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The effect of lithium ions on the hydrophobic effect: does lithium affect hydrophobicity differently than other ions?☆

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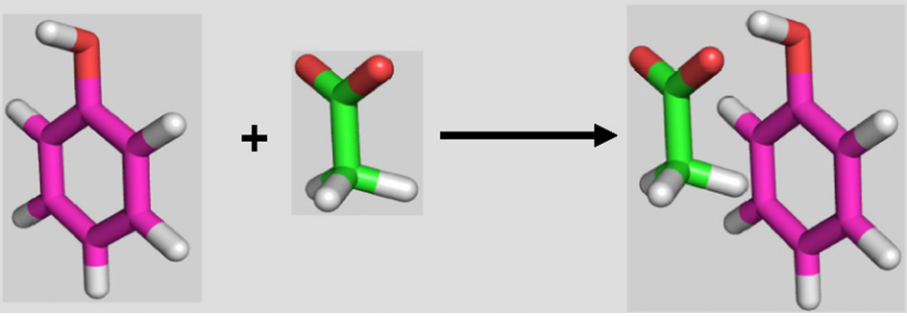
HIGHLIGHTS

- We determine how cations influence the hydrophobic effect.
- Cations with high charge density are shown to enhance the hydrophobic effect.
- Cations with low charge density are shown to diminish the hydrophobic effect.
- Lithium cations influence the hydrophobic effect as expected from their ionic size.
- No lithium anomaly is observed in the methyl-phenyl ring model system.

GRAPHICAL ABSTRACT



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ABSTRACT

Ionic species have been shown to significantly perturb the interactions between non-polar solutes in aqueous solution. These perturbations are often analyzed in terms of the interactions existing between hydrophobic surfaces and ions. It has been known for some time, that ions with a high charge density are repelled from hydrophobic surfaces while ions with a low charge density tend to stick to these surfaces. Therefore, from a continuum model standpoint, small monovalent ions promote hydrophobicity by minimizing the exposed hydrophobic surface area, while "sticky" large monovalent ions interact with the hydrophobic surfaces and discourage aggregation. However, the charge-dense lithium ion often exhibits anomalous behaviour different from these predicted trends: instead of enhancing, the addition of lithium ions often seems to weaken the hydrophobic effect and on the contrary help dissolve hydrophobic molecules. This weakening of apparent hydrophobicity is considered to be one of the reasons for the protein denaturing properties of lithium salts. Recent theoretical and experimental results however have shown that lithium cations can interact with a variety of molecular functional groups. This suggests that this apparent lithium-induced lowering of hydrophobicity, that is often reported in the literature may be a result of specific interactions between these molecular functional groups and lithium, rather than weakening the interaction between hydrophobic surfaces. This work examines these possibilities by studying the effect of various cations on the simple hydrophobic interaction existing between methyl and phenyl contact-pairs and demonstrates that the effect of lithium cations on the hydrophobic effect follows the trend predicted by continuum models. In other words, the influence of an ion on the hydrophobic interaction between two non-polar surfaces is a function of the interaction of that ion and each non-polar surface.

1. Introduction

The hydrophobic effect is perhaps one of the most biologically important solvent-induced interactions between non-polar aqueous

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solutes [1]. The magnitude of this interaction has been shown to be significantly influenced by the presence of electrolyte co-solutes. This influence is not merely a function of ionic strength, but is also highly dependent on the nature of the ionic species as well. For example, the solubility of proteins (the Hofmeister Effect) [2], the critical micelle concentration of surfactants [3], catalytic activity of enzymes [4], salt-induced stability of proteins [5,6], the modulation of protein–ligand interactions [7] and the salting-out of non-polar molecules [8] from aqueous solution are all dependent on the nature of the salt co-solute.

These salt specific influences on hydrophobicity have originally been interpreted from a continuum electrostatic model standpoint [9]. In this case, the aqueous solvent is represented by a high dielectric continuum, the salt ions are represented by spherical charges and the hydrophobic surface is a region with a low dielectric constant. Based on this model, the interaction between the salt ion and the hydrophobic moiety is a sum of two opposing forces: first, the repulsive "image force" that exists between an ion in a region of high dielectric constant and a nearby region of lower dielectric constant which results from the preference of the electric field of the ion to remain in a region of high dielectric constant [6,10–12]; second, the cohesive force of water which results from the strong interaction between water molecules and tends to minimize the water-exposed surface area of dissolved solutes [11,13]. From these considerations it can be inferred that small monovalent ions are repelled from the vicinity of hydrophobic moieties, while large monovalent ions should preferentially accumulate in the hydrophobic neighbourhood.

This interpretation has been extended to the molecular level by Collins [11], in which he provides a microscopic ruler for the interaction between ions and hydrophobic surfaces: monovalent anions having a radius smaller than 1.78 Å and monovalent cations having a radius smaller than 1.06 Å are repelled by hydrophobic surfaces, while larger monovalent ions are sticky and adsorb to the hydrophobic surface. For the specific case of alkali ions, this translates into lithium ions being strongly and sodium ions being weakly repelled from hydrophobic surfaces; while the capacity of potassium, rubidium and cesium ions to absorb on hydrophobic surfaces increases with the ion radius. The influence of ionic species on the hydrophobic effect is therefore a function of the interaction between ions and hydrophobic surfaces. Solutions that contain a large number of small monovalent ions are more stable if image "repulsion" forces are reduced by hydrophobic surface minimization (i.e. the hydrophobic effect is enhanced). Conversely, the adsorption of large monovalent ions on hydrophobic surfaces discourages the aggregation of hydrophobes. More recent molecular level calculations also follow the trend predicted by continuum models [14–19]. These calculations also indicate that large monovalent ions are attracted to non-polar moieties in aqueous solutions and that the presence of ions with high charge density in solution enhances hydrophobicity, while the presence of ions with low charge density disrupts hydrophobic aggregation.

