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Molecular Aspects of the Hydration Process in a Globular Protein

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In past years we have been interested in some general problems of enzyme dynamics [1], and we have considered the possibility that random changes in the hydration of the globular protein may induce conformational fluctuations relevant toward catalysis. We have also obtained experimental evidence [2] of a critical hydration value (at about 20% mg water/mg dry weight) for the onset of the enzymatic activity in lysozyme powder. We have also been able to follow in detail the hydration dependent events of this macromolecule by gravimetric and infrared techniques [3]. However, there are still some existing problems at the molecular level, particularly in the region of low coverage (below 10% hydration) which we believe are appropriate for discussion here. More exactly, we would like to understand the nature of the rather sharp transition that occurs in this range, and that is displayed by several physical properties, notably by the profile of the IR stretching band of the adsorbed D_2O.

Let us try to discuss the nature of this low hydration transition in terms of the current order–disorder and nucleation theories of embryonic droplets [4]. According to nucleation theory it is impossible to condense water on an insoluble particle with a radius smaller than about 100 Å, unless the pressure is higher than the equilibrium vapor pressure of liquid water. Therefore, a globular protein dry powder could not become hydrated under normal laboratory conditions if its surface is made of amide backbone and of nonionizable residues. Actually it has just occurred for "soluble particles" like the ionizable residues that water vapor condenses on a protein macromolecule, much in the same way as in the clouds, namely, the condensation of water droplets is induced by small salt particles. Of course this is because the solution process decreases the free energy to compensate for the free energy increase due to the surface tension of the droplet. As a matter of fact in our experiments [2], the first detectable event—when the dry specimen of protein gets hydrated—is correct for the ionization of the acidic side chains. It is conceivable that these strong hydration sites will continue to grow even connecting together similar side chains of different close macromolecules. However, while the hydration continues to increase, the surface backbone and the nonionizable side chains finally get involved in a process dominated by the surface tension of these hydrogen bonded species.

From an empirical study of the surface entropy of a large number of liquids,

it is known that a kind of ordering must occur in the surface of the hydrogen bonded liquids. This fact, in the case of water, is also supported by direct calculations. However, when the surface is not flat but spherical with a small radius there is an extra positive term in the free energy because of the work that destroys the parallel orientation of the surface dipoles. For this reason it seems that a very small droplet of H_2O should be a disordered network of dipoles, arranged as in ice, and that only for larger droplets the ordered surface structure should become stable. Actually it has been found that a disorder-to-order transition should take place when increasing the size of the droplet, the critical size consisting of about 100 water molecules. Of course this argument holds for an homogeneous nucleation process, but we may speculate that a similar picture should be shown in the heterogeneous nucleation of water on the protein surface amide backbone, because of the close hydrogen bonding energies of the water–water, amide–amide, and water–amide species. Therefore the transition observed in the IR data [3] below 10% hydration should reflect a change from the initial icelike structure of the H_2O molecules around the ionizable groups to the final liquidlike structure of the H_2O molayer adsorbed on the macromolecule.

At the present time we are working on a better computer analysis of our IR data in the low hydration region. It appears that the surface amide backbone (detected by the amide I band) is also involved. This is quite possible, because the two hydrogen bonded species (water and amides) must be accommodated in a common network, the water–protein interphase. It is hard to assess a value for the surface tension of this interphase.

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Discussion

C. Decoret: I would like to attract attention to one remark that is connected with the idea that the chemical bond concept is strictly related to chemical reactivity, i.e., to the cleavage and formation of bonds.

If I have understood correctly, water molecules more or less strongly bound to the amide function, as well as the latter itself, are in incessant relative motion; they can be imagined to be linked, so to speak, by little springs, and those motions should be taken into account for a clear understanding of how a new molecule can approach and eventually react. Those motions are not only susceptible to making room for the newcomer, but, if I have understood, they modify the electronic charges of the amide function, until conditions favorable to the for-

mation of a new bond are created. This space–time aspect of things is so natural that it may seem trivial; but theoreticians reasoning with static models tend to miss it.

G. Del Re: I would like to express my agreement with the idea that the dynamical point of view is fundamental for understanding chemical facts and concepts. In particular, I agree that the very motion of a chemical bond is associated with the idea that along the axis joining the two partners, vibrations are very difficult to excite: It seems to me that the value of the force constant is the real distinguishing feature of a chemical (covalent) bond. To adopt a dynamical picture is, of course, even more important in the case of weak associations, which may change continuously because of temperature effects.

However, a static picture may be less inadequate than one would think. Regarding ordinary bonds, a large force constant normally also corresponds to a deep energy minimum. In weaker binding, we must distinguish three cases: (a) The motion along the reaction coordinate is markedly slower than the vibrations of the centers involved; then the latter can be treated as fixed in space–time with properties practically corresponding to averages around equilibrium positions, possibly on account of the Boltzmann distribution. (b) If the motion along the reaction coordinate is markedly faster than the motions of the centers involved, the time development of the physical situation should be described in terms of the instantaneous properties of the various centers. (c) Finally, if the motions along the various degrees of freedom take place at many different rates, then case (a) applies to the fastest ones, case (b) to the slowest ones, and the others must be treated together, giving a sort of cooperative effect. It seems likely that in typical chemical reactions (say, addition of a carbocation to benzene) the first case applies, with thermal averages very close to fixed-nuclei properties computed at the energy minimum; this is why I think that the static picture can be accepted in many cases. Of course, it is by no means true that it holds in the case of solvation effects.

Y. Smeyers: In the problem of lysozyme hydrolysis, a possible conformation change should be taken into account for the formation of a $-COO^-$ and/or of an $-NH_3^+$ ion inducing internal rotations. Such conformational changes could mask the hybridization process and even be responsible for the phase change observed at 7% hydration.