

Surface tension measurements show that chaotropic salting-in denaturants are not just water-structure breakers

(guanidinium chloride/lithium perchlorate/benzene solubility)

RONALD BRESLOW* AND TAO GUO

Department of Chemistry, Columbia University, New York, NY 10027

Contributed by Ronald Breslow, October 17, 1989

ABSTRACT Since the salting-in agents guanidinium chloride, urea, and lithium perchlorate increase the surface tension of water, the salting-in phenomenon does not reflect easier cavity formation in water. Therefore, these salting-in agents must be directly contributing to the solvation of a solute such as benzene in water, probably by a direct solvation interaction. The increased surface-tension effects do not overbalance these solvation effects since they are smaller than the large surface-tension increases with lithium chloride, a typical salting-out agent. The salting-in agent tetra-*n*-butylammonium chloride differs in that it lowers the surface tension of water. Thus, it probably contributes both to easier cavity formation and to direct solvation of the substrate. The previous findings that most salting-in agents switch to become salting-out agents in other polar solvents such as ethylene glycol and formamide but that tetra-*n*-butylammonium chloride does not switch in these solvents can be understood in terms of relative polarities.

Substances such as urea and guanidinium chloride (GdmCl) are well-known denaturants of proteins and nucleic acids; because of the disordering they cause they are often referred to as chaotropic agents. Extensive studies (1-26) show that they act principally by decreasing the hydrophobic effect that contributes to the structuring of proteins and nucleic acids. More fundamentally, these agents increase the water solubility of hydrocarbons such as benzene. For example, in contrast to the well-known salting-out effect of LiCl, which decreases the water solubility of benzene by electrostriction of the solvent, GdmCl increases the water solubility of benzene (Fig. 1). It is thus a salting-in agent. Similarly, LiCl causes benzaldehyde to salt out of water, but LiClO₄ increases the water solubility of benzaldehyde and is thus a salting-in agent (27).

We have used the contrasting salting-out effect of LiCl and the salting-in effect of GdmCl (28-31) or of LiClO₄ (27) to detect hydrophobic packing in the transition states of Diels-Alder reactions (28-31) and of the benzoin condensation (27) in water solution. We have also investigated other polar solvents (27, 28). Solvophobic packing effects were seen in ethylene glycol and in formamide solutions, although of smaller magnitude than the effects in water. Strikingly, however, we found that in these solvents GdmCl is now a salting-out agent, similar in effect to LiCl (28). By contrast, tetra-*n*-butylammonium chloride (Bu₄NCl) and to a lesser extent tetramethylammonium chloride (Me₄NCl) are salting-in agents in all three solvents: water, ethylene glycol, and formamide (28).

These solvent-dependent salt effects prompted us to investigate further the reasons for the chaotropic salting-in effects of denaturants in water. In a solution of benzene in water with added salting-out or salting-in agent, we refer to

the water as solvent, the benzene as solute, and the other agent as a solubility modifier. In principle, solubility modifiers can affect two different energy terms (Eq. 1). One is the energy required to produce a solvent cavity into which the solute (such as benzene) can go, and the other is the energy of solute-medium solvation interaction. Which of these terms do salting-in or salting-out agents affect, and how does this effect occur?

$$\delta(\Delta G^\circ)_{\text{solvation}} = \delta(\Delta G^\circ)_{\text{cavitation}} + \delta(\Delta G^\circ)_{\text{solute solvation}}. \quad [1]$$

Salting out apparently affects the $(\Delta G^\circ)_{\text{cavitation}}$ by electrostriction that squeezes out free space and makes cavity creation harder (18). This effect is reflected in the internal pressure of the solvent (18, 23). At high concentrations of LiCl it is also possible that the solvation term could be affected if the bulk of the water were solvating the ions. The salting-in agents, including urea, have been called "structure breakers." It was assumed that they disrupt the structure of water by setting up new hydrogen bonds with water when a cavity is produced to accommodate the solute. Our finding that they are salting-out agents in ethylene glycol or formamide is consistent with this, if the unique water structure were the only one they can break. This was our first interpretation, but we now believe that it is too simple.