The work cited above demonstrates that theory quite clearly predicts how various monobasic salts should affect hydrophobicity. However, experimental studies of the effects of cationic species on hydrophobicity often yield results that are in apparent contradiction with theoretical predictions. In particular lithium salts, which theoretically ought to enhance hydrophobic aggregation, often seem to weaken interactions that are usually thought to be the result of the hydrophobic effect. This is seen most notably in experimental studies of salt effects on the aqueous solubility of aliphatic and aromatic compounds [20–23]. For these compounds, the salting out capability of chlorides decrease from NaCl to CsCl, in other words, the strength of the hydrophobic effect is reduced as the size of the cation co-solute is increased, [20,21,23] this is consistent with theory. However, for the same aliphatic and aromatic compounds listed above, LiCl does not behave like a strong salting-out agent at all but exhibits behaviour similar to that of KCl or RbCl. [20,21,23] In other words, although the lithium cation has a large charge density, it behaves like a much larger ion and enhances hydrophobicity to a much more modest degree than that predicted by theory. Examples of this lithium anomaly can be seen in other biophysical phenomena. Studies of salt effects on micelle formation indicate that the addition of salt species promote the formation of micelles, however, although the efficiency of micelle promotion increases from CsCl to NaCl, it abruptly drops with LiCl [24–29]. Salt studies of cyclodextrin inclusion complexes indicate that LiCl salts promote the incorporation of hydrophobic hosts to a much smaller degree than NaCl and KCl [30]. Protein folding studies have also shown that in contrast to sodium chlorides that stabilize the protein folded state, lithium chloride stabilizes protein structure to a much more modest degree [5,6,31,32]. This anomalous behaviour of lithium cation has been explained by invoking the small radius and the lower water coordination number of the lithium cation [33,34]. However, other studies indicate that this anomalous behaviour of lithium is not universal and in certain cases LiCl has been shown to actually enhance hydrophobic interactions between non-polar moieties to a greater degree than other chloride salts [35–39]; thereby, ruling out the hydration properties of lithium cation as the only reason for its "anomalous" behaviour.

A closer examination of the experimental data cited above indicates that many of the model systems studied above are too complex and the interactions between lithium ion and these molecules can not be simply represented as that of a spherical charge and a hydrophobic surface. Lithium ions can interact with amide moiety of the polypeptide chain [40–43], therefore it is possible that any stabilizing effect that the cation has on protein hydrophobicity may be neutralized by interactions with the polypeptide backbone. The polar head groups of non-ionic surfactants [44–50] as well as cyclodextrin [51] have also been shown to interact with lithium cations, preventing a simple interpretation of the critical micelle concentration (or cyclodextrin incorporation) results in terms of the ion-hydrophobic surface interaction. Lithium ions have also been shown to interact with aromatic groups [52–55] and perhaps even with methyl moieties [56]. These interactions will contribute favourably to the solvation of these groups in water, complicating the interpretation of the solubility data. These results point to a previously unnoticed flaw in the model systems used to quantify the effects of ions on hydrophobicity: namely, that most of these model systems contain moieties that can potentially interact with lithium. Therefore, the observed discrepancy observed between theoretical predictions and experimental data may indeed be the result of the interaction of lithium ions with specific segments of hydrophobic molecules. On the other hand, in the instances cited above where the influence of lithium cation on hydrophobicity is in agreement with the predictions of continuum models, the hydrophobic molecules are simple and are less likely to interact with the lithium cation.

This work studies the effects of ionic co-solutes on the hydrophobic aggregation of methyl and phenyl moieties, a hydrophobic interaction that is often observed in the interior of proteins [57,58]. The model system chosen is the contact pair that forms between the phenol molecule and the acetate ion [59,60]. This system is simple enough to allow the complete isolation of the contribution of hydrophobicity to contact pair formation. The phenol molecule shows appreciable fluorescence in the excited state [61], allowing for low concentrations of phenol to be used and therefore the study of contact pair formation is not complicated by the formation of larger phenyl aggregates. The formation of contact pairs between phenol and acetate can be adequately probed via fluorescence because acetate quenches excited phenol via a reaction controlled mechanism [59,60]. In other words, the quenching of phenol by acetate initially involves the formation of an encounter complex, followed by rearrangement of the encounter complex to facilitate quenching via a proton transfer mechanism [62]. The formation of the encounter complex between phenol and acetate ion is dependent on a variety of

interactions including the hydrophobic interaction between the phenyl ring and the methyl group of the acetate ion.

In this work we have successfully isolated the contribution of hydrophobicity to the interaction between methyl and phenyl groups in aqueous solutions and we have also unambiguously determined how ions influence the aggregation of these simple hydrophobic moieties in solution. The data are in full agreement with the predictions of dielectric continuum models: namely, that lithium ion significantly enhances the magnitude of the hydrophobic methyl-benzene interactions: sodium slightly enhances these interactions; while potassium and rubidium weaken these interactions to a sizable degree. Thus providing experimental evidence that shows salt effects on interactions between hydrophobic moieties can be interpreted through considering the interactions of constituent ions with the hydrophobic surfaces. This emphasizes that a large number of deviations from continuum model predictions that are observed in the literature may be the result of alternate non-hydrophobic interactions between solutes and the lithium ion, rather than being the result of any kind of "unusual hydration" of the lithium cation.

