Activity and dynamics of an enzyme, pig liver esterase, in near-anhydrous conditions

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ABSTRACT  Water is widely assumed to be essential for life (1), although the exact molecular basis of this requirement is unclear (2-4). Water facilitates protein motions (5-9), and although enzyme activity has been demonstrated at low hydrations in organic solvents (10-13), such non-aqueous solvents may allow the necessary motions for catalysis. To examine enzyme function in the absence of solvation and bypass diffusional constraints we have tested the ability of an enzyme, pig liver esterase, to catalyse alcoholysis as an anhydrous powder, in a reaction system of defined water content (16) and where the substrates and products are gaseous (14, 15). At hydrations of 3 (±2) molecules of water per molecule of enzyme, activity is several orders of magnitude greater than non-enzymatic catalysis. Neutron spectroscopy indicates that the fast (<nanosecond) global anharmonic dynamics of the anhydrous functional enzyme are suppressed. This indicates that neither hydration water nor fast anharmonic dynamics are required for catalysis by this enzyme, implying that one of the biological requirements of water may lie with its role as a diffusion medium rather than any of its more specific properties.

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An understanding of the role of hydration in enzyme activity is a central question in molecular biophysics. Previous work has indicated that the hydration required for activity is below the monolayer coverage (10, 15, 17, 18). For instance, pig liver esterase studies here (PLE; molecular weight of monomeric form ~60070 Da) has been found to have hydrolytic activity at a hydration level of 0.03 gm water/gm enzyme (h) at room temperature; i.e. ~100 water molecules per molecule of protein. PLE is useful for low hydration studies because water is neither a substrate nor a product in the alcoholysis reactions catalysed. Here, the acyl transfer between methyl butyrate and propanol was followed by headspace analysis. The isotopic labelling of water molecules (19) and its quantification by mass spectrometry is one of the most sensitive (16) methods of water determination and is used here to quantify low levels of PLE hydration, and accompanied by activity measurements and neutron spectroscopic experiments has allowed the correlation of protein hydration with flexibility and activity. The role of
water as a reactant or as a diffusion medium for the products and substrates of the reaction is precluded here by the use of a gas phase transesterification catalytic system. Figure 1 shows that enzyme activity is observed at all hydration levels investigated.

The lowest hydration achieved (see the inset to Figure 1) is 3 (±2) water molecules per molecule of protein. This hydration level may relate to the presence of internal water molecules that cannot be removed by the method we have used, but with current analytical methods this is difficult to verify experimentally, and there is a significant possibility that the enzyme is actually anhydrous at this reported hydration. The hydration level at which activity is observed is thus very much lower than the 0.2 grams of water per gram of protein (h), i.e. a mole ratio of over 600, conventionally taken to be necessary for enzyme activity, and represents a qualitatively lower hydration regime. The first stage of any protein sorption isotherm consists of the hydration of the ionised groups at the protein surface, up to about 0.05 h (20). The data here show that enzyme activity occurs and increases up to this level of PLE hydration. Although the enzyme rates are low, they are at least one order of magnitude higher than the un-catalysed rate. At very low hydrations there is no clear correlation between activity and hydration, so although completely anhydrous enzyme may not have been achieved, enzyme activity at zero hydration seems likely. Water that interacts directly with the protein surface has been generally thought to play a major role in protein function (4). Since a water content as low as 3 ± 2 water molecules per molecule of PLE represents an insignificant coverage of the charged groups of the protein surface the evidence here indicates that surface hydration water is not essential for PLE activity, although it may facilitate it.

PLE being active at hydration levels close to zero, any motions required for the onset of enzyme activity are not likely to be dependent on hydration. Although water seems to play a major role in protein dynamics, previous work on xylanase in cryosolvent has revealed that this enzyme may be active while apparently rigid (21).

Thus, any correlation between enzyme hydration, dynamics and activity is still not clear (22, 23). To examine the fast motions of the enzyme, the average internal atomic mean-square displacement of PLE, \( \langle u^2 \rangle \) was determined by neutron scattering with the IN5 time-of-flight spectrometer (24) and the IN16 backscattering spectrometer (refer to supplementary material) at the Institut Laue-Langevin, Grenoble, France. These measurements were performed on dried or hydrated powders as for activity measurements.

In Figure 2 \( \langle u^2 \rangle \) is shown as a function of temperature for three different hydrations.

Figure 1: Enzyme activity with respect to propyl butyrate (lines) and methanol (dashed lines) production in the gas phase, as a function of the protein hydration. The inset is a blow-up of the very low hydration region of the plot.

Figure 2: \( \langle u^2 \rangle \) of PLE as a function of the temperature for the three hydrations measured and obtained from data collected with IN16 and IN5 (24).

The curve for the “fully hydrated” control, (0.5 h), exhibits a change in slope at ~220K – this is the so-called “dynamical transition” or “glass transition” of the protein, where the protein motions apparently pass out of the timescale window of the instrument (25, 26). The activation of motions at the dynamical transition has been associated with protein function. For the two other lower-hydration samples, the anharmonic motions that are reflected in the increased slope above the dynamical transition, are strongly suppressed, consistent with their being largely solvent driven (7, 27, 28). These results are consistent with an interpretation that water decreases the energy barriers between local minima, as is required for the onset of
diffusive motions of the protein atoms (7, 29). However, we note that NMR has shown that the inherent inhomogeneous temperature dependence of motion predicts the dynamical transition, consistent with it not being a product of solvent slaving per se (30). Because of the differing energy resolutions of the respective instruments, IN16 (Figure 2) probes motions on a nano-second timescale while IN5 (Figure 2) probes motion on a pico-second timescale. With IN16, a steeper change in slope with hydration is observed than for IN5 (24), indicative of the effect of the energy resolution on the mean-square displacement (MSD): IN16 has a finer resolution and thus incorporates additional, slower motions into the MSD.

CONCLUSIONS

The present work shows clear evidence that the activity of PLE does not necessarily require that the enzyme be significantly hydrated: within the limits of the water detection method used, activity at very near zero hydration has been observed. It is important to realize that the hydration level of 3+/~ 2 is an average, and that those enzyme molecules in the sample exhibiting the residual activity might be significantly more highly hydrated. Whether the present results can be generalized to all enzymes is an open question. Perhaps pig liver esterase is comparatively rigid, requiring only stabilization of the transition state of the catalyzed reaction, consistent with the idea that electrostatic preorganization accounts for the observed catalytic effects of enzymes, rather than dynamical effects (31). Other enzymes such as those involving mechanical displacements may require higher hydration levels.

The present results raise general questions concerning the role of surface hydration in enzyme activity. Clearly hydrolysis reactions require the participation of water molecules, and some proteins contain strongly-bound structurally-important water molecules that may be difficult to remove by drying. However, the results show that in principle, although hydration facilitates activity, probably due to the dynamical effects manifested above the “glass transition” in the neutron spectra in Figure 2, significant solvation is not an absolute requirement.

Given that water is the only readily-available terrestrial liquid solvent, it is unsurprising to find its incorporation in proteins, and dependence upon it as diffusion medium. However, the present results are consistent with the main role of water in enzymology being as a (non-specific) solvent and diffusion medium rather than as a chemically-unique essential component.

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