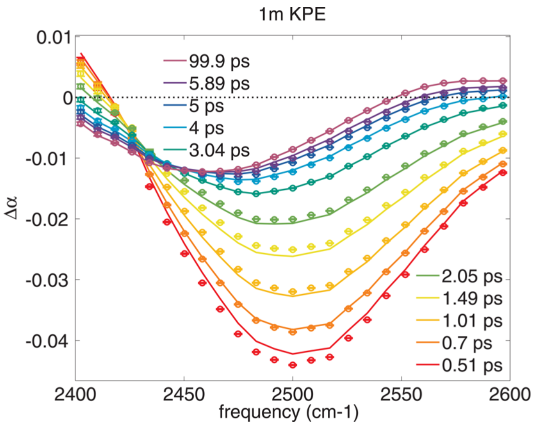


Figure 2. Transient absorption spectra at different delay times for a 1 mol/kg solution of KPE. The solid lines are the results of a fit to the data of the kinetic model described in the text.

spectrum. To determine the time constant of the vibrational relaxation, we fit a kinetic model to the data in which the excited state relaxes to the thermalized, hot ground state through an intermediate state. We assumed the spectral response of the intermediate state to be the same as that of the ground state, meaning that this state does not possess a differential absorption spectrum. This model provides an accurate description of the vibrational decay and thermalization of neat 8% $HDO:H_2O$.[26] The solid lines in Figure 2 represent the fit of the model to the 1 mol/kg KPE data. For all the different salts and concentrations the vibrational relaxation times obtained are very close to the value of 1.8 ps obtained for neat 8% $HDO:H_2O$.

The fit yields the dynamics of the rise of the thermal difference spectrum. We can thus subtract its contribution at all delay times from both the parallel and perpendicular probed signals to obtain the polarization dependent absorption changes that only result from the excited OD oscillators. Using these signals in equation eq 2 provides us with the anisotropy parameter $R(t)$ of the excitation of the OD vibrations.

The anisotropy decay for different concentrations of NaPE are presented in Figure 3. The reorientation dynamics of neat 8% $HDO:H_2O$ decays monoexponentially for delay times $t >$ 0.4 ps with a time constant of 2.5 ps, similar to what was found in previous work.[26] The reorientation dynamics for pump−probe delay times shorter than 0.4 ps were not fitted. Hence, we do not include the ultrafast decay component of the anisotropy due to librational motions. For increasing concentrations of NaPE, the anisotropy decay becomes slower. The curves in the logarithmic plot of Figure 3 clearly show that the decay is not single exponential. A free biexponential fit to the reorientation dynamics of three independently measured data sets of 2 mol/kg NaPE yielded a fast reorientation time of 2.3 ± 0.2 ps and a slow reorientation time of 20 ± 5 ps.

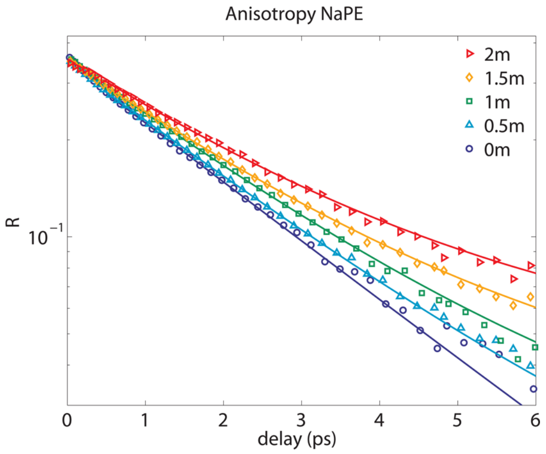


Figure 3. Anisotropy decays for different concentrations of NaPE. With increasing concentration a larger fraction of the OD groups shows slower reorientation.

Figure 4 shows the anisotropy decays obtained for 1 mol/kg solutions of NaPE, KPE, NaI and KI. The curves of all measured salts and concentrations are very well described by a biexponential function with a fast and a slow component. Previous work on aqueous solutions of osmolytes also found a biexponential behavior of the anisotropy dynamics.[31−33] It was shown that even in concentrated solutions a fast relaxation component persists with a time constant similar to the bulk water reorientation time. For such concentrated solutions it is very unlikely that there are many water molecules in an environment that is like bulk water. However, at high solute concentrations there is still a considerable amount of OD groups that have the freedom to reorient on a time scale like in bulk water.