2. Experimental

2.1. Materials

Lithium chloride and sodium acetate were purchased from Fisher Scientific (Fair Lawn, NJ); lithium formate was purchased from GFS Chemicals Inc. (Columbus, OH); lithium acetate, sodium formate and sodium chloride were purchased from Sigma (St-Louis, MO); rubidium chloride, rubidium acetate and rubidium formate were respectively purchased from Alfa Aesar (Ward Hill, MA), Strem Chemicals (New Bury Port, MA) and Science Lab Inc. (Houston, TX). The phenol was purchased from Baker Chemical Co. (Phillispburg, NJ). All experiments were performed in 10 mM bis-tris purchased from Sigma (St-Louis, MO) at pH 7.0. The concentration of phenol in all samples is 200 μM.

2.2. Methods

Steady state fluorescence experiments were carried out on a Varian Cary Eclipse Fluorescence Spectrophotometer at room temperature (23.5 °C). The fluorescence was measured in $10\times3mm^2$ quartz cuvettes. All samples were prepared in triplicate. Fluorescence spectra were collected using an excitation wavelength set to 270 nm, excitation and emission slits were set to 5 nm band pass resolution. Quenching studies were performed by monitoring changes in the fluorescence intensity at the maximum emission of 297 nm as a function of quencher concentration.

3. Results and discussion

3.1. Results

In order to understand the effects of various salts on the magnitude of hydrophobicity we have used fluorescence quenching methods to study the hydrophobic contribution to the formation of contact-pairs between phenol and acetate ions. The fluorescence spectra of phenol are given in the presence of varying amounts of sodium acetate and sodium formate (Fig. 1). The addition of acetate and formate quenches the fluorescence of phenol and the quenching profiles (Fig. 2) follow the Stern–Volmer plot given by Eq. (1):

$$\frac{F_0}{F} = 1 + K_{sv}[Q] \tag{1}$$

where F_0 is the fluorescence of phenol in the absence of quencher, F is the fluorescence of phenol in the presence of Q molar of quencher and K_{sv} is the Stern–Volmer constant. [63] All of our plots exhibit linearity; we have checked for static quenching using the modified Stern–Volmer equation:

$$\frac{F_0}{F} = (1 + K_{sv}[Q])\exp(V[Q]) \tag{1a}$$

In which V is the volume of action around the fluorophore. Using the modified Stern–Volmer equation does not significantly improve the fits and leads to large random variations of V (between 0.3 and 0.2 for acetate and 0.2 and 0.1 for formate in all measurements). Therefore, the quenching contribution from the exponential factor is minor and Eq. (1) shall be used throughout this work.

The effects of alkaline chlorides on the quenching of phenol fluorescence by acetate and formate have been investigated in this work (cesium is not used because it is a strong quencher itself). In order to minimize the contribution of quencher to ionic strength, salt concentrations above 1.5 M have been investigated. In Table 1 the values of the Stern–Volmer quenching constant K_{sv} for the phenol fluorescence quenchers (acetate and formate) measured in chloride solutions are given. The values of K_{sv} for formate and acetate all uniformly depend on the chloride co-solute salt concentration (Fig. 3). The dotted lines in (Fig. 3) represent the best fit of the K_{sv} values to the following straight line:

$$K_{SV} = a[S] + b \tag{2}$$

Where "a" and "b" are constants that depend on the nature of co-solute and [S] is the concentration of co-solute. In Table 2 the values obtained from fitting the data in (Fig. 3) are listed. As a check we have also measured the effect of the monoprotic chlorides on phenol fluorescence. The addition of salt slightly enhances the phenol fluorescence in the case of NaCl, LiCl and KCl; while RbCl quenches the fluorescence of phenol moderately. The fact that Rb^+ unlike the other ions also quenches phenol fluorescence causes the value of b of RbCl to be significantly (more than 10%) different from that of the other salts. In the subsequent analysis we will use the correlation parameters of Table 2 to calculate values of K_{SV} at any given salt concentration.

3.2. Identifying the contribution of contact pair formation to the quenching data

Fluorescent molecules are quenched via two processes: dynamic and static [64]. Static quenching is caused by the presence of a quencher in the near vicinity of the fluorophor prior to excitation, while dynamic quenching is the result of the diffusion of the quencher into the action-sphere of the fluorescent molecule during the lifetime of the excited state. Static quenching often manifests itself as nonlinearities in the Stern–Volmer equation and the usual interpretation of this nonlinearity is to assume that the fluorophor and quencher form an optically dark ground state complex with an association constant of K_a. In this case the Stern–Volmer equation becomes:

$$\frac{F_0}{F} = (1 + K_{sv}[Q])(1 + K_a[Q]) \tag{1b}$$

However, as Lakowicz [64]and Webber [63] point out, the nonlinearity observed at very high quencher concentrations (greater than 0.25 M) is not due to the formation of quencher-fluorophor complexes, but is rather due to the finite probability of finding a quencher in the action sphere of the protein at these high concentrations. Under these conditions applying Eq. (1b) is erroneous and the modified Stern–Volmer form (Eq. (1a)) must be used. Because the nonlinear contribution to quenching of phenol is observed at acetate and formate concentrations above 0.5 M, we contend that Scheraga's application of Eq. (1b) to measure phenol-carboxylate contact pair