If LiClO₄, GdmCl, or urea do function chiefly to affect $(\Delta G^\circ)_{\text{cavitation}}$ by decreasing the energy cost of producing a new surface in the water, one would expect this to show up in their effects on surface tension (26). To check this, we have now investigated the effects on the surface tension of water and of ethylene glycol caused by salting-in and salting-out agents. The results indicate that salting-in by chaotropic agents is due in significant part to a change in $(\Delta G^\circ)_{\text{solute solvation}}$, as happens with typical cosolvents (32) such as ethanol.

METHODS

LiCl (Baker analyzed reagent, 99.8%), LiClO₄ [Alfa, anhydrous, 99.5% (Alfa-Ventron, Danvers, MA)], ethylene glycol (Aldrich spectrometric grade, 99+%), formamide (Aldrich spectrometric grade, 99+%), and water (Aldrich glass-distilled HPLC grade) were used without further purification. Guanidinium chloride (Aldrich, 99%) was recrystallized from absolute ethanol and then dried under high vacuum >15 hr; benzene (Aldrich 99+%, thiophene free) was freshly distilled from CaH₂ before use.

Surface tension was measured by the maximum bubble pressure method (33). Air bubbles were blown slowly (*ca.* 50 per min) through a glass capillary into solutions whose temperature was controlled at 20.00 ± 0.05°C. The maximum pressures were read on a manometer connected to the top of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GdmCl, guanidinium chloride; Bu₄NCl, tetra-*n*-butylammonium chloride.

*To whom reprint requests should be addressed.

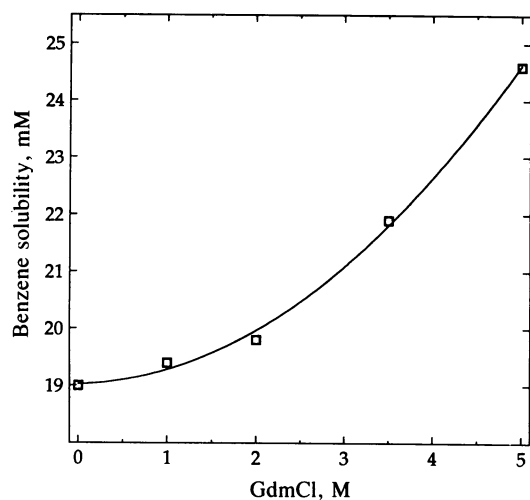


FIG. 1. Solubility of benzene in water at $20.00 \pm 0.1^\circ\text{C}$ as a function of the concentration of added GdmCl.

the capillary. Surface tensions (γ) were calculated by the standard treatment (33) and calibrated with pure water [$\gamma = 72.75$ dynes/cm at 20°C (34)]. The accuracy is better than $\pm 0.3\%$. The results are listed in Table 1 and partly plotted in Fig. 2.

The solubility of benzene was determined by measuring the absorbance of benzene-saturated solutions at 253.7 nm with a 2-mm cell in a Beckman DU-8B spectrophotometer. Aqueous solutions and benzene were equilibrated in the cell at $20.00 \pm 0.1^\circ\text{C}$ for 20 hr (half-saturation time is *ca.* 3 hr). We found by independent measurements that the change of solvents produced a negligible change in the benzene absorptivity. The solubility results were reproducible within ± 0.2 mM. The results are listed in Table 2 and plotted in Fig. 1.

RESULTS AND DISCUSSION

The results of our surface-tension measurements are listed in Table 1, and the data for water are plotted in Fig. 2. It is apparent that the salting-out agent LiCl and the salting-in agents GdmCl and LiClO_4 all increase the surface tension of water, although the effects of GdmCl and of LiClO_4 at high concentration are significantly smaller than the effect of LiCl.