Here we also find a component of the anisotropy decay that has a bulk-like reorientation time. This component includes both water molecules with a bulk-like reorientation mechanism, and the wobbling motion of OD groups that are hydrogen-bonded to the negatively charged oxygens of the phosphate group of PE^-. Wobbling motions[34,35] can lead to a relatively fast but incomplete reorientation of water and have been observed by many researchers.[16,20,36−38] It has recently been shown that the wobbling motion of OD groups hydrogen-bonded to halide anions happens with a time-constant of 2 ps.[16] Since we expect the same components for the solutions of lower concentrations, we fix the time constant of 2.5 ps in all fits. The slow component represents OD groups for which the reorientation is severely hindered. For all concentrations we fix this time constant to 20 ps according to the value we found from a biexponential fit to the 2 mol/kg anisotropy decays of KPE and NaPE. It should be noted that the error margin on the fitted time constants is quite large due to the fact that we can

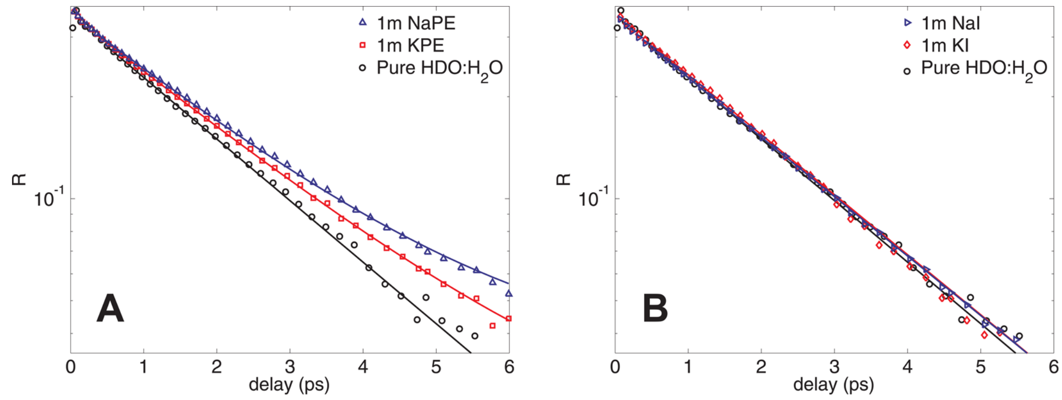


Figure 4. Comparison of the anisotropy decay of different salt solutions, all at a concentration of 1 mol/kg. (A) NaPE has a stronger effect of immobilization of water molecules than KPE. (B) This difference is absent in the case of the corresponding iodide salts and therefore finds its origin in a cooperative effect between the cation and the PE^- ion.

only probe the anisotropy over a time interval of 8 ps, due to the short vibrational lifetime (1.8 ps). The fraction of slowly reorienting water $f_{slow} = A_{slow}/(A_{slow}+A_{fast})$ follows from the fitted fast and slow amplitudes, A_{fast} and A_{slow} respectively. The biexponential fits are represented by the solid lines in Figure 3 and Figure 4. The slow water fraction f_{slow} is shown in Figure 5 for all data (salt solutions and concentrations). We obtain the hydration number Z, the number of slow OD groups per solute molecule, from the slope of a straight line fitted through the points (solid lines in Figure 5). The hydration number decreases in the cation series $Ca^{2+} > Na^{+} > K^{+} \approx Cs^{+} > NH_4^{+}$.

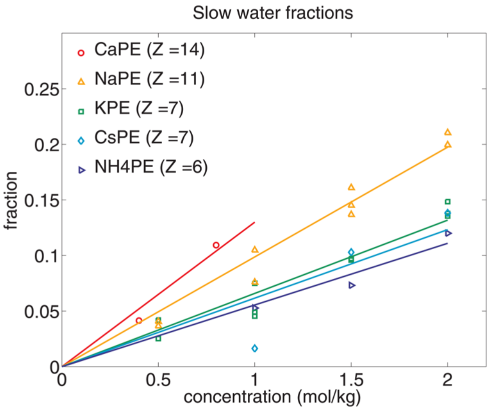


Figure 5. Slow fraction of water ($\tau_{slow} \sim 20$ ps) for PE solutions with different cations, as inferred from fs-IR experiments. The slope of the lines represents the hydration number Z. Cations with a larger surface charge density are seen to slow down a larger number of water molecules.

GHZ-DR RESULTS

Figure 6 shows the real (blue, circles) and imaginary (red, squares) part of the dielectric function for different concentrations of NaPE. As already apparent from the raw data, the spectra show an intense relaxation mode (peak in ε'' and accompanying dispersion in ε') at frequencies ~0.3 to 1 GHz. Its amplitude increases with concentration, apparent from the increase of the peak in the dielectric loss upon the addition of solute. The electronic structure of the PE^- anion is such that the negative charge is located at the phosphate group and the positive charge is centered at the ammonium fragment. This charge separation leads to an intrinsically large dipole moment and the observed lower frequency relaxation can thus be assigned to the rotation of the PE^- ion.

