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The Lubricant of Life: A Proposal That Solvent Water Promotes Extremely Fast Conformational Fluctuations in Mobile Heteropolypeptide Structure†

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ABSTRACT: Recent observations using the novel technique of Raman optical activity suggest that individual residues in unfolded proteins and in disordered loop regions of molten globule-like states cluster in the α-helix, β-structure, and PPII-helix regions of the Ramachandran surface and that they "flicker" between these regions at rates $\sim 10^{12}$ s^{-1} at room temperature. It is proposed that these rapid motions, which occur on the same picosecond time scale as rearrangements of the hydrogen bond network in bulk water, are promoted by solvent water molecules via a repertoire of transient hydrated reverse turn conformations. Some implications of this proposal for protein folding and function are discussed.

The great speed and efficiency of the dynamic processes involved in protein folding and function continue to amaze. New experimental techniques have recently started to provide detailed information about early events in protein folding (Williams et al., 1996; Pascher et al., 1996; Ballew et al., 1996; Hagen et al., 1996; McCammon, 1996). For example, observations by Hagen et al. (1996) using time-resolved laser spectroscopy suggest a rate of $\sim 10^6$ s^{-1} as the upper speed limit for protein folding based on the diffusion-controlled rate of collision of sequentially distant segments of an unfolded protein, which is assumed to determine the shortest time for the initial collapse to a compact structure. These experiments provide valuable information about the rate-limiting slopes of energy landscapes within the many-dimensional folding funnels that are starting to provide a new view of protein folding kinetics (Wolynes et al., 1995; Dill & Chan, 1997). But there remains a dearth of information about the very earliest stages of protein folding, "the view from the high vistas" of the folding funnel in the words of Dill and Chan (1997). A glimpse from these high vistas has now been provided by Wilson et al. (1996a) using a form of laser spectroscopy that is sensitive to the chirality (handedness) of the peptide backbone: the view, although hazy, reveals processes within non-native protein states which are 6 orders of magnitude faster still and which appear to be mediated by solvent water.

The central role of water in protein science has long been appreciated (Rupley & Careri, 1991; Gregory, 1995; Jeffrey & Saenger, 1994). In particular, its ability to promote conformational changes was emphasized by Sundaralingham and Sekharudu (1989) following an analysis of the hydration of α-helices in a set of protein X-ray crystal structures. They found that the hydration can be external by a water molecule hydrogen bonding to the backbone carbonyl oxygen atom, to both the carbonyl oxygen and amide nitrogen atoms with the amide proton involved in a three-center hydrogen bond (i.e., the amide proton is bound to three centers comprising the amide nitrogen, the carbonyl oxygen, and the water oxygen), or internal by a water molecule inserting into the α-helix 5 → 1 hydrogen bond and forming a hydrogen-bonded bridge between the backbone carbonyl oxygen and amide nitrogen atoms. The structures with three-center hydrogen bonds can be regarded as intermediates in the

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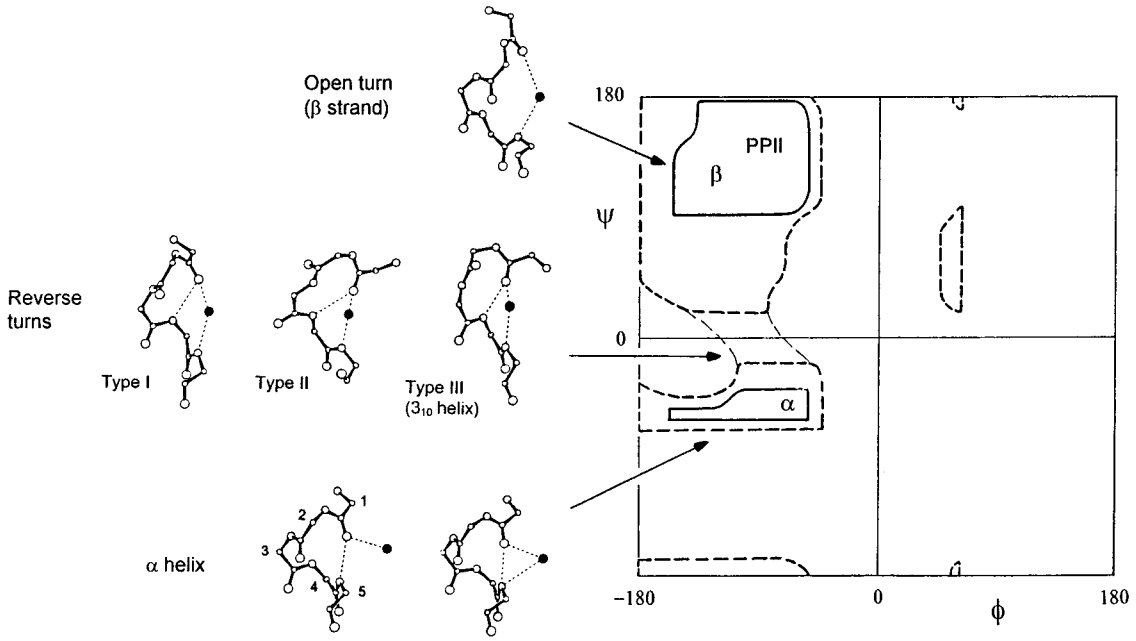