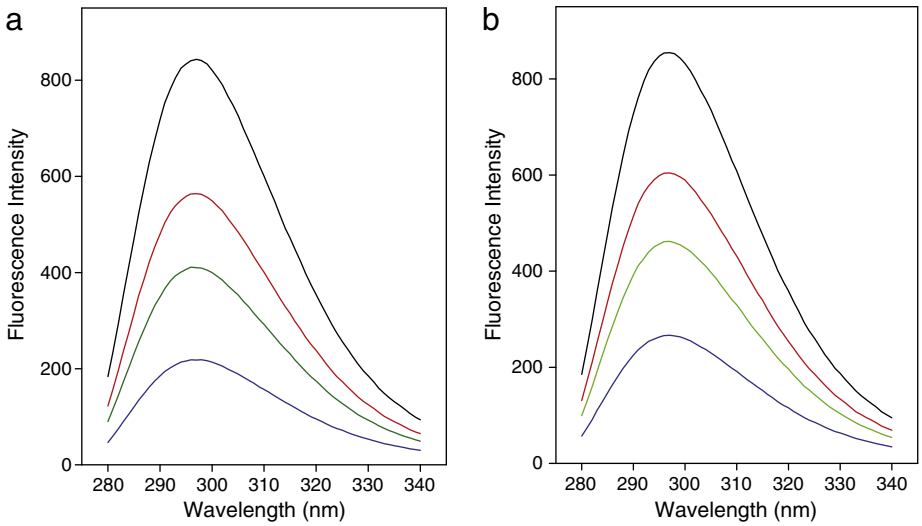

Fig. 1. The quenching of phenol fluorescence by acetate (a) and formate (b) ions, quencher concentrations are: 0 M (black), 0.1 M (red), 0.2 M (green) and 0.4 M (blue).

formation [59,60] is incorrect and an alternate mathematical description for this process is required.

The results above indicate that the major contributors to phenol quenching (at the quencher concentrations studied) are dynamic processes. In this case, the Stern–Volmer quenching constant K_{sv} of a quencher is related to the fluorescence lifetime of its associated fluorophor via the following equation [63]:

$$K_{sv} = k_q \tau \tag{3}$$

Where k_q is the rate of quenching and τ is the lifetime of the fluorophor in the absence of quencher. The quenching process can be represented by the following scheme [65]:

$$F^* + Q \underset{\leftarrow}{\rightarrow} [F^* \bullet Q] \xrightarrow{k_{et}} F + Q \qquad \text{Scheme} - 1$$

In this scheme F^* is the excited fluorophor, Q is the quencher, $[F^* \bullet Q]$ is the encounter complex formed between the quencher and fluorophor, k_{et} is the rate of energy transfer between the quencher and excited fluorophor within the encounter complex and F is the fluorophor in the ground state. Previous work has established that the quenching of phenol by acetate and formate is "reaction controlled" [59,60]: in other words, the equilibrium between the excited fluorophor, quencher and encounter complex is formed much faster than the transfer of energy. In this case Eq. (3) can be re-written as: [62]

$$K_{sv} = k_q \tau = K_{ec} * k_{et} * \tau \tag{4}$$

Where the parameter K_{ec} is the equilibrium constant associated with the formation of the encounter complex. This is the contribution of contact pair formation to the quenching process.

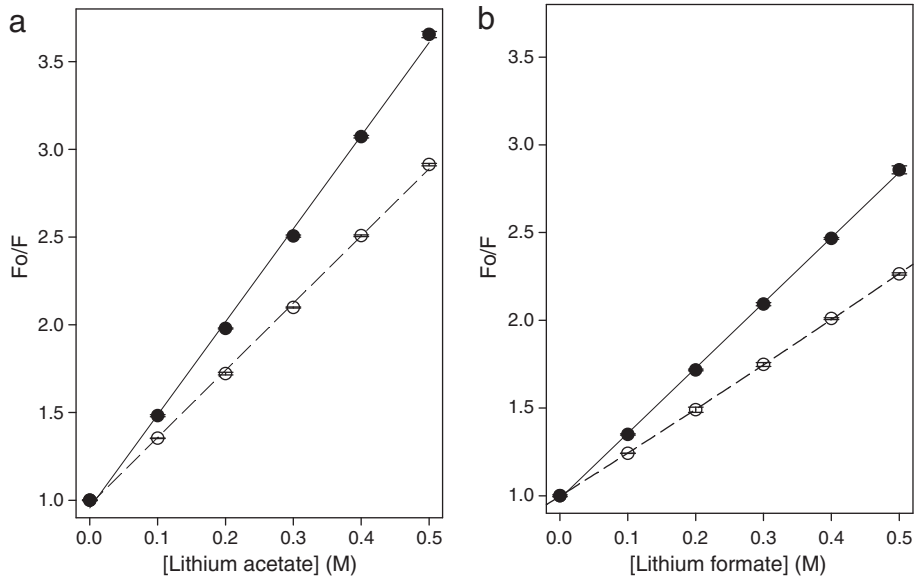

Fig. 2. Stern–Volner plots for the quenching of phenol by acetate (a) and formate (b); the concentration of LiCl is kept constant at 1.5 M (filled symbols) and 4 M (open symbols), solution is buffered at pH 7.

Table 1
Stern–Volmer constants (in units of M^{-1}) measured for the quenching of phenol fluorescence by acetate and formate in saline solutions at different concentrations.