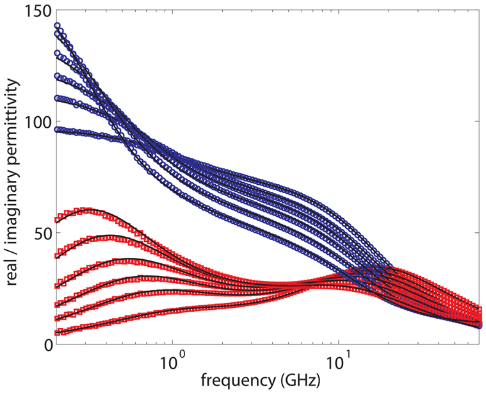


Figure 6. Real (blue, circles) and imaginary (red, squares) part of the dielectric function for solutions of 0.24, 0.48, 0.71, 0.93, 1.33, and 1.75 mol/kg of NaPE in water. The bulk water mode at 20 GHz is decreasing with concentration and a mode at low frequencies grows in with concentration. The latter mode is assigned to the reorientation of the zwitterion PE^-.

The observed frequency corresponds to a reorientation time of the solute on the order of 200 ps, increasing with increasing solute concentration to over 400 ps. We derive the effective dipole of the PE^- ions from the fitted mode amplitudes using the Cavell equation eq 4. For all salt solutions except $Ca_{0.5}PE$ the effective dipole is independent of both cation type and its concentration (Figure 8). The obtained dipole moments of 25 ± 1 D correspond well to the results of an *ab initio* calculation of a PE^- zwitterion in a dielectric continuum.[59] Interestingly, the effective dipole of PE^- in solutions of $Ca_{0.5}PE$ shows a significant decrease with concentration.

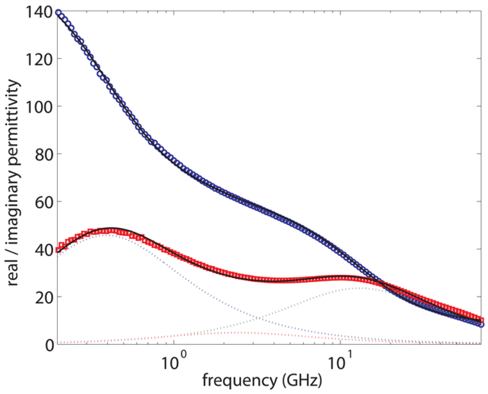


Figure 7. Results of a fit (solid line) of three Debye modes to the real (blue, circles) and imaginary (red, squares) part of the dielectric function of a 1.75 mol/kg solution of NaPE in water. The dotted lines represent the imaginary part of the three resolved modes. The amplitude and time constant of the slow water mode (middle) are obtained from the fs-IR experiments.

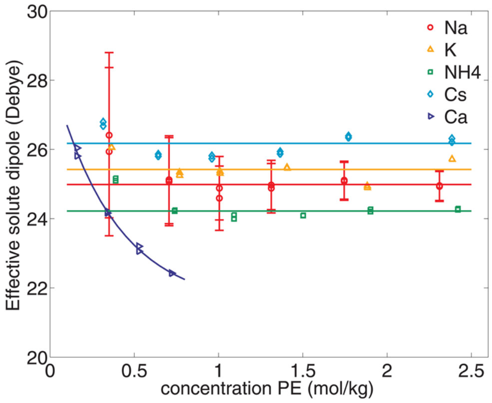


Figure 8. Effective dipole moment of PE^- as obtained from the amplitude of the low frequency mode using the Cavell equation (eq 4). The obtained values are in good agreement with an ab initio calculation of the PE^- dipole in a constant medium, which gives μ_{eff} = 24−33 D, depending on the conformation. The error bars for the NaPE data points are exemplary, the error bars for the other data points are of similar magnitude. The dipole moments of PE^- show no significant dependence on concentration, except if Ca^{2+} forms the counterion.

The data in Figure 6 shows another relaxation mode that is centered at ∼20 GHz. The amplitude of this relaxation decreases with increasing concentration and can be readily assigned to the reorientation of bulk-like water.[49]