FIGURE 1: Helix−coil unfolding promoted by insertion of a water molecule (●) into the α-helix 5 → 1 hydrogen bond via initial formation of a transient three-center hydrogen bond leading to the formation of a repertoire of hydrated reverse turn conformations. These turn conformations populate the neck connecting the α- and β-regions and so might facilitate the extremely rapid α ↔ β "flickering", as sensed by ROA, between individual residues in unfolded proteins by serving as low energy intermediates along the interconversion pathway. Adapted from Sundaralingham and Sekharudu (1989).

transition between the externally and internally bound states. As illustrated in Figure 1, these water-inserted α-helical segments display a range of reverse turn conformations including type III, type II, and type I all with 4 → 1 hydrogen bonds, plus an open turn with no 4 → 1 hydrogen bond and tending toward the extended β-strand. Since these turn conformations tend to occupy the neck connecting the α-helix and β-structure regions on the Ramachandran potential energy surface, Sundaralingham and Sekharudu suggested that the hydrated segments could represent intermediates in the unfolding process from the α-helix to the extended chain or random coil in aqueous solution. This process is initiated by water molecules prying open the helix hydrogen bond via initial formation of a three-center hydrogen bond, with the conformational reaction coordinate represented by the structures that differ least in conformation and therefore energy lying in the contiguous regions of the ϕ,ψ space that are both stereochemically and energetically permitted. This use of X-ray crystal structures to map reaction pathways falls within the scope of a method pioneered earlier for small molecules by Bürgi and Dunitz (1983). Subsequently, molecular dynamics simulations of α-helix unfolding in water broadly confirmed the importance of helix destabilization by water insertion (DiCapua et al., 1990; Soman et al., 1991; Tobias & Brooks, 1991; Tirado-Rives & Jorgensen, 1991). Jeffrey and Saenger (1994) discussed how three-center hydrogen bonds can lower the potential energy barrier in general dynamic processes associated with hydration and were led by the results of Sundaralingham and Sekharudu (1989) to propose that "the combination of water molecules and three-center hydrogen bonds may be considered as "lubricants" in the dynamic processes of macromolecular folding, unfolding and interaction which are so important for life."

Further support for these ideas is provided by some work of Griebenow and Klibanov (1996), which showed that, in contrast to aqueous−organic mixtures which usually induce denaturation, the inherent restrictions on protein conformational mobility in anhydrous organic media stabilize protein secondary structure. They suggested that, even though the thermodynamic tendency of a protein to denature increases as the organic solvent content in the medium is raised, as the water content decreases the protein conformational mobility and hence its ability to acquire the thermodynamically dictated conformation decreases; in other words, the conformation becomes kinetically trapped in anhydrous solvents.

New Spectroscopic Evidence for Extremely Fast Conformational Fluctuations. Recent studies of non-native protein states in aqueous solution (Wilson et al., 1996a,b) using the novel technique of Raman optical activity (ROA),[1] which measures vibrational optical activity via a small difference in the intensity of Raman scattering from chiral molecules in right and left circularly polarized incident light (Barron et al., 1996), have provided further insight into the role of water as a "lubricant" in conformational changes. Protein ROA spectra contain bands from loops and turns in addition to secondary structure and, hence, provide information about the tertiary backbone fold which, together with a special sensitivity to conformational mobility and the very short timescale of $\sim 10^{-14}$ s for the Raman scattering process, makes ROA valuable for studying non-native states. Specifically, ROA has revealed that the individual amino acid residues in unfolded (reduced) lysozyme and ribonuclease A appear to cluster in regions of Ramachandran space corresponding to the ϕ,ψ angles found in α-helix, β-sheet, and possibly poly(L-proline) II (PPII)-helix, in agreement with current ideas on the "random coil" state (Smith et al., 1996); but in addition, the ROA data suggest that the