Table 1. Surface tension γ at $20.00 \pm 0.05^\circ\text{C}$

Solvent	Salt	Salt, M	γ , dynes/cm
Water	None		72.75
	LiCl	1.00	73.8
		2.00	75.2
		3.50	77.4
		5.00	79.4
	GdmCl	1.00	73.5
		2.00	74.0
		3.50	74.7
		5.00	74.9
		LiClO_4	1.00
		2.00	73.4
		3.00	73.5
		4.00	73.9
Formamide	None		59.3
	LiCl	1.00	60.5
	GdmCl	1.00	60.3
Ethylene glycol	None		48.4
	LiCl	1.00	49.6
	GdmCl	1.00	49.8

γ values were obtained by the maximum bubble pressure method; their error is no more than 0.3%. Dynes/cm = mN/m.

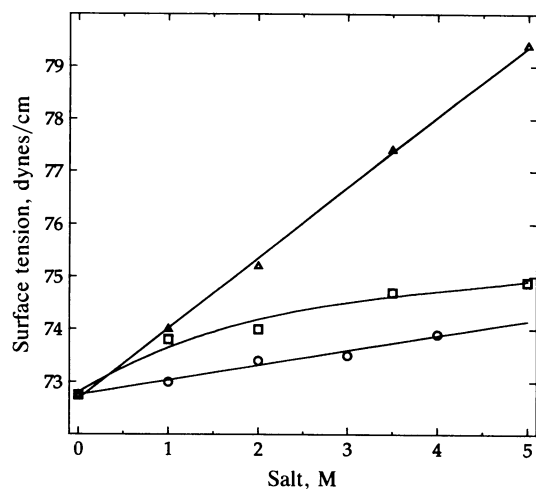


FIG. 2. Surface tension of water at $20.00 \pm 0.05^\circ\text{C}$ as a function of added LiCl (Δ), GdmCl (\square), or LiClO_4 (\circ) (dynes/cm = mN/m).

It has been reported previously that urea also increases the surface tension of water (25). By contrast, Bu_4NCl is known to decrease the surface tension of water, while Me_4NCl has almost no effect (24). In ethylene glycol and in formamide we find again (Table 1) that both LiCl and GdmCl increase the surface tension. In these solvents we had found (28) that they are both salting-out agents.

If the major salting-in effect of GdmCl, LiClO_4 , or urea in water were due to a decrease in $(\Delta G^\circ)_{\text{cavitation}}$, one would have expected to see a decrease in the surface tension; this is actually measured by examining the pressure required to produce an air bubble in the solution. The only way to avoid this conclusion is to postulate that these agents make it more difficult to produce large bubble-sized cavities but easier to produce small molecular-sized cavities. Although several theories predict a rescaling of macroscopic surface tensions with molecule-sized curved cavities (22, 35–37), there seems to be no evidence or strong argument for a size-related reversal of the effect. Of course a bubble of air is not an empty cavity into which a solute molecule can be placed, but there is no serious chance that our solubility modifiers solvate air. If they did, the $\delta(\Delta G^\circ)_{\text{cavitation}}$ would be even more positive than the estimate ignoring this unlikely solvation.

Since their effect on the surface tension of water should have made all these chaotropes act as salting-out agents, with a positive value for $\delta(\Delta G^\circ)_{\text{cavitation}}$, their salting-in behavior [a negative value for $\delta(\Delta G^\circ)_{\text{solute solvation}}$] must reflect a larger counterbalancing negative value for $\delta(\Delta G^\circ)_{\text{solute solvation}}$. The effect could be indirect, if these agents somehow promoted a better solvating interaction of the solute with water; it seems more likely that they act as typical cosolvents (20, 32). That is, chaotropic agents enter the solvation sphere of the solute and act as a bridge between the solutes and the water, just as a cosolvent like ethanol can do. The upward curvature in the solubility plot of Fig. 1 may reflect increasing solvation by the

Table 2. Solubility of benzene in aqueous GdmCl solutions at $20.0 \pm 0.1^\circ\text{C}$

GdmCl, M	Solubility,* mM
0	19.0
1.00	19.4
2.00	19.8
3.50	21.9
5.00	24.6

*Values were obtained by the determination of the UV absorbance of solutions saturated with benzene (error *ca.* ± 0.2 mM).