[NaCl] (M)	K_{SV} (acetate)	K_{SV} (formate)
1.5	5.55 ± 0.07	4.27 ± 0.04
2.0	5.41 ± 0.09	4.17 ± 0.04
3.0	5.29 ± 0.07	4.11 ± 0.03
4.0	5.07 ± 0.07	3.86 ± 0.04
[KCl] (M)		
1.5	6.19 ± 0.09	4.25 ± 0.05
2.0	6.10 ± 0.1	4.32 ± 0.04
2.5	6.1 ± 0.1	4.32 ± 0.03
3.0	6.06 ± 0.08	4.33 ± 0.03
3.5	6.0 ± 0.1	4.37 ± 0.01
[LiCl] (M)		
1.5	5.3 ± 0.1	3.72 ± 0.03
2.0	5.0 ± 0.1	3.44 ± 0.04
2.5	4.72 ± 0.08	3.18 ± 0.03
3.0	4.42 ± 0.08	2.97 ± 0.03
3.5	4.11 ± 0.05	2.76 ± 0.02
4.0	3.83 ± 0.06	2.54 ± 0.02
[RbCl] (M)		
1.5	4.27 ± 0.08	2.17 ± 0.07
2.0	3.78 ± 0.01	2.1 ± 0.2
2.5	3.6 ± 0.1	2.06 ± 0.03
3.0	3.41 ± 0.07	1.97 ± 0.05

3.3. Isolating the contribution of the hydrophobic effect to contact pair formation

If the focus is now on the thermodynamic properties of the encounter complex, the standard free energy of formation of the encounter complex can be defined as:

$$\Delta G^{\circ}_{ec} = -RT \ln K_{ec} \tag{5}$$

At any given salt concentration [S], the free energy of formation of the encounter complex $\Delta G^{\circ}_{ec}([S])$ is a sum of the following contributions:

$$\Delta G^{\circ}_{ec}([S]) = \Delta G^{\circ}_{sol} + \Delta G^{\circ}_{es} + \Delta G^{\circ}_{hb} + \Delta G^{\circ}_{hydrophobic} \tag{7}$$

The first term, ΔG°_{sol}, is the dielectric solvation contribution to the free energy. The second term, ΔG°_{es}, is the contribution of electrostatic interactions between phenol and quencher to the free energy. The third term and fourth terms, ΔG°_{hb} and $\Delta G^{\circ}_{hydrophobic}$ are the contributions

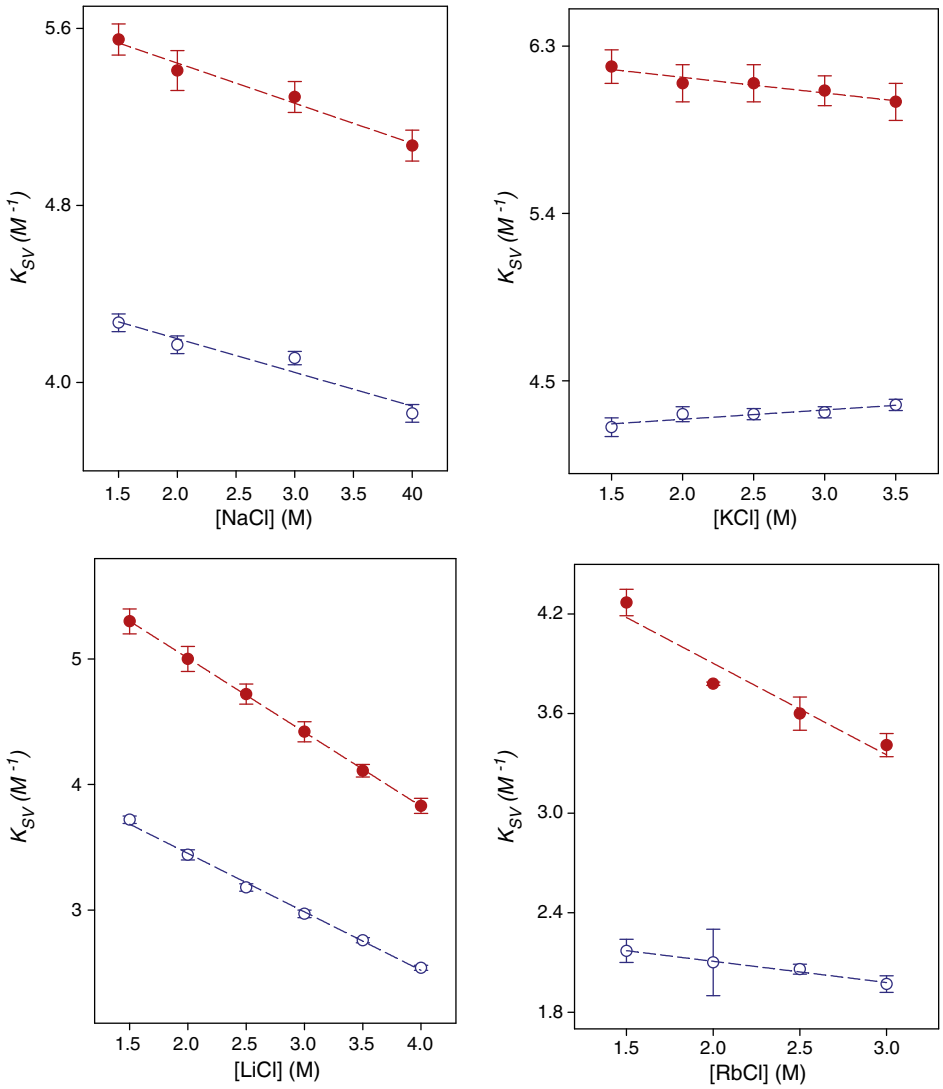

Fig. 3. Co-solute salt concentration dependence of the Stern–Volmer constants (K_{SV}) associated with the quenching of phenol fluorescence by acetate (filled symbols) and formate (open symbols). The lines are the best fits of the data to Eq. (2).