[1] Abbreviations: ROA, Raman optical activity; NMR, nuclear magnetic resonance; PPII, poly(L-proline) II.

individual residues "flicker" between these regions at rates $\sim 10^{12}$ s^{-1} at room temperature, which is close to the maximum allowed by simple chemical kinetics theory. These conclusions were based on the observation of sharp positive amide III ROA peaks at ~1300 and 1314 cm^{-1} assigned to α- and β-structures, respectively, which coalesce with increasing temperature in a manner reminiscent of chemical exchange effects in NMR, and a negative peak at ~1237 cm^{-1} assigned to PPII-helix (Wilson et al., 1996a). (Coalescence of vibrational bands occurs in the fast exchange limit where the rate of exchange of the two conformers, which is inversely proportional to their lifetimes, is faster than the angular frequency difference $2\pi\delta\nu$ between the vibrational bands characteristic of the conformers; hence, the observed wavenumber difference ($\delta\nu/c$) of ~14 cm^{-1} corresponds to a rate of $\sim 2.6 \times 10^{12}$ s^{-1}, which is on the picosecond time scale and so is easily detectable on the time scale of Raman spectroscopy, which is 2 orders of magnitude faster.)

Since only weak ROA signals characteristic of α-helix and β-sheet were observed in the backbone $C^{\alpha}-C$ and $C^{\alpha}-N$ stretch region ~870−1150 cm^{-1} of unfolded lysozyme at room temperature, it was concluded that very little extended secondary structure itself is present. Unfolded ribonuclease A appears to contain rather more secondary structure.

In another study, this time on the non-native molten globule state adopted by α-lactalbumin at low pH, ROA revealed that, while much native-like secondary structure persists over the temperature range 2−45 °C, the loop structure defining the tertiary backbone fold is completely disordered at 35 °C but becomes increasingly native-like with decreasing temperature (Wilson et al., 1996b). Of relevance here is that, at the higher temperatures where the loop structure is disordered, similar amide III ROA signals to those observed in unfolded lysozyme and ribonuclease A were seen, which indicates that individual residues within the mobile loop regions have similar dynamic behavior to the residues in unfolded proteins. The same might be true for some mobile regions in native lysozyme at room temperature (Wilson et al., 1997), but the ROA data are not so clear in this case. This last possibility is particularly interesting in view of the recent demonstration that two human lysozyme variants responsible for amyloid disease have unstable β-domains (Booth et al., 1997).

Solvent Water Might Promote α↔β Flickering. The results of Sundaralingham and Sekharadu (1989), of which we were not aware at the time of writing Wilson et al. (1996a), provide a simple explanation for such rapid rates: the solvent water molecules might actually "catalyze" (in a loose sense since the water also affects the equilibrium constants) the interconversions by means of a repertoire of transient hydrated reverse turn conformations, which provide a low-energy pathway between the α- and β-regions of the Ramachandran surface (Figure 1). Such a mechanism would be similar to the "polyfunctional catalysis" by solvent molecules observed in concerted proton transfers of simple organic reactions in accordance with Jencks' principle (Schowen, 1997).