Gdm⁺ ion as it becomes a more important component of the medium.

The situation with Bu₄NCl is more ambiguous. Since it decreases the surface tension of water (24), as detergents also do, the salting-in properties could simply reflect this effect. However, if relatively polar materials such as Gdm⁺ cation and ClO₄⁻ anion solvate the benzene solute, it seems likely that the more organic Bu₄N⁺ ion will also do this. Thus, for Bu₄NCl we assign a negative value to both terms of Eq. 1, the first by measurement and the second by structural reasoning.

Finally, we must explain why LiClO₄ and GdmCl are salting-in agents in water but salting-out agents in ethylene glycol or formamide, while Bu₄NCl is a salting-in agent in all three solvents (28). We do this by considering the extent of hydrocarbon solvating ability of these three agents relative to the solvents.

Bu₄N⁺ ion is so nonpolar that it can preferentially solvate benzene in all three solvents, being less polar than any of them. Thus, it can have a negative $\delta(\Delta G^\circ)_{\text{solute solvation}}$ in all three. However, Gdm⁺ ion and ClO₄⁻ ion are more polar. We propose the perhaps surprising idea that they can solvate benzene better than water can, so $\delta(\Delta G^\circ)_{\text{solute solvation}}$ is negative in water. However, they are more polar than is ethylene glycol or formamide. In these solvents the second term of the equation is not negative, since the solubility modifiers do not solvate benzene better than the organic solvents do. Now the surface-tension effects we detect in these solvents (Table 1) produce an uncompensated $\delta(\Delta G^\circ)_{\text{cavitation}}$. The result is salting-out.

CONCLUSIONS

(i) Since the salting-in agents GdmCl, urea, and LiClO₄ increase the surface tension of water, the salting-in phenomenon does not reflect easier cavity formation in water.

(ii) Therefore, these salting-in agents must be directly contributing to the solvation in water of a solute such as benzene, probably by a direct interaction.

(iii) The surface tension effects do not overbalance these solvation effects. They are smaller than the large surface-tension increases with LiCl, a typical salting-out agent.

(iv) The salting-in agent Bu₄NCl differs in that it lowers the surface tension of water. Thus, it probably contributes both to easier cavity formation and to direct solvation of the substrate.

(v) The previous findings that most salting-in agents switch to become salting-out agents in other polar solvents such as ethylene glycol and formamide but that Bu₄NCl does not switch in these solvents can be understood in terms of the relative polarities of solvents and additives.

We thank the National Institutes of Health for support of this work and Prof. K. Eisenthal for use of the surface tension equipment.

1. Long, F. A. & McDevit, W. F. (1952) *Chem. Rev.* **51**, 119–169.
2. Gordon, J. E. (1975) *The Organic Chemistry of Electrolyte Solutions* (Wiley, New York).