Table 2
Parameters associated with Eq. (2), demonstrating the salt dependence of the Stern–Volmer constants obtained from quenching studies of phenol fluorescence. These parameters result from fitting the quenching data depicted in Fig. (3); R^2 is the coefficient of determination for the fit.

Cosolute (quencher)	A	B	R^2
NaCl (acetate)	−0.18 ± 0.02	5.81 ± 0.05	0.982
NaCl (formate)	−0.15 ± 0.03	4.50 ± 0.08	0.934
KCl (acetate)	−0.08 ± 0.01	6.30 ± 0.04	0.919
KCl (formate)	0.05 ± 0.01	4.19 ± 0.03	0.836
LiCl (acetate)	−0.590 ± 0.004	6.19 ± 0.02	0.999
LiCl (formate)	−0.47 ± 0.01	4.38 ± 0.04	0.996
RbCl (acetate)	−0.6 ± 0.1	5.01 ± 0.02	0.932
RbCl (formate)	−0.13 ± 0.01	2.36 ± 0.03	0.980

of hydrogen bonding and the hydrophobic effect to the free energy. If we now subtract the free energy of formation of the phenol-formate encounter complex from the phenol-acetate encounter complex:

$$\begin{aligned}&(\Delta G^{\circ}_{ec}([S]))_{acetate-phenol}-(\Delta G^{\circ}_{ec}([S]))_{formate-phenol}\\&=\left\{(\Delta G^{\circ}_{sol})_{acetate-phenol}-(\Delta G^{\circ}_{sol})_{formate-phenol}\right\}_{[S]}\\&\quad+\left\{(\Delta G^{\circ}_{es})_{acetate-phenol}-(\Delta G^{\circ}_{es})_{formate-phenol}\right\}_{[S]}\\&\quad+\left\{(\Delta G^{\circ}_{hb})_{acetate-phenol}-(\Delta G^{\circ}_{hb})_{formate-phenol}\right\}_{[S]}\\&\quad+\left\{\left(\Delta G^{\circ}_{hydrophobic}\right)_{acetate-phenol}-\left(\Delta G^{\circ}_{hydrophobic}\right)_{formate-phenol}\right\}_{[S]}\end{aligned}\tag{7b}$$

The formate contribution to hydrophobicity can be assumed to be negligible. Measurements of hydrogen bonding between neutral and charged species have been shown to be weakly dependent on ionic strength [66,67]; in addition, the pKa values of carboxylic acids do not change more than 0.3 units in the salt concentration range studied [66,67]. Therefore, the hyd'rogen bonding contribution in Eq. (7b), $\{(\Delta G^{\circ}_{hb})_{acetate-phenol}-(\Delta G^{\circ}_{hb})_{formate-phenol}\}_{[S]}$, is unlikely to be significantly perturbed by changes in ionic strength. Therefore, the salt-induced change in free energy of formation at any salt concentration relative to that measured at the 1.5 M is:

$$\begin{aligned}&\left\{(\Delta G^{\circ}_{ec}([S]))_{acetate-phenol}-(\Delta G^{\circ}_{ec}([S]))_{formate-phenol}\right\}\\&-\left\{(\Delta G^{\circ}_{ec}([1.5\ M]))_{acetate-phenol}-(\Delta G^{\circ}_{ec}([1.5\ M]))_{formate-phenol}\right\}\\&=\left\{(\Delta G^{\circ}_{sol})_{acetate-phenol}-(\Delta G^{\circ}_{sol})_{formate-phenol}\right\}_{[S]}\\&\quad-\left\{(\Delta G^{\circ}_{sol})_{acetate-phenol}-(\Delta G^{\circ}_{sol})_{formate-phenol}\right\}_{[1.5\ M]}\\&\quad+\left\{(\Delta G^{\circ}_{es})_{acetate-phenol}-(\Delta G^{\circ}_{es})_{formate-phenol}\right\}_{[S]}\\&\quad-\left\{(\Delta G^{\circ}_{es})_{acetate-phenol}-(\Delta G^{\circ}_{es})_{formate-phenol}\right\}_{[1.5\ M]}\\&\quad+\left\{\left(\Delta G^{\circ}_{hydrophobic}\right)_{acetate-phenol}\right\}_{[S]}-\left\{\left(\Delta G^{\circ}_{hydrophobic}\right)_{acetate-phenol}\right\}_{[1.5\ M]}\end{aligned}\tag{7c}$$

Because salts induce a gradual change in the free energy we can assume the ΔG° functions to be both smooth and continuous. Therefore, we can expand each ΔG° function as a Taylor series around the salt concentration of 1.5 M and each term of Eq. (7c can be thus represented as:

$$\begin{aligned}&\left\{(\Delta G^{\circ}_{sol})_{acetate-phenol}-(\Delta G^{\circ}_{sol})_{formate-phenol}\right\}_{[S]}\\&-\left\{(\Delta G^{\circ}_{sol})_{acetate-phenol}-(\Delta G^{\circ}_{sol})_{formate-phenol}\right\}_{[1.5\ M]}\\&=m*[S-1.5]+n*[S-1.5]^2+\ldots\left\{(\Delta G^{\circ}_{es})_{acetate-phenol}-(\Delta G^{\circ}_{es})_{formate-phenol}\right\}_{[S]}\\&\quad-\left\{(\Delta G^{\circ}_{es})_{acetate-phenol}-(\Delta G^{\circ}_{es})_{formate-phenol}\right\}_{[1.5\ M]}\\&=p*[S-1.5]+q*[S-1.5]^2+\ldots\left\{\left(\Delta G^{\circ}_{hydrophobic}\right)_{acetate-phenol}\right\}_{[S]}\\&\quad-\left\{\left(\Delta G^{\circ}_{hydrophobic}\right)_{acetate-phenol}\right\}_{[1.5\ M]}=\alpha*[S-1.5]+\beta*[S-1.5]^2+\ldots\end{aligned}$$