A rough, but instructive, representation of the potential energy profile through the neck connecting the α- and β-structure regions of the Ramachandran surface may be obtained in terms of the torsional energy function calculated by McCammon et al. (1980) in the context of the helix−coil transition within a virtual bond model using the geometric and energy parameters for polyvaline. This energy function was chosen to approximate the potential of mean force corresponding to a thermal average at 300 K over polypeptide and solvent degrees of freedom. They defined a virtual torsion angle Φ_i, related to $\phi + \psi$, in terms of the interaction centers R_i (taken at the C^{β} positions) of residues i, $i + 1$, $i + 2$, and $i + 3$ with $\Phi_i = 0$ for the eclipsed conformation. This angle is a variant of the virtual torsion angle defined by Levitt (1976) in terms of the positions of four adjacent C^{α} atoms in the chain [see also Peticolas and Kurtz (1980)]. The continuous curve in Figure 2 shows the potential energy calculated for a residue at the helix−coil interface using only near-neighbor atomic nonbonded interactions, whereas the broken curve shows the complete potential energy which includes bond and bond angle interactions, attractive van der Waals and solvent interactions E_{sol}, a repulsive excluded volume term E_{ev}, and a helix stabilization term which includes free energy changes associated with the formation of a backbone hydrogen bond and the freezing of rotational motions of the amide plane. Since ROA indicates that very little extended α-helix is present in unfolded lysozyme at room temperature, we can use the continuous curve to visualize the profile of the potential energy surface (including both E_{sol} and E_{ev}, which approximately cancel in the range ~20−240°) over which the α ↔ β conformational flickering of individual residues occurs at room temperature in this sample. There are two shallow wells with minima at ~55 and 200° which can be identified with the general α- and β/PPII-regions in which the individual residues appear to cluster. The β-region is slightly more stable than the α, and the two regions are separated by an energy barrier of ~0.5 kcal/mol, which within the simplest version of absolute reaction rate theory (Creighton, 1993), gives a rate constant at 45 °C of $\sim 3.0 \times 10^{12}$ s^{-1}. This is remarkably close to the observed value of 2.7×10^{12} s^{-1} at 45 °C (Wilson et al., 1996a), which suggests that the continuous potential energy profile is not too unrealistic. It can be seen from the broken curve in Figure

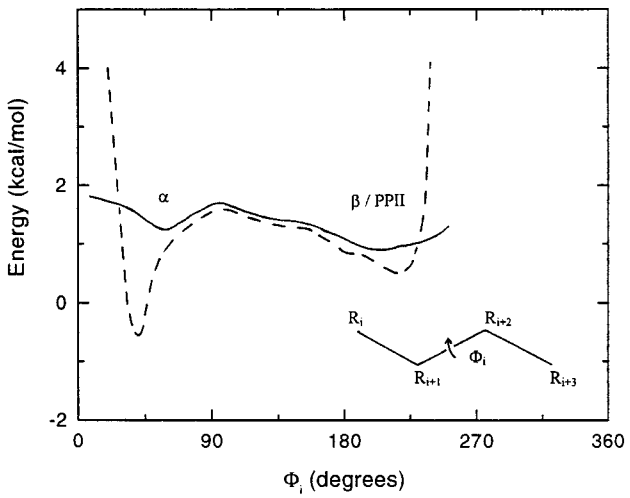

FIGURE 2: Approximate potential energy profiles for changes of an individual residue i in a heteropolypeptide between the general α- and β/PPII-conformational regions as a function of the virtual torsion coordinate Φ_i. The continuous curve is for near-neighbor atomic nonbonded interactions only, and the broken curve is for the complete energy including helix stabilization interactions. Adapted from the calculated curves of McCammon et al. (1980) for a residue at the helix−coil interface.

2 that, when the stabilization forces present in extended α-helix are included, a narrow potential well with a barrier of ~2 kcal/mol for the helix−coil transition results. However, as discussed above, formation of extended α-helix is inhibited by interactions with water molecules which destabilize the nascent helices so that, from the ROA evidence of α ↔ β flickering and the absence of significant amounts of extended α-helix, a potential energy curve like the continuous one in Figure 2 is more appropriate for reduced lysozyme at room temperature. The broken curve would be more appropriate at reduced temperatures where, as expected (Tirado-Rives & Jorgensen, 1991; Creighton 1993), ROA shows more extended secondary structure to be present (Wilson et al., 1996a). Of course an accurate calculation on reduced lysozyme itself should automatically generate the correct curve for a given temperature. The fact that, according to ROA (Wilson et al., 1996a), reduced ribonuclease A contains roughly 50% of its native-like secondary structure at room temperature suggests that the temperature dependence of these potential energy curves is very sensitive to the details of the sequence.

In bulk water, collective motions and energy fluctuations occur, which are associated with rearrangements of the hydrogen bond network, with hydrogen bond breaking and making occurring on the picosecond time scale (Ohmine & Tanaka, 1993). Studies of Raman band widths have shown that vibrational motions within polynucleotides may be perturbed by such water dynamics (Terpstra et al., 1997). Furthermore, Chen et al. (1994) have shown from resonance Raman studies that the motions of water molecules hydrogen bonded to the model peptide *N*-methylacetamide in aqueous solution are coherently vibrationally coupled to motions of the nuclei of the peptide and suggested that such coupling could be important in the dynamics of peptides and proteins. Hence, some sort of cooperative proton motion, coupled to changes in the torsional reaction coordinate Φ_i and driven by picosecond dynamics of bulk liquid water, might be involved in the α ↔ β flickering process in the unfolded protein in aqueous solution.

