SOLUBILIZATION OF PARTICULATE PROTEINS AND NONELECTROLYTES BY CHAOTROPIC AGENTS*

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Abstract.—Chaotropic ions (those ions which favor the transfer of apolar groups to water) provide a highly effective means for the resolution of membranes and multicomponent enzymes and for increasing the water solubility of particulate proteins and nonelectrolytes. The action of chaotropic agents is related to their effect on the structure and lipophilicity of water.

The meager water solubility of particulate proteins and many biological compounds such as hemes, purines and pyrimidines, nucleosides, certain vitamins, and various structures of pharmacological interest has posed considerable difficulty in the study of their chemical and biological properties. Also, because of the predominance of hydrophobic bonds in membranes and multicomponent enzymes, the stability of these systems in aqueous media has been a major impediment in attempts at their resolution and unraveling of their molecular organization and mechanism of action.

Among the forces contributing to the stability of these structures in aqueous media, hydrophobic attractions are most significant. This is because van der Waals attractions between apolar groups are weak and hydrogen bonds of the C=O⋅⋅⋅H—N and C=O⋅⋅⋅H—O type are, according to Klotz and co-workers,1 thermodynamically unstable if not protected from water. Since hydrophobic attraction is in essence a water-repulsion force, a consideration of the factors that influence the expulsion of most nonelectrolytes and the apolar regions of particulate proteins from water might provide significant clues to methods for increasing the solubility of such structures in aqueous media.

According to Kauzmann,2 apolar groups form hydrophobic bonds mainly as a result of their thermodynamically unfavorable interaction with water, rather than as a consequence of interaction with each other. Thus, the transfer of an apolar molecule from a lipophilic surrounding to water is endergonic by 2–6 kcal per mole because of an associated unitary entropy2 decrease of 10–20 entropy units (eu). (See, for example, the negative ΔH and ΔS values in Table 1 for the transfer of a variety of simple nonelectrolytes from benzene to water.) This large, negative entropy difference appears to be almost entirely related to the structure of water3–5 and can be diminished by changing the structure of water in the direction of greater disorder. Our studies suggest that such a condition can be created in the presence of certain inorganic anions.

As shown in Table 2, the hydrated forms of anions such as SCN−, ClO4−, I−, Br−, and Cl− are associated with large, positive entropies. A simple interpretation of this large entropy increase (see also the entropies of hydration in Table 2) is the possible effect of these ions on the structure of water. This interpretation is in agreement with the conclusions of Hamaguchi and Geiduschek,6

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Table 1. Thermodynamic changes in the transfer of small nonelectrolytes from benzene to water at 25°.

<table>
<thead>
<tr>
<th>Anion</th>
<th>$S_{40}$ (m)</th>
<th>$S_{i,\text{hydr}}$</th>
<th>Salt</th>
<th>$\delta$</th>
<th>Ion</th>
<th>$\Delta H$ (cal/mole)</th>
<th>$\Delta S$ (eu)</th>
<th>$\Delta H$ (cal/mole)</th>
<th>$\Delta S$ (eu)</th>
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<tbody>
<tr>
<td>He</td>
<td>3550</td>
<td>16.9</td>
<td>N$_2$</td>
<td>3100</td>
<td>17.7</td>
<td>CH$_4$ 2820</td>
<td>18.3</td>
<td>CH$_4$ 2820</td>
<td>18.3</td>
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<tr>
<td>Ne</td>
<td>4440</td>
<td>19.5</td>
<td>O$_2$</td>
<td>3370</td>
<td>18.5</td>
<td>C$_2$H$_6$ 2180</td>
<td>19.5</td>
<td>C$_2$H$_6$ 2180</td>
<td>19.5</td>
</tr>
<tr>
<td>A</td>
<td>3590</td>
<td>18.9</td>
<td>CO</td>
<td>4470</td>
<td>17.1</td>
<td>C$_2$H$_4$ 1610</td>
<td>15.2</td>
<td>C$_2$H$_4$ 1610</td>
<td>15.2</td>
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<tr>
<td>HCl</td>
<td>2740</td>
<td>14.5</td>
<td>N$_2$O</td>
<td>2670</td>
<td>15.7</td>
<td>C$_4$H$_4$ 0</td>
<td>14</td>
<td>C$_4$H$_4$ 0</td>
<td>14</td>
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</tbody>
</table>