3. Bunton, C. A. & Robinson, L. (1968) *J. Am. Chem. Soc.* **90**, 5965–5971.
4. Goldschmidt, H. & Stunde, F. (1906) *Z. Physik. Chem.* **56**, 1–42.
5. Hatefi, Y. & Hanstein, W. G. (1969) *Proc. Natl. Acad. Sci. USA* **62**, 1129–1136.
6. von Hippel, P. H. & Wong, K. Y. (1964) *Science* **145**, 577–580.
7. von Hippel, P. H. & Schleich, T. (1969) *Acc. Chem. Res.* **2**, 257–265.
8. Wetlaufer, D. B., Malik, S. K., Stollere, L. & Coffin, R. L. (1964) *J. Am. Chem. Soc.* **86**, 508–514.
9. Barone, G., Rizzo, E. & Vitagliano, V. (1970) *J. Phys. Chem.* **74**, 2230–2232.
10. Thomas, J. & Evans, D. F. (1970) *J. Phys. Chem.* **74**, 3812–3819.
11. Ray, A. (1969) *J. Am. Chem. Soc.* **91**, 6511–6512.
12. Ray, A. (1971) *Nature (London)* **231**, 313–315.
13. Collins, K. D. & Washabaugh, M. W. (1985) *Q. Rev. Biophys.* **18**, 323–422.
14. Evans, D. F., Chen, S.-H., Schriver, G. W. & Arnett, E. M. (1981) *J. Am. Chem. Soc.* **103**, 481–482.
15. Sergeeva, V. F. (1965) *Russ. Chem. Rev. (Engl. Transl.)* **34**, 309–318.
16. Sergeeva, V. F. & Kaluzhinova, G. P. (1961) *Zh. Obshch. Khim.* **31**, 2448–2450.
17. Sergeeva, V. F., Chumachenko, T. G. & Glybovskaya, V. A. (1972) *Zh. Obshch. Khim.* **42**, 1459–1462.
18. McDevit, W. F. & Long, F. A. (1952) *J. Am. Chem. Soc.* **74**, 1773–1777.
19. Saylor, J. H., Whitten, A. I., Claiborne, I. & Gross, P. M. (1952) *J. Am. Chem. Soc.* **74**, 1778–1781.
20. Janado, M. & Yano, Y. (1985) *Bull. Chem. Soc. Jpn.* **58**, 1913–1917.
21. Greco, F. A. (1984) *J. Phys. Chem.* **88**, 3132–3133.
22. Tanford, C. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4175–4179.
23. Dack, M. R. J. (1975) *Chem. Soc. Rev.* **4**, 211–229.
24. Tamaki, K. (1974) *Bull. Chem. Soc. Jpn.* **47**, 2764–2767.
25. Siskova, M., Hejtamankova, J. & Bartovska, L. (1985) *Coll. Czech. Chem. Commun.* **50**, 1629–1635.
26. Aveyard, R. (1982) *Can. J. Chem.* **60**, 1317–1326.
27. Kool, E. T. & Breslow, R. (1988) *J. Am. Chem. Soc.* **110**, 1596–1597.
28. Breslow, R. & Guo, T. (1988) *J. Am. Chem. Soc.* **110**, 5613–5617.
29. Breslow, R. & Rideout, D. (1980) *J. Am. Chem. Soc.* **102**, 7816–7817.
30. Breslow, R., Maitra, U. & Rideout, D. (1983) *Tetrahedron Lett.* **24**, 1901–1904.
31. Breslow, R. & Maitra, U. (1984) *Tetrahedron Lett.* **25**, 1239–1240.
32. Haak, J. R. & Engberts, J. B. (1986) *J. Am. Chem. Soc.* **108**, 1705–1706.
33. Alexander, E. A. & Hayter, J. B. (1972) in *Physical Methods of Chemistry 1*, (eds. Weissberger, A. & Rossiter, B. W.) (Wiley-Interscience Publishers, New York), Part 5, p. 501.
34. (1989) *Handbook of Chemistry and Physics* (CRC, Boca Raton, FL), 69th Ed., p. F33.
35. Sinanoglu, O. (1968) in *Proceedings of the International Conference on Mol. Assoc. Biol.*, ed. Pullman, B. (Academic, New York).
36. Tolman, R. C. (1949) *J. Chem. Phys.* **17**, 333–337.
37. Defay, R., Prigogine, I., Bellemans, A. & Everett, D. H. (1966) *Surface Tension and Adsorption* (Longmans, London).