The Entropic Cost of Bound Water in Crystals and Biomolecules

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Most structural chemists and biochemists would agree that some entropic cost must be associated with the binding of water molecules to proteins or other macromolecules, but they have been undecided about how large this cost might be. Comparison of standard entropies (at 298 K) for anhydrous and hydrated inorganic salts (see table) (*1*) indicates that each bound water molecule in a crystalline hydrate contributes roughly the same amount (about 10 cal $\mathrm{mol^{-1}\ K^{-1}}$) to the standard entropy, almost the same as the standard entropy of ice at its freezing point (9.9 cal $\mathrm{mol^{-1}\ K^{-1}}$). This temperature difference does not change the conclusion that the entropy associated with each immobilized water molecule in a hydrated crystal is relatively insensitive to the detailed crystal environment. As the standard entropy (298 K) of liquid water is 16.7 cal $\mathrm{mol^{-1}\ K^{-1}}$, the entropic cost of transferring a single water molecule to a site in which it is relatively immobile in ice or in a crystalline salt is thus at most 7 cal $\mathrm{mol^{-1}\ K^{-1}}$, corresponding to a free energy cost of about 2 kcal $\mathrm{mol^{-1}}$ $\mathrm{K^{-1}}$ at 300 K.

This information has been available for a long time, but science has become so specialized that its practitioners in one branch are all too often unaware of what is common knowledge in another. More than 40 years ago, Latimer assigned the value 9.4 cal $\mathrm{K^{-1}}$ to the entropy contribution of a mole of water in solid hydrates (*2*). This has evidently not been lost on geochemists and mineralogists for, more recently, a value of 9.6 cal $\mathrm{mol^{-1}\ K^{-1}}$ has been proposed for the contribution of tightly bound structural water in estimates of the entropies of minerals (*3*). For the loosely bound water in the channels of zeolites and related minerals, the entropy contribution is larger, about 14 to 15 cal $\mathrm{mol^{-1}\ K^{-1}}$, almost as large as for liquid water.

Although the structures of the hydrates in our table differ widely, they have in common that the water molecules are strongly associated with the cations through ion-dipole interactions and hence adopt definite equilibrium positions and orientations.

The frequencies of the three normal vibrations of the water molecule are so high that these vibrations do not contribute to the entropy at 298 K. In the solid hydrates, the relatively constant 10 cal $\mathrm{mol^{-1}\ K^{-1}}$ per water molecule must therefore arise from hindered translations and rotations (librations), that is, from lattice vibrations, which will have frequencies between about 200 and 500 $\mathrm{cm^{-1}}$. From the Einstein formula (*4*), each such vibration would contribute about 1 to 2 cal $\mathrm{mol^{-1}\ K^{-1}}$ to the standard entropy. As there are six such vibrations (three translations and three librations), the constant value of around 10 cal $\mathrm{mol^{-1}\ K^{-1}}$ is of the expected magnitude. For ice, and possibly also for some of the salt hydrates, there is also the residual entropy due to proton disorder. But this only amounts to 0.81 cal $\mathrm{mol^{-1}\ K^{-1}}$ for ice (*5*) and hardly affects the above semiquantitative estimates.

So what does all this have to do with the hydration of proteins and other biomolecules? It sets limits. Since a water molecule in a crystalline protein can hardly be bound more firmly than a water molecule in a crystalline hydrate salt, its entropy contribution can hardly be less than our overall figure of around 10 cal $\mathrm{mol^{-1}\ K^{-1}}$. On the other hand, the corresponding contribution of the most weakly bound water molecules can hardly be greater than 16 to 17 cal $\mathrm{mol^{-1}\ K^{-1}}$, the value of liquid water. Thus, we have a range of between 0 and 7 cal $\mathrm{mol^{-1}}$ $\mathrm{K^{-1}}$ for the entropy cost of transferring a water molecule from the liquid to the protein, corresponding to a free energy cost of between 0 and 2 kcal $\mathrm{mol^{-1}}$ at 300 K. The higher value will apply only to those few water molecules that are most firmly bound (to metal centers or to polar groups); these can have Debye-Waller temperature factors (B values) of around 5 to 10 Å^2, comparable to values found for well-localized main-chain atoms and not much larger than those for water molecules in salt hydrates at room temperature. Most of the remaining water content, including much of the surface water, is likely to cost only a small fraction of this upper limit. It will behave more like liquid water, similar to the loosely bound nonstructural water in zeolites.

Salt	$S°$	$\Delta S°/n$
$ZnSO_4$	29.8	
1	34.9	5.1
6	86.8	9.5
7	92.4	8.9
$CdCl_2$	28.3	
1	40.8	12.5
5/2	55.6	10.9
$CdBr_2$	31.9	
4	74.7	10.7
$CdSO_4$	32.8	
1	41.1	8.3
8/3	57.9	9.4
$CuSO_4$	27.1	
1	35.8	8.7
3	53.8	8.9
5	73.0	9.2
$NiCl_2$	25.6	
6	75.2	8.3
$NiSO_4$	18.6	
6	73.1	9.1
$Al_2(SO_4)_3$	57.2	
6	112.1	9.2
$NH_4Al(SO_4)_2$	51.7	
12	166.6	9.6
$MgCl_2$	21.4	
1	32.8	11.4
2	43.0	10.8
4	63.1	10.4
6	87.5	11.0
$Ca(NO_3)_2$	46.2	
2	64.3	9.1
3	74	9.3
4	81	8.7
Na_2SO_4	35.73	
10	141.7	10.6
$KAl(SO_4)_2$	48.9	
12	164.3	9.6

Standard entropies (298.16 K) of anhydrous salts and their corresponding hydrates (*1*). For each hydrate, the number *n* of water molecules per formula unit is given below the formula of the anhydrous salt.

Although this discussion has not led to any new information, it shows how extensive thermodynamic data in one area can be used to derive approximate values for nonexistent thermodynamic data in another. The transfer entropies discussed here are not to be confused with the "entropies of hydration" of polar and nonpolar groups in proteins, which are relevant to the thermodynamics of protein folding. These involve entropies of transfer of model compounds from the gas phase to water, and not the entropies of transfer of water from the liquid to the protein. Even if we have merely limits for such processes, that is better than nothing.

References

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