* Compiled from refs. 2 and 3.

von Hippel and Wong, and Frank and Evans regarding the possible structure-breaking effect of such ions on water. According to Frank and Evans, all the alkali and halide ions, except Li$^+$ and F$^-$, appear to have a net structure-breaking effect. Thus in ionic solutions there is thought to be "too much" entropy, whereas in rare gas solutions there is "too little." The probable explanation is that around the ions, beyond the first 'saturated' region of water molecules, there is a region or belt in which the water structure is broken down, or melted, or depolymerized as compared to ordinary water. Furthermore, as mentioned above, we find that the transfer of nonelectrolytes to solutions of such ions (as listed in Table 2) involves a negative entropy change that is smaller than that involved in transfer to water. Thus, the transfer of nitromethane from water to 1 M solutions of HClO$_4$, LiClO$_4$, and NaClO$_4$ is accompanied by an entropy increase of 1.1 e.u., and the transfer entropies of argon from water to 1 M KCl, KBr, and KI are all positive (+0.77, +0.83, and +1.30 e.u., respectively). The data of columns 5 and 7 of Table 2 on proton-NMR shifts and water self-diffusion are also in agreement with the above interpretation. Thus, both the increased shielding of water protons and the increased mobility of water molecules suggest breaking of water structure.

Another factor, which further contributes to the effectiveness of the anions

Table 2. Effect of ions on water.

<table>
<thead>
<tr>
<th>Anion</th>
<th>$S_{40}$ (m)</th>
<th>$S_{i,\text{hydr}}$</th>
<th>Salt</th>
<th>$\delta$</th>
<th>Ion</th>
<th>$\Delta H$ (cal/mole)</th>
<th>$\Delta S$ (eu)</th>
<th>$\Delta H$ (cal/mole)</th>
<th>$\Delta S$ (eu)</th>
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</thead>
<tbody>
<tr>
<td>SCN$^-$</td>
<td>36</td>
<td>8.1</td>
<td></td>
<td></td>
<td>I$^-$</td>
<td>$\Delta H$ (cal/mole)</td>
<td>$\Delta S$ (eu)</td>
<td>$\Delta H$ (cal/mole)</td>
<td>$\Delta S$ (eu)</td>
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<tr>
<td>ClO$_4^-$</td>
<td>43.2</td>
<td>8.3</td>
<td>NaClO$_4$</td>
<td>1.32</td>
<td>NO$_3^-$</td>
<td>0.08</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>I$^-$</td>
<td>25.3</td>
<td>10.1</td>
<td>NaI</td>
<td>0.92</td>
<td>Br$^-$</td>
<td>0.07</td>
<td></td>
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<tr>
<td>Br$^-$</td>
<td>19.7</td>
<td>14.4</td>
<td>NaBr</td>
<td>0.78</td>
<td>ClO$_4^-$</td>
<td>0.03</td>
<td></td>
<td>0.03</td>
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<tr>
<td>Cl$^-$</td>
<td>13.5</td>
<td>18.2</td>
<td>NaCl</td>
<td>0.58</td>
<td>Cl$^-$</td>
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<td>0.0</td>
<td></td>
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<tr>
<td>F$^-$</td>
<td>2.3</td>
<td>31.2</td>
<td>(CH$_2$)$_2$NCl</td>
<td>0.0</td>
<td>F$^-$</td>
<td>0.11</td>
<td></td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>4.4</td>
<td></td>
<td></td>
<td></td>
<td>SO$_4^{2-}$</td>
<td>0.18</td>
<td></td>
<td>0.18</td>
<td></td>
</tr>
</tbody>
</table>