From the fs-IR data, it is apparent that not all water molecules show bulk-like dynamics, and that a subensemble of water molecules exhibits distinctively slower rotational dynamics. Accordingly, we fit a superposition of three Debye modes to the experimental permittivity spectra, representing the solute rotation, the relaxation of slow water molecules, and the orientational relaxation of bulk-like water molecules. As the relaxation of the slow water subensemble is located in the spectrum between the intense bulk water relaxation and the lower frequency solute mode, it is difficult to unambiguously determine its amplitude and central frequency. Thus, we reduce the number of adjustable parameters and fix the fraction of slow water molecules in these fits to that obtained from our fs-IR experiments. Further, we fix the relaxation time of the slow water molecules. GHz-DR measures the first order correlation function of the macroscopic polarization, whereas fs-IR measures the second order correlation function of the transition dipole moments of the OD stretch vibration. The rotational correlation times of the two different experiments are found to be related to each other by a factor of 3.4.[50] We use a value of τ = 68 ps correspondsing to the value of 20 ps used in the fs-IR analysis. As can be seen in Figure 6 the fits are in excellent agreement with our experimental spectra. The good quality of the spectral decomposition into the three Debye modes is exemplified for the spectrum of a 1.75 mol/kg solution of NaPE in Figure 7.

After correction for kinetic depolarization[22] the sum of the amplitudes of the two water relaxation modes (slow water + bulk-like water) reproduce the total analytical water concentrations for KPE, NH_4PE and CsPE within experimental accuracy. For the samples with Ca^{2+} and Na^+ as counterions, the water concentration extracted from the sum of the amplitudes of the two water relaxation modes, is lower than the analytical water concentration, indicative of the presence of irrotationally bound water molecules within the hydration shell shells of those cations.[22]

■ DISCUSSION

The hydration numbers obtained from the analysis of the fs-IR data for solutions with low charge density cations like K^+, Cs^+ and NH_4^+ all close to $Z \approx 7$ (as shown in Figure 5). Considering that these three cations have a low surface charge density this suggests that the hydration number of KPE, CsPE, and NH_4PE is entirely due to the solvation of the PE^- anion. This hydration water likely corresponds to water molecules that are strongly hydrogen-bonded to the phosphate group or in the hydrophobic hydration shell of the C_2H_4 backbone of PE^-.

Figure 5 shows that a marked difference in hydration number exists between NaPE ($Z = 11 \pm 1$) and KPE ($Z = 7 \pm 1$). This means that there are more slow water molecules for NaPE than for KPE. This suggests that for NaPE not only the PE^- ion, but also the cation is responsible for the slower dynamics of water. Na^+ has a higher surface charge density than K^+, which indeed points to a stronger interaction of Na^+ with its solvation shell. The data on NaI and KI, however, are contradicting the hypothesis that the stronger retardation around NaPE compared to KPE is just caused by the cation. The anisotropy decays shown for the solutions of NaI and KI in Figure 4B reflect the reorientation of only the OD groups hydrogen-bonded to other water molecules (OD···O, see also ref 16), including those in the solvation shell of the cations. The anisotropy decays are identical to that of neat 8% HDO:H_2O, implying no retardation of the reorientation of OD groups of water molecules around either K^+ or Na^+. The difference between NaPE and KPE can therefore not be explained by a cationic effect alone, but implies a cooperative effect between the cation and the PE^- ion, present for sodium, but not for potassium. This observation is indicative of the formation of weak solvent separated ions pairs for sodium, similar to the longer ranged cooperative effects that were recently found in highly charged ion combinations such as $MgSO_4$.[15]

Positively and negatively charged ions affect the dynamics of their solvating water molecules in different ways. Cations tend to align the static dipoles of their hydrating water molecules through coulomb interaction.[15] In this configuration the water molecule can rotate around the dipolar axis, so that OD groups (or hydroxyls) can still reorient in a bulk-like fashion. In the vicinity of the PE^- ions, however, the OD groups of the cation hydration layer can form hydrogen-bonds to the negatively charged phosphate oxygens at the same time, thus decreasing the mobility of the OD groups. Such a hydration complex can be defined as a solvent separated ion pair.

This cooperative locking of water molecules in between PE^- and the cation only occurs if both the cation and the anion strongly interact with water. In the case of NaI and KI solutions, the iodide ions have a too low surface charge density to cooperatively lock water molecules with their cosolvated cations. When iodide is replaced by the PE^- anion, a distinct difference between the Na^+ and K^+ solutions becomes apparent: In the case of NaPE about three water molecules show slower dynamics in addition to the slow water molecules that were associated with the hydration of PE^-. In case the cation is Ca^{2+}, with a yet higher surface charge density than Na^+, the effect is even more pronounced ($Z = 14 \pm 2$). Both the PE^- ion and a cation with large surface charge density are thus required to form such a solvent separated ion pair. The obvious molecular group in PE^- to participate in these hydration complexes is the negatively charged phosphate group. It is noteworthy that we observe the rotation time of water molecules located between the phosphate group and the cation of the solvent separated ion-pair to be in the order of 20 ps. This time constant implies that the lifetime of the Ca^{2+}–PE^- and Na^+–PE^- ion-pairs must exceed 20 ps in order to observe a noticeable retardation of the water dynamics.