Based on dielectric continuum models, the constants m and p will be independent of the nature of the salt co-solute. Eq. (7c) must therefore have the form of:

$$\begin{aligned}&\left\{(\Delta G^{\circ}_{ec}([S]))_{acetate-phenol}-(\Delta G^{\circ}_{ec}([S]))_{formate-phenol}\right\}\\&-\left\{(\Delta G^{\circ}_{ec}([1.5\ M]))_{acetate-phenol}-(\Delta G^{\circ}_{ec}([1.5\ M]))_{formate-phenol}\right\}\\&=(m+p+\alpha)[S-1.5]+(n+p+\beta)[S-1.5]^2+\ldots\end{aligned}\tag{7d}$$

Based on this analysis, we define the parameter H:

$$H_{[S]}=-RT\ln\left(\frac{(K_{sv})_{acetate}}{(K_{SV})_{formate}}\right)_{[S]}$$

we obtain:

$$\begin{aligned}H_{[S]}&=-RT\ln\left(\frac{(K_{sv})_{acetate}}{(K_{SV})_{formate}}\right)_{[S]}\\&=-RT\ln\left(\frac{(K_{ec})_{acetate-phenol}*\tau_{phenol}*(k_{et})_{acetate-phenol}}{(K_{ec})_{formate-phenol}*\tau_{phenol}*(k_{et})_{formate-phenol}}\right)_{[S]}\end{aligned}\tag{8}$$

As it can be observed, this ratio corrects variations in the fluorescence lifetime. If we substitute the free energy in Eq. (8) we now obtain:

$$\begin{aligned}H_{[S]}&=\left\{(\Delta G^{\circ}_{ec}([S]))_{acetate-phenol}-(\Delta G^{\circ}_{ec}([S]))_{formate-phenol}\right\}\\&\quad-RT\ln\left(\frac{(k_{et})_{acetate-phenol}}{(k_{et})_{formate-phenol}}\right)\end{aligned}\tag{8a}$$

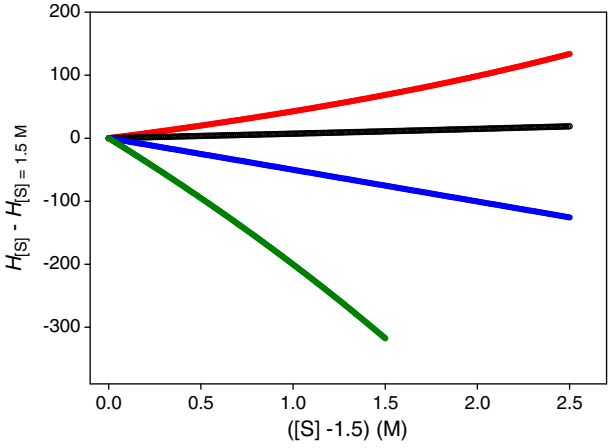

Fig. 4. Concentration dependence of $H_{[S]}-H_{[S]=1.5M}$ as defined by Eq. (8) for different salts; the various salts are: red (LiCl), black (NaCl), blue (KCl) and green (RbCl).

If we subtract the value of H at a concentration of 1.5 M from $H_{[S]}$ we obtain:

$$H_{[S]} - H_{[1.5\ M]} = (m+p+\alpha)[S-1.5] + (n+p+\beta)\,[S-1.5]^2 + \ldots - RT\ln\left(\frac{\left(\frac{(k_{et})_{acetate-phenol}}{(k_{et})_{formate-phenol}}\right)_{[S]}}{\left(\frac{(k_{et})_{acetate-phenol}}{(k_{et})_{formate-phenol}}\right)_{[1.5\ M]}}\right) \quad (8b)$$

The kinetics of proton transfer reactions are well correlated with the thermodynamics via linear free-energy relationships [59,60,62]. In the case of acetate and formate, the pKa values change by approximately the same amount as the ionic strength of the solution is changed from 1.5 M to 4 M [66,67]. Therefore, the final term of Eq. (8b) can be assumed to be negligible and Eq. (8b) can be rewritten as:

$$H_{[S]} - H_{[S]=1.5M} = (m+p+a)[S-1.5] + (n+q+\beta)[S-1.5]^2 + \ldots\ldots \quad (8c)$$

In this equation, the first order coefficient ($\eta = m+p+\alpha$) contains the constant α that characterizes how each individual salt affects the hydrophobic contribution to the formation of the encounter complex.

3.4. Salt effects on hydrophobic contact-pair formation

Eq. (8c) can now be used to characterize the effects of salts on the formation of the hydrophobic contact-pair between phenol and acetate. Using the correlation lines determined in Fig. (3), the values of K_{SV} at any given salt concentration can be determined. These values are used to plot $H_{[S]} - H_{[S]=1.5M}$ against ($[S]-1.5$) in Fig. (5) for each salt species. It can be observed that the data for each monoprotic chloride has a different initial slope. Because m and p do not depend on the nature of the salt, differences in the slope values are due to variations in α (the hydrophobic parameter); the large changes observed in η indicate that α is the major contributor. Each salt therefore affects the hydrophobicity (as characterized by η) differently: the lithium and sodium chloride data exhibit positive η values while in contrast, potassium and rubidium chloride data are associated with negative η values. Because in all cases the concentration of anion is constant, this change in slope η must be due to changing the cation.