$S_{40}(m)$ is the partial molar entropy of the aqueous ion (cal/mole X degree) at 25°. $S_{i,\text{hydr}}$ is the partial molar entropy of hydration (cal/mole X degree) at 25°. $\delta$ is the proton chemical shift in 1 M solutions of electrolytes compared to water as standard (ppm). $\Delta_1$ is $(D_1-D_0)/D_0$, where $D_0$ is the self-diffusion coefficient for pure water, and $D_1$ is the self-diffusion coefficient for a 1 M solution of the ions in water. $\Delta_1$ is surface tension increase (dyn/cm). $\Delta_1$ is surface potential (mv).
listed in Table 2 (except F\textsuperscript{−} and SO\textsubscript{4}\textsuperscript{2−}) in increasing the water solubility of particulate proteins and nonelectrolytes, is their tendency to make water more lipophilic. Unlike cations, anions decrease the polarity of the surrounding water. In the hydration shell of a cation the hydrogen atoms of water are directed out, whereas with the anions they are directed in, and this qualitative difference is in all probability not confined to the first hydration shell. Greyson\textsuperscript{16} suggests that “the proton of the solvated water molecule polarizes the large halide ions and leads to a water-anion bond less polar than the OH...O of water itself, while the less polarizable cations form hydrate bonds in similar way to that of water” (see also ref. 11). Thus, as shown in Table 2, the increase in surface potential (Δχ) and the diminution of surface-tension rise (Δγ) in solutions of these compounds indicate that water around anions is more lipophilic than water around cations. In going from F\textsuperscript{−} to SCN\textsuperscript{−}, the anions are found in increasing concentrations near the interphase between the solution and the gas phase, which (according to the general treatment of Miller and Hildebrand\textsuperscript{22}) is “nonwetting,” or hydrophobic. Cations are generally excluded from the surface.

The above considerations suggest, therefore, that by making water more disordered and lipophilic in the presence of the appropriate ions listed in Table 2, it should be possible to weaken the hydrophobic bonds of membranes and multicomponent enzymes and increase the water solubility of particulate proteins and nonelectrolytes. This conclusion is supported by available data in the literature as well as by the rigorous tests performed in this laboratory. Before describing these tests, we might point out that the nature and the order of the ions listed in the first column of Table 2 are reminiscent of the Hofmeister series and of Buchner’s order of the lyotropic number of anions.\textsuperscript{20} Hamaguchi and Geiduschek\textsuperscript{4} have published a somewhat similar series of anions, which at neutral pH lower the thermal denaturing temperature of DNA by as much as 60°, and Dandliker and co-workers\textsuperscript{21} have used SCN\textsuperscript{−}, ClO\textsubscript{4}\textsuperscript{−}, and I\textsuperscript{−} for the first time to dissociate antigen-antibody complexes without destroying the immunospecific activity of the antibody. Hamaguchi and Geiduschek\textsuperscript{6} applied the term “chaotropic” to anions which tend to disorder the structure of DNA. In view of the above considerations, however, this term will be applied to those inorganic anions which favor the transfer of apolar groups to water.

Solubilization of Small Organic Molecules in Water.—It is well known that salts decrease the solubility of most nonelectrolytes in water. The impressive list provided by Long and McDevit\textsuperscript{22} shows that most compounds are salted out by alkali salts. However, we find that the order of increasing salting-out effect of anions is essentially:

\[ \text{SCN}^- < \text{ClO}_4^- < \text{NO}_3^- < I^- < \text{Br}^- < \text{Cl}^- < \text{SO}_4^{2-}, \text{CH}_3\text{COO}^-, \text{F}^- \].

Table 3 shows that in fact SCN\textsuperscript{−}, ClO\textsubscript{4}\textsuperscript{−}, and NO\textsubscript{3}\textsuperscript{−} can be used to increase the water solubility of a variety of organic compounds which are sparingly soluble in water. Thus, approximately 1 M concentrations of NaClO\textsubscript{4} and NaSCN augment the solubility of adenine, menadione (2-methyl-naphthoquinone), and riboflavin in water, while similar concentrations of NaF and NaCl have the opposite effect. It is seen that the order of effectiveness of these ions is the
TABLE 3. *Per cent solubility of small organic molecules in solutions of neutral salts as compared to water.*

<table>
<thead>
<tr>
<th>Salt</th>
<th>Adenine</th>
<th>Menadione</th>
<th>Riboflavin</th>
<th>Sorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaSCN</td>
<td>126</td>
<td>197</td>
<td>338</td>
<td>103</td>
</tr>
<tr>
<td>NaClO₄</td>
<td>130</td>
<td>169</td>
<td>261</td>
<td>79</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>105</td>
<td>162</td>
<td>143</td>
<td>98</td>
</tr>
<tr>
<td>NaCl</td>
<td>83</td>
<td>77</td>
<td>114</td>
<td>69</td>
</tr>
<tr>
<td>NaF</td>
<td>70</td>
<td>54</td>
<td>45</td>
<td>—</td>
</tr>
</tbody>
</table>

Solutions are 0.91 M in salt and 0.091 M in Tris-HCl, pH 8.3, at 23°C, except for sorbic acid, where solutions are 1 M and unbuffered.