We thus find strong evidence for the formation of solvent separated ion pairs for NaPE and $Ca(PE)_2$ solutions. Dielectric spectroscopy is in particular powerful in detecting such ion pair species as an ion aggregate possesses an intrinsic electrical dipole moment. In case the lifetime of such an ion aggregate is comparable or longer than its rotational correlation time, the rotation of the aggregate will be observed as a strong low frequency relaxation in the DR spectrum.[51]

In the present case the PE^- anion itself has an electrical dipole owing to its zwitterionic chemical structure. The formation of an ion-pair via interaction of the cation with the negatively charged phosphate group of PE^- would balance part of the negative charge located at the phosphate group. An M^+PE^- ion pair would therefore have a smaller electrical dipole than the PE^- anion itself.

For all studied samples (except for Ca^{2+}), we observe μ_{eff} to be constant at all studied concentrations. This observation implies that ion pairs between PE^- and the monovalent alkalimetal ions (including Na^-) are too short-lived (<200 ps) to be detected with DRS. This provides an upper boundary for the persistence times of the PE^- cation contacts as inferred from our fs-IR results. Only for Ca^{2+} we observe μ_{eff} to decrease with increasing concentration. This indicates that only for Ca^{2+} the solvent separated ion pair exists longer than 200 ps. The observation of a reduced PE^- dipole in solutions with co-solvated Ca^{2+} cations is in line with recent phase-sensitive SFG results.[13] A reduced ordering of water was found at water-membrane interfaces containing Ca^{2+} cations.[13] Such an effect can be explained by the binding of Ca^{2+} ions to the phosphate groups of membrane lipids that lead to a reduced dipole-induced alignment of the water solvating the headgroups.

Our findings agree with previous theoretical work and molecular dynamics simulations that showed that the interaction between membrane lipids and co-solvated ions is highly dependent on the charge density of the ions. Cations with a high surface charge density like sodium and calcium were found to have a higher propensity at the lipid surface.[8,12,14] The binding of Na^+ and Ca^{2+} to particular anionic groups may also play a role in the denaturation of biomolecules (e.g., proteins and DNA), as cations with a higher surface charge density typically destabilize protein conformation.[52] To explain denaturation effects, most recent work favors the picture of direct ion-protein interaction rather than a long-range structure making or breaking effect on water.[53−58] Our observation of solvent separated ion pairs between phosphate and Na^+ or Ca^{2+} is strong support for direct ion−protein interaction.

CONCLUSIONS

We studied the dynamics of water in solutions of phosphorylethanolamine (PE^-) and different types of cations using femtosecond infrared (fs-IR) pump−probe spectroscopy. We find that cations with a large surface charge density like sodium and calcium form a hydration complex or solvent separated ion pair with PE^-. For larger cations like potassium or ammonium such ion pairs are not observed. The dielectric spectra in the region of 200 MHz to 70 GHz are consistent with these results. In these spectra we find a low frequency mode that we assign to the reorientation of the PE^- ion. From this mode we determined the effective dipole moment of PE^- to be 25 ± 1 D, consistent with ab initio calculations. The effective dipole shows a concentration dependence in the case of Ca^{2+} as counterion, which is indicative of the formation of ion pairs with lifetimes exceeding 200 ps. Since such a concentration dependence is not observed for solutions of NaPE, but the fs-IR results provide evidence for the formation of ion pairs, the lifetime of Na^+-PE^- complexes is in the range 20−200 ps.

AUTHOR INFORMATION

Corresponding Author

*(H.J.B.) E-mail: bakker@amolf.nl.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work is part of the research program of the “Stichting voor Fundamenteel Onderzoek der Materie (FOM)”, which is financially supported by the “Nederlandse organisatie voor Wetenschappelijk Onderzoek (NWO)”.

REFERENCES

(1) Berkowitz, M.; Bostick, D.; Pandit, S. Aqueous solutions next to phospholipid membrane surfaces: Insights from simulations. *Chem. Rev.* **2006**, *106*, 1527−1539.

(2) Hakizimana, P.; Masureel, M.; Gbaguidi, B.; Ruysschaert, J.; Govaerts, C. Interactions between phosphatidylethanolamine headgroup and LmrP, a multidrug transporter. *J. Biol. Chem.* **2008**, *283*, 9369−9376.

(3) Bursing, H.; Kundu, S.; Vohringer, P. Solvation Dynamics at Aqueous Lipid Membrane Interfaces Explored by Temperature-Dependent 3-Pulse-Echo Peak Shifts:? Influence of the Lipid Polymorphism. *J. Phys. Chem. B* **2003**, *107*, 2404−2414.