In Fig. (4), the values of η as a function of ΔS_{II} in cal K^{-1} M^{-1}, as reported in the literature [11]) have been plotted. ΔS_{II} is defined as the difference between the partial molar entropy of the ion and that for water in water [68]; thus, ΔS_{II} is the local entropy change for turning a water molecule into an ion. Negative values of ΔS_{II} indicate tightly bound cationic water that is less mobile than bulk water, while positive values of ΔS_{II} indicate that the waters of the cation hydration are more mobile than that of bulk water. The results demonstrate that the effect of cations on the hydrophobic interaction between phenyl and methyl groups is related to their interactions with the hydrophobic surface. Ions with positive ΔS_{II} values have been shown to have a tendency to "stick" to hydrophobic surfaces, while ions with negative ΔS_{II} values are repelled from hydrophobic surfaces. The salt effects on hydrophobicity, as characterized by η follow this tendency: a clear-cut difference is observed between the effects of lithium which has a negative ΔS_{II} and thus promotes hydrophobic aggregation through minimizing the repulsion surface area (positive η values), and, that of potassium and rubidium which have positive ΔS_{II} values and by sticking to hydrophobic surfaces discourage the aggregation of hydrophobic surfaces (negative η values). The sodium cation, whose ΔS_{II} is close to zero, slightly enhances hydrophobicity. It must also be noted that (in contrast to the ion "stickiness" data of Collins) the η values are not linearly well correlated with ΔS_{II}, but are better correlated by the function:

$$\eta = \frac{(-59\pm15) + (103\pm3)\Delta S_{II}}{(-23.3\pm0.3) + \Delta S_{II}} \quad (9)$$

This apparent nonlinearity may be the result of the fact that as the size of cations approach the size of the fluorophor and quencher moieties, modeling the hydrophobic surfaces as macroscopic low-dielectrics becomes less realistic. Indeed the point of discontinuity ($\Delta S_{II} = 23.4$), corresponds to a cationic radius greater than 2.5 which is very close to the average of the radii of phenol and acetate [69].

Finally, we would also like to point out that our analysis corrects for any effects resulting from the specific interaction of lithium with the aromatic ring. In the case of the phenol-acetate contact pair formation, the encounter complex is a molecular entity. If we show the formation of the encounter complex schematically as:

$$\text{acetate} + (\text{phenol}^{\circ\circ\circ}\text{Li}^{+}) \rightleftarrows (\text{acetate}\bullet\text{phenol}^{\circ\circ\circ}\text{Li}^{+})$$
$$\text{formate} + (\text{phenol}^{\circ\circ\circ}\text{Li}^{+}) \rightleftarrows (\text{formate}\bullet\text{phenol}^{\circ\circ\circ}\text{Li}^{+})$$

It can be observed that the analysis above corrects for any specific interactions of lithium with the benzene ring. Thereby, allowing us to isolate the effects of cations on the hydrophobic interaction between the methyl and phenyl moieties.

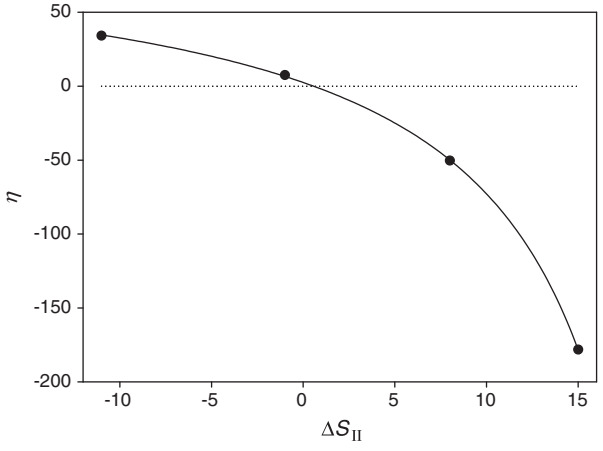

Fig. 5. The values of η, the initial slopes of the curves in Fig. 4, as a function of ΔS_{II} local entropy of the cation hydration sphere as reported in the literature [11].

4. Conclusions

The addition of lithium salts to aqueous solutions often appears to lower the hydrophobic effect. This behaviour of lithium has sometimes been explained by invoking the small size and hydration properties of the cation. However, our survey of the literature has shown that lithium ions can interact with a variety of molecular moieties and in a large number of the model systems used to study hydrophobicity, such moieties exist and interactions between these moieties and lithium can compensate for hydrophobic interactions between non-polar molecules. This can lead to an apparent weakening of the hydrophobic effect.

We have re-visited this problem by studying the effects of salts on the simple hydrophobic contact pair formed between a methyl group and a benzene ring. The system is simple enough to eliminate factors that complicate the analysis of salt effects in other hydrophobic systems and we have been successful in isolating the effect of salts on the methyl–phenyl hydrophobic interaction. The data show full agreement with continuum model predictions: ions that stick to hydrophobic surfaces reduce hydrophobic interactions between non polar surfaces, while ions that are repelled from non polar surfaces enhance hydrophobicity. In other words, there is nothing special about the way lithium affects hydrophobicity: the way ions affect the hydrophobic interaction between non-polar molecules, depends

on how these ions interact with the hydrophobic surface of these molecules.

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