The reverse of the order of the increasing salting-out effect of the ions depicted above, and is in agreement with the data of Table 2. Figure 1 shows the salting-in effect of NaClO₄ and NaSCN on menadione, adenine, adenosine, and riboflavin. It is seen that, unlike NaClO₄, NaCl and sodium acetate salt-out menadione and that in 7 M thiocyanate, riboflavin is 660 times as soluble as in buffer alone. That this spectacular salting-in effect of NaSCN on riboflavin is not due to a chemical alteration of the riboflavin structure has been ascertained from the

![Graph A](image_url)

![Graph B](image_url)

![Graph C](image_url)

**Fig. 1.—**The effect of chaotropic ions on the water solubility of (A) 2-methyl-naphthoquinone, (B) adenine and adenosine, and (C) riboflavin at 23°C. $S_1,$ solubility in salt solution; $S_{H_2O},$ solubility in water.
spectrum of riboflavin in thiocyanate solution and from the recovery of riboflavin after removal of thiocyanate by dialysis.

Guanidine and urea are also capable of increasing the solubility of the above molecules in water and behave very much like chaotropic ions in effecting the solubilization of membrane proteins. In fact, adenine, menadione, and sorbic acid are more soluble in 1 M guanidine hydrochloride than in comparable concentrations of NaClO₄ and NaSCN. Therefore, the solubilizing effect of guanidine and urea will also be described, even though their effect on water structure may not be the same as that of the particular ions discussed above.²³

Solubilization of Membrane Proteins.—Table 4 shows the effect of NaSCN, NaClO₄, guanidine hydrochloride, and urea in solubilizing membrane-bound proteins at pH 8.0. It is seen that in the presence of 2 M NaSCN, as much as 40 per cent electron-transport particle protein is solubilized. By comparison, 2 M NaClO₄ results in 24 per cent protein extraction, 2 M NaCl in about 10 per cent, and 2 M CsF in only 4–5 per cent. Thus, once again, the order of effectiveness of these anions under the same conditions of temperature, pH, and ionic strength of the solution is the same as might be predicted from Table 2. Table 4 also shows protein extraction by the above compounds from complex I (DPNH-coenzyme Q reductase)²⁴ of the respiratory chain, Bacillus subtilis plasma membranes,²⁵ erythrocyte ghosts, and beef-liver microsomes.²⁶ Again, in all these cases the order of effectiveness is SCN⁻ > ClO₄⁻, guanidine > urea > Cl⁻ > F⁻. Except for complex I, which has been studied in detail, the extractions shown in Table 4 were not necessarily performed under optimal conditions. Thus, the data of this table are only for illustrating the phenomenon and do not represent maximal protein extraction from each source.

Sodium thiocyanate, NaClO₄, guanidine, and urea have also been used for the resolution of complexes I, II, and III²⁴ of the mitochondrial electron-transport system, and NaClO₄ has afforded a 30 per cent purification of complex IV²⁷ to an active cytochrome oxidase containing 12 nmoles of heme a per milligram of protein. Furthermore, it has been shown that the resolution of complex I by the

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Chaotropic agent</th>
<th>Per cent of protein solubilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron-transport particle</td>
<td>NaSCN, 2 M</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Guanidine-HCl, 2 M</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>NaClO₄, 2 M</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Urea, 2 M</td>
<td>6.5</td>
</tr>
<tr>
<td>Complex I</td>
<td>NaClO₄, 0.5 M</td>
<td>30</td>
</tr>
<tr>
<td>Complex I</td>
<td>Guanidine-HCl, 0.5 M</td>
<td>30</td>
</tr>
<tr>
<td>Complex I</td>
<td>Urea, 2.25 M</td>
<td>20</td>
</tr>
<tr>
<td>B. subtilis membranes</td>
<td>NaSCN, 2 M</td>
<td>24</td>
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<tr>
<td>B. subtilis membranes</td>
<td>NaClO₄, 2 M</td>
<td>21</td>
</tr>
<tr>
<td>Erythrocyte ghosts</td>
<td>NaClO₄, 2 M</td>
<td>27.5</td>
</tr>
<tr>
<td>Beef-liver microsomes</td>
<td>NaSCN, 4 M</td>
<td>31</td>
</tr>
</tbody>
</table>