(4) Lee, A. G. How lipids affect the activities of integral membrane proteins. *Biochim. Biophys. Acta* **2004**, *1666*, 62−87.

(5) Berntsen, P.; Svanberg, C.; Swenson, J. Interplay between Hydration Water and Headgroup Dynamics in Lipid Bilayers. *J. Phys. Chem. B* **2011**, *115*, 1825−1832.

(6) Clarke, R.; Lupfert, C. Influence of Anions and Cations on the Dipole Potential of Phosphatidylcholine Vesicles: A Basis for the Hofmeister Effect. *Biophys. J.* **1999**, *76*, 2614−2624.

(7) Böckmann, R. A.; Hac, A.; Grubmüller, H. Effect of Sodium Chloride on a Lipid Bilayer. *Biophys. J.* **2003**, *85*, 1647−1655.

(8) Garcia-Celma, J. J.; Hatahet, L.; Kunz, W.; Fendler, K. Specific Anion and Cation Binding to Lipid Membranes Investigated on a Solid Supported Membrane. *Langmuir* **2007**, *23*, 10074−10080.

(9) Leontidis, E.; Aroti, A.; Belloni, L. Liquid Expanded Monolayers of Lipids As Model Systems to Understand the Anionic Hofmeister Series: 1. A Tale of Models. *J. Phys. Chem. B* **2009**, *113*, 1447−1459.

(10) Leontidis, E.; Aroti, A. Liquid Expanded Monolayers of Lipids As Model Systems to Understand the Anionic Hofmeister Series: 2. Ion Partitioning Is Mostly a Matter of Size. *J. Phys. Chem. B* **2009**, *113*, 1460−1467.

(11) Casillas-Ituarte, N.; Chen, X.; Castada, H.; Allen, H. Na^+ and Ca^{2+} Effect on the Hydration and Orientation of the Phosphate Group of DPPC at Air-Water and Air-Hydrated Silica Interfaces. *J. Phys. Chem. B* **2010**, *114*, 9485−9495.

(12) Vacha, R.; Jurkiewicz, P.; Petrov, M.; Berkowitz, M.; Bockmann, R.; 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.

(13) Chen, X.; Hua, W.; Huang, Z.; Allen, H. Interfacial Water Structure Associated with Phospholipid Membranes Studied by Phase-Sensitive Vibrational Sum Frequency Generation Spectroscopy. *J. Am. Chem. Soc.* **2010**, *132*, 11336−11342.

(14) Jurkiewicz, P.; Cwiklik, L.; Vojtiskova, A.; Jungwirth, P.; Hof, M. Structure, Dynamics, and Hydration of POPC/POPS Bilayers Suspended in NaCl, KCl, and CsCl Solutions. *Biochim. Biophys. Acta: Biomembr.* **2012**, *1818*, 609−616.

(15) Tielrooij, K.-J.; Garcia-Araez, N.; Bonn, M.; Bakker, H. Cooperativity in Ion Hydration. *Science* **2010**, *328*, 1006−1009.

(16) van der Post, S.; Bakker, H. The Combined Effect of Cations and Anions on the Dynamics of Water. *Phys. Chem. Chem. Phys.* **2012**, *14*, 6280−6288.

(17) Kropman, M. F.; Bakker, H. J. Dynamics of Water Molecules in Aqueous Sovation Shells. *Science* **2001**, *291*, 2118−2120.

(18) Omta, A. W.; Kropman, M. F.; Woutersen, S.; Bakker, H. J. Negligible Effect of Ions on the Hydrogen-bond Structure in Liquid Water. *Science* **2003**, *301*, 347−349.

(19) Moilanen, D. E.; Fenn, E. E.; Wong, D.; Fayer, M. D. Water Dynamics at the Interface in AOT Reverse Micelles. *J. Phys. Chem. B* **2009**, *113*, 8560−8568.

(20) Ji, M.; Odelius, M.; Gaffney, K. Large Angular Jump Mechanism Observed for Hydrogen Bond Exchange in Aqueous Perchlorate Solution. *Science* **2010**, *328*, 1003−1005.

(21) Barthel, J.; Bachhuber, K.; Buchner, R.; Hetzenauer, H. Dielectric Spectra of some Common Solvents in the Microwave Region. Water and Lower Alcohols. *Chem. Phys. Lett.* **1990**, *165*, 369−373.

(22) Buchner, R.; Hefter, G. T.; May, P. M. Dielectric Relaxation of Acqueous NaCl Solutions. *J. Phys. Chem. A* **1999**, *103*, 1−9.

(23) Buchner, R.; Hölzl, C.; Stauber, J.; Barthel, J. Dielectric Spectroscopy of Ion-pairing and Hydration in Aqueous Tetra-n-alkylammonium Halide Solutions. *Phys. Chem. Chem. Phys.* **2002**, *4*, 2169−2179.