Some Implications for Protein Folding and Function. The possibility that solvent water actually catalyzes extremely rapid interconversions between residue conformations lying in the α- and β-regions of the Ramachandran potential energy surface in mobile heteropolypeptide structure provides new insight into the dynamic processes that facilitate the rapid flow down the folding funnel toward the native structure. To start with, the transient conformations of the individual residues in the unfolded protein are already clustering in the same regions of ϕ,ψ space where they are mostly found stabilized in native folded proteins. As the open coil starts to collapse, connected residues in parts of the sequence destined to coalesce into segments of, e.g., α-helix, can, if not already in the correct part of the ϕ,ψ surface, adjust in the shortest possible time. Furthermore the expulsion of water, the agent that catalyzes rapid conformational fluctuations, from hydrophobic regions might help to stabilize transient secondary structure during collapse. It could be significant for protein function that, as mentioned above, the same type of dynamic behavior of individual residues found in the unfolded state survives in solvated loop regions in the band of molten globule states well down the funnel, and even perhaps in certain regions of native folded proteins near the bottom.

The functional importance of residual mobility in certain regions of native folded proteins has been highlighted by an ROA study of cyclodextrins, which revealed evidence for considerable mobility in the glycosidic links, thereby providing new insight into the analogous behavior of enzymes and cyclodextrins with respect to ligand binding and catalytic activity (Bell et al., 1997). In both cases, residual mobility within the links connecting a framework of relatively rigid elements (secondary structure elements in proteins, glucose residues in cyclodextrins) allows rapid sampling of a range of conformations without loss of the overall three-dimensional integrity of the structure. The recent discovery that zeolites have similar characteristics, with rigid SiO_4 and AlO_4 tetrahedra connected by very flexible links through shared oxygen atoms (Hammonds et al., 1997), suggests that some inorganic catalysts share a common modus operandi with enzymes and enzyme mimics. Further refinement of this concept is provided by a low-temperature X-ray crystallographic study of α-lytic protease, which revealed the existence of "dynamic close packing" from correlated motions of residues in the binding pocket facilitated by an ensemble of conformational substates (Rader & Agard, 1997). That water lubricates this machinery is inferred from the requirement that a certain critical hydration level must be reached before initially anhydrous enzymes become catalytically active (Rupley & Careri, 1991).

The idea of solvent water promoting rapid conformational fluctuations also helps to cast light on the role of 3_{10}-helix in protein folding and function, a subject of considerable current interest (Millhauser, 1995; Smythe et al., 1995a,b; Sheinerman & Brooks, 1995). Since a 3_{10}-helix turn is equivalent to a type III reverse turn, the absence of characteristic 3_{10}-helix signals in the ROA spectra of unfolded proteins (Wilson et al., 1996a) suggests that, in unfolded states, 3_{10}-helical conformations have only a virtual existence (i.e., their lifetimes are comparable to the period of vibrations associated with the torsional reaction coordinate) as transient hydrated intermediates on the α ↔ β interconversion pathway. Thus, there appears to be zero propensity for individual residues to take up 3_{10}-helical ϕ,ψ angles in fully solvated mobile heteropolypeptide structure in aqueous solution at room temperature, which reinforces conclusions from earlier protein and peptide ROA studies that 3_{10}-helix is finely tuned and can only exist in a relatively structured environment (Wilson et al., 1996b,c; 1997).

Interestingly, ROA does not sense the same type of dynamic disorder in unordered *homo*polypeptides, such as poly-L-lysine (Wilson et al., 1996c), as that sensed in unfolded proteins. This fits with the idea of water catalyzing the α ↔ β interconversion pathway because homopolypeptides cannot support the full repertoire of required turn conformations. As well as indicating that small homopolypeptides are not good models for studying the fastest processes in proteins, this has implications for discussions of the chemical origin of life, for, if based on amino acids, the homochiral polymers thought to have been the necessary precursors (Cline, 1996) are likely to have been *hetero*- rather than homopolypeptides since they must surely have exploited the remarkable dynamic efficiency unique to the former.

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