In addition to the chaotropic agents indicated, the suspensions also contained 50 mM Tris-HCl, pH 8.0, except for electron-transport-particle and complex I suspensions which also contained 0.1 M sucrose. Electron-transport particles and microsomes were extracted at ice-bath temperature; others were incubated 5–10 min at 30° with the chaotropic agents. Proteins were considered soluble in the media indicated above if not sedimented at 105,000 g in 1.5–2 hr.
above reagents can be very carefully controlled (i.e., accelerated, slowed down, or interrupted before completion). This degree of control, not duplicable by any method devised to date for comminution of membranes and enzyme complexes, has permitted a study of the kinetics of the resolution process and the various factors that might influence it.

In addition, it has been found that urea, guanidine, KI, NaClO₄, and NaSCN induce a very rapid oxidation of lipids in mitochondria, microsomes, and the complexes of the electron-transport system.²⁸ Depending on the nature of the particle used, 350–950 nmoles of oxygen are taken up per milligram of protein, and the rates of electron-transport particle oxidation by 2 M solutions of urea, KI, NaClO₄, and NaSCN at 30° and pH 8.0 are respectively 0.6, 5, 10, and 23 nmoles O₂/min × mg protein (note once again the order of potency of these compounds). Membrane lipid oxidation as a consequence of aging or ionizing radiation has been studied by others.²⁹ These reactions are generally slower than the oxidations induced by chaotropic agents, and in these instances it has not been determined whether membrane breakdown precedes or follows lipid oxidation. In the case of chaotropic agents, however, mitochondrial lipid oxidation is definitely a consequence of the breakdown of membrane structure and appears to be catalyzed by the components of iron-sulfur proteins, which are also destroyed under aerobic conditions. It is known that ferrous ions and mixtures of oxidized and reduced thiols, such as glutathione, can induce membrane lipid oxidation.²⁹ The chaotropic-induced lipid oxidation of electron-transport particles, microsomes, and the complexes of the respiratory chain is considerably inhibited by substrates. This finding is in accord with the increased resistance of the substrate-treated form of the complexes to resolution, and indicates a significant difference between the conformation of the oxidized and the reduced states of these particulate systems. Another important finding, which also relates membrane lipid oxidation to structural breakdown, is that in complex III antimycin A inhibits²⁹ both lipid oxidation²⁸ and resolution of the complex as induced by chaotropic agents.³¹

The phenomenon of lipid oxidation induced by the above reagents has not only provided a sensitive and convenient probe for monitoring membrane breakdown and studying the factors that influence it but has also revealed the irreversible changes that can occur during the resolution of membranes. However, it has been shown in this laboratory that lipid oxidation and iron-sulfur protein destruction are not necessary for the chaotropic-induced resolution of complex I.

**Summary and Conclusions.**—All the phenomena described above occur in water at neutral pH values, moderate temperatures, and not too extreme concentrations of the appropriate neutral salts (and urea). The effective ions are, by and large, chemically inert under the conditions used and have in common a fairly large size and a single (mostly negative) charge. As shown in Table 5, they also effect the dissociation and solubilization of antigen-antibody complexes, denaturation of proteins, depolymerization of protein polymers, and thermal stability of DNA. In each case the trend for the potency of various ions is essentially the same as depicted above for solubilization of nonelectrolytes and particulate proteins. Thus, depending on the conditions and the nature of the biopolymer used, SCN⁻ is the most denaturing, depolymerizing, and solubilizing
anion, while sulfate, fluoride, and acetate have the least effect. Table 5 further illustrates that the effect of simple, aqueous anions on solutes as different as oxygen, benzene, and proteins follows the same pattern as the effect of these ions on the structure and lipophilicity of water (see Table 2).