(24) Tielrooij, K.-J.; Hunger, J.; Buchner, R.; Bonn, M.; Bakker, H. Influence of Concentration and Temperature on the Dynamics of Water in the Hydrophobic Hydration Shell of Tetramethylurea. *J. Am. Chem. Soc.* **2010**, *132*, 15671−15678.

(25) Zasetsky, A. Y. Dielectric Relaxation in LiquidWater: Two Fractions or Two Dynamics? *Phys. Rev. Lett.* **2011**, *107*, 117601.

(26) Rezus, Y. L. A.; Bakker, H. J. On the Orientational Relaxation of HDO in Liquid Water. *J. Chem. Phys.* **2005**, *123* (114502), 1−7.

(27) Ensing, W.; Hunger, J.; Ottosson, N.; Bakker, H. On the Orientational Mobility of Water Molecules in Proton and Sodium Terminated Nafion Membranes. *J. Phys. Chem. C* **2013**, *117*, 12930−12935.

(28) Tielrooij, K.-J.; van der Post, S.; Hunger, J.; Bonn, M.; Bakker, H. Anisotropic Water Reorientation around Ions. *J. Phys. Chem. B* **2011**, *115*, 12638−12647.

(29) Buchner, R.; Chen, T.; Hefter, G. Complexity in "Simple" Electrolyte Solutions: Ion Pairing in $MgSO_4(aq)$. *J. Phys. Chem. B* **2004**, *108*, 2365−2375.

(30) Myller, A.; Karhe, J.; Pakkanen, T. Preparation of Amino-functionalized TiO2 Surfaces by Binding of Organophosphates. *Appl. Surf. Sci.* **2010**, *257*, 1616−1622.

(31) Rezus, Y. L. A.; Bakker, H. J. Observation of Immobilized Water Molecules around Hydrophobic Groups. *Phys. Rev. Lett.* **2007**, *99* (148301), 1−4.

(32) Rezus, Y. L. A.; Bakker, H. J. Strong Slowing Down of Water Reorientation in Mixtures of Water and Tetramethylurea. *J. Phys. Chem. A* **2008**, *112*, 2355−2361.

(33) Petersen, C.; Bakulin, A. A.; Pavelyev, V. G.; Pshenichnikov, M. S.; Bakker, H. J. Femtosecond Midinfrared Study of Aggregation Behavior in Aqueous Solutions of Amphiphilic Molecules. *J. Chem. Phys.* **2010**, *133* (164514), 1−8.

(34) Wang, C. C.; Pecora, R. Time-correlation Functions for Restricted Rotational Diffusion. *J. Chem. Phys.* **1980**, *72*, 5333−5340.

(35) Lipari, G.; Szabo, A. Effect of Librational Motion on Fluorescence Depolarization and Nuclear Magnetic Resonance Relaxation in Biomolecules and Membranes. *Biophys. J.* **1980**, *30*, 489−506.

(36) Sahu, K.; Mondal, S. K.; Ghosh, S.; Roy, D.; Sen, P. Temperature Dependence of Solvation Dynamics and Anisotropy Decay in a Protein. ANS in Bovine Serum Albumin. *J. Chem. Phys.* **2006**, *124* (124909), 1−7.

(37) Piletic, I. R.; Moilanen, D. E.; Spry, D. B.; Levinger, N. E.; Fayer, M. D. Testing the Core/Shell Model of Nanoconfined Water in Reverse Micelles Using Linear and Nonlinear IR Spectroscopy. *J. Phys. Chem. A* **2006**, *110*, 4985−4999.

(38) Son, H.; Jin, H.; Choi, S.; Jung, H.; Park, S. Infrared Probing of Equilibrium and Dynamics of Metal-Selenocyanate Ion Pairs in *N,N*-Dimethylformamide Solutions. *J. Phys. Chem. B* **2012**, *116*, 9152−9159.

(39) Becke, A. Density-functional Thermochemistry. III. The Role of Exact Exchange. *J. Chem. Phys.* **1993**, *98*, 5648−5652.

(40) Stephens, P.; Devlin, F.; Chabalowski, C.; Frisch, M. Ab Initio Calculation of Vibrational Absorption and Circular Dichroism Spectra Using Density Functional Force Fields. *J. Phys. Chem.* **1994**, *98*, 11623−11627.

(41) Perdew, J.; Wang, Y. Accurate and Simple Analytic Representation of the Electron-gas Correlation Energy. *Phys. Rev. B* **1992**, *45*, 13244−13249.

(42) Perdew, J.; Burke, K.; Ernzerhof, M. Generalized Gradient Approximation Made Simple. *Phys. Rev. Lett.* **1996**, *77*, 3865−3868.

(43) Neese, F. The ORCA Program System. *Wiley Interdiscip. Rev.: Comput. Mol. Sci.* **2012**, *2*, 73−78.

(44) Schafer, A.; Horn, H.; Ahlrichs, R. Fully Optimized Contracted Gaussian Basis Sets for Atoms Li to Kr. *J. Chem. Phys.* **1992**, *97*, 2571−2577.

(45) Weigend, F.; Ahlrichs, R. Balanced Basis Sets of Split Valence, Triple Zeta Valence and Quadruple Zeta Valence Quality for H to Rn: Design and Assessment of Accuracy. *Phys. Chem. Chem. Phys.* **2005**, *7*, 3297−3305.

(46) Vahtras, O.; Almlof, J.; Feyereisen, M. Integral Approximations for LCAO-SCF Calculations. *Chem. Phys. Lett.* **1993**, *213*, 514−518.

(47) Neese, F. An Improvement of the Resolution of the Identity Approximation for the Calculation of the Coulomb Matrix. *Comput. Chem.* **2003**, *97*, 2571−2577.

(48) Sinnecker, S.; Rajendran, A.; Klamt, A.; Diedenhofen, M.; Neese, F. Calculation of Solvent Shifts on Electronic g-Tensors with the Conductor-Like Screening Model (COSMO) and Its Self-Consistent Generalization to Real Solvents (Direct COSMO-RS). *J. Phys. Chem. A* **2006**, *110*, 2235−2245.

(49) Fukasawa, T.; Sato, T.; Watanabe, J.; Hama, Y.; Kunz, W.; Buchner, R. Relation between Dielectric and Low-Frequency Raman Spectra of Hydrogen-Bond Liquids. *Phys. Rev. Lett.* **2005**, *95* (197802), 1−4.

(50) Tielrooij, K. J.; Petersen, C.; Rezus, Y. L. A.; Bakker, H. J. Reorientation of HDO in Liquid H_2O at Different Temperatures: Comparison of First and Second Order Correlation Functions. *Chem. Phys. Lett.* **2009**, *471*, 71−74.

(51) Buchner, R. G. Hefter Interactions and Dynamics in Electrolyte Solutions by Dielectric Spectroscopy. *Phys. Chem. Chem. Phys.* **2009**, *11*, 8984−8999.

(52) Marcus, Y. Effect of Ions on the Structure of Water: Structure Making and Breaking. *Chem. Rev.* **2009**, *109*, 1346−1370.

(53) Mason, P.; Neilson, G.; Dempsey, C.; Barnes, A.; Cruickshank, J. The Hydration Structure of Guanidinium and Thiocyanate Ions: Implications for Protein Stability in Aqueous Solution. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4557−4561.

(54) Smith, J.; Saykally, R.; Geissler, P. The Effects of Dissolved Halide Anions on Hydrogen Bonding in Liquid Water. *J. Am. Chem. Soc.* **2007**, *129*, 13847−13856.

(55) Mancinelli, R.; Botti, A.; Bruni, F.; Ricci, M.; Soper, A. Hydration of Sodium, Potassium, and Chloride Ions in Solution and the Concept of Structure Maker/Breaker. *J. Phys. Chem. B* **2007**, *111*, 13570−13577.

(56) Das, A.; Mukhopadhyay, C. Urea-Mediated Protein Denaturation: A Consensus View. *J. Phys. Chem. B* **2009**, *113*, 12816−12824.

(57) Schmidt, D. A.; Birer, O.; Funkner, S.; Born, B. P.; Gnanasekaran, R.; Schwaab, G. W.; Leitner, D. M.; Havenith, M. Rattling in the Cage: Ions as Probes of Sub-picosecond Water Network Dynamics. *J. Am. Chem. Soc.* **2009**, *131*, 18512−18517.

(58) Lin, Y.; Auer, B.; Skinner, J. Water structure, Dynamics, and Vibrational Spectroscopy in Sodium Bromide Solutions. *J. Chem. Phys.* **2009**, *131* (144511), 1−13.

(59) Quantum mechanical geometry optimizations for several conformers of the PE^- anion were performed using density functional theory (B3LYP[39,40] and PBE[41,42]) as provided by the ORCA program package.[43] For all calculations, the TZVPP basis set[44,45] was used within the resolution of identity.[46,47] Solvation effects were taken into account by the COSMO model,[48] taking the dielectric constant of water $\varepsilon_s = 80$ as the permittivity of the medium. Dipole moments were calculated assuming the geometric center as the pivot. These calculations give values for μ_{eff} = 24−33 D, depending on the conformation.