In conclusion, it has been shown that chaotropic ions provide an extremely simple, effective, and controllable means for resolution of membranes and multi-component enzymes and for increasing the water solubility of biopolymers and small organic molecules. The thermodynamic basis of chaotropic action as related to hydrophobicity of solutes and the structure and lipophilicity of water has been discussed. Moreover, some of the hazards and complications attending chaotropic action, such as induced lipid oxidation in membranes, have been reported. It is envisaged that the application of chaotropic agents will provide considerable insight into the molecular organization and mechanism of action of biological membranes and enzyme complexes.

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**Table 5. Order of increasing potency of simple aqueous anions on denaturation, depolymerization or solubility of biopolymers and small organic and inorganic molecules.**

<table>
<thead>
<tr>
<th>Effects</th>
<th>SO₄⁻</th>
<th>F⁻</th>
<th>AcO⁻</th>
<th>Cl⁻</th>
<th>Br⁻</th>
<th>NO₃⁻</th>
<th>ClO₄⁻</th>
<th>I⁻</th>
<th>SCN⁻</th>
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<td>Denaturation:</td>
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<tr>
<td>Collagen-gelatin</td>
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<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<td>9</td>
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<tr>
<td>Rabbit muscle aldolase</td>
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<td>DNA</td>
<td>4</td>
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<td>Actomyosin</td>
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<td>7</td>
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<td>34</td>
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<td>F-actin</td>
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<td>9</td>
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<tr>
<td>Solubility:</td>
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* This work.
additional effects of salts which might influence protein
ular hydrogen bonds, is also decreased by the
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by these ions. In this regard it may be added, however,
14), 24 (1965). See also Shrier, E. E., and E. B. Shrier, J.
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weaken hydrophobic bonds by changing the structure and
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aqueous
membrane systems as mitochondria in the presence of various ions.
extracted in soluble form (studies in collaboration
90,
Soc.,
Biochemistry,
Shapiro,
Zh.
Kleinschmidt,
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Robinson, D. R., and
Hofmeister, F., Arch.
Nagy, B., and
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Wolff,
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Ca~stellenio,
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Packer,
Hatefi,
Fowler,
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Mtshuller, A.
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Frank, H. S., and
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4Elev,
D. D., Trans.
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von
Stern,
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Hamaguchi,
Ben-Naim,
Dandliker,
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Shoolery,
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Mtshuller, A.
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Although the data presented here strongly support the
the basis of ultrasonic attenuation, Hammes and colleagues conclude that urea and
guanidinium chloride also have solvent structure-breaking properties: Hammes, G. G., and
SOC., 90, 7119 (1968).

Hatefi, Y., in Comprehensive Biochemistry, ed. M. Florkin and E. H. Stotz (Amsterdam:

Among other proteins, UDPG-teichoic acid glucosyl transferase is almost completely
extracted in soluble form (studies in collaboration with F. E. Young and colleagues).

By disintegrating membranes, chaotropic ions also cause a rapid decrease in turbidity of
aqueous suspensions of these particles. This effect might be relevant to the swelling of such
membrane systems as mitochondria in the presence of various ions.

Hatefi, Y., and W. G. Hanstein, manuscript in preparation.

Antimycin A reacts with complex III in amounts equimolar to cytochrome c1 and inhibits
electron transfer in this complex from cytochrome b and coenzyme Q to cytochrome c1.


Although the data presented here strongly support the possibility that chaotropic ions
weaken hydrophobic bonds by changing the structure and lipophilicity of water, other
attractive forces (especially in proteins) such as ionic and hydrogen bonds might also be influenced
by these ions. In this regard it may be added, however, that the "denaturation" (insolubilization)
temperature of polyvinylmethylloxazolidinone, a polymer incapable of making intramolecu-
lar hydrogen bonds, is also decreased by the compounds under consideration in this article in the
order NaSCN, LiBr, NaCl, urea NaF, (NH4)2SO4 (Klotz, I. M., Federation Proc., 24 (Suppl.
additional effects of salts which might influence protein solubilization.

Miller and Hildebrand have shown that the degree of entropy loss of inert molecules and atoms is proportional to the "nonwetting" surfaces of the solutes.
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Shapiro, Biochemistry, 6, 1460 (1967).
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