A Catalytic Role for Protic Solvents in Conformational Interconversion

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The role of solvent in protein structural stability and dynamics is very important. It has been hypothesized that protic solvents play fundamentally important roles in destabilizing electrostatic interactions in macromolecular structure and in inducing dynamics.[1] Recently, spectroscopic methods have been developed to observe solvent bound on the surface of proteins and polypeptides.[2] However, the structural, dynamic, and functional implications for bound solvent have been less actively pursued. Here gramicidin A, which forms several well-defined conformers in organic solvents, is studied. Conformer distribution and interconversion rates for these dimers are solvent dependent; in particular, polar solvents have been reported to enhance these interconversion rates.[3,4] More specifically, we show that protic solvents enhance this rate, and furthermore, we show the location of solvent molecules bound to the surface of the polypeptide. From this we suggest that the protic solvent is catalyzing the interconversion between conformational states by facilitating hydrogen bond exchange in an otherwise low dielectric environment.

Gramicidin A is a 15 amino acid polypeptide that forms dimeric helical structures in organic solvents and in a lipid environment.[3,5] These structures in organic solvents have been characterized by solution NMR[6−8] and X-ray crystallography[9] as parallel and antiparallel dimeric structures varying in helical sense and as to whether the monomers are staggered or optimally hydrogen bonded. In alcohol solvents the interconversion rate between conformers is slow compared to the NMR time scale ($100\ s^{-1}$),[7] but fast compared to thin layer chromatographic separation time ($10^{-3}\ s^{-1}$).[3] In aprotic solvents such as dioxane or tetrahydrofuran, the interconversion rate slows so that individual conformations can be separated by HPLC[6] and thin layer chromatography.[3] In fact, the individual conformers isolated by normal phase HPLC are stable in dioxane for more than 2 weeks ($>10^{-6}\ s^{-1}$).[6] However, at equilibrium in dioxane and other low dielectric solvents, only a single conformer (95%), the antiparallel left-handed (APLH) intertwined helix, is present.[10] Therefore, the other conformations represent trapped metastable states that are in non-minimum energy conformations for this solvent. The lipid bilayer, as another low dielectric medium, has also been shown to trap a metastable conformational state of gramicidin.[11]

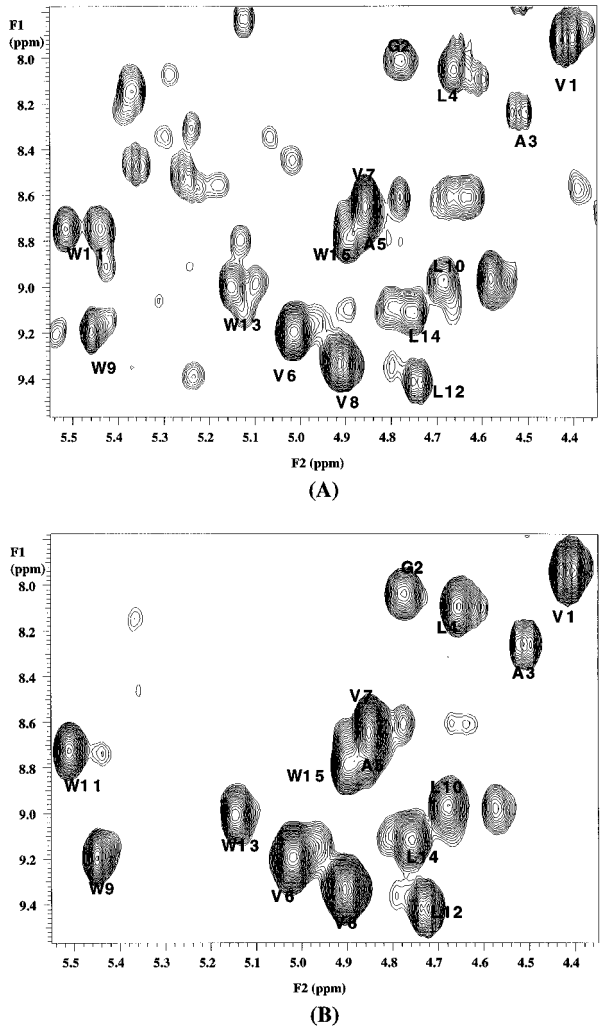

Figure 1. TOCSY spectra of 10 mM synthetic gramicidin A produced by solid phase peptide synthesis on an ABI model 430A peptide synthesizer using Fmoc blocking chemistry. These spectra were obtained on a Unity Plus 500 MHz spectrometer with a 60 ms mixing time and a 2 s recycle delay. The spectra were processed with the same window functions and plotted on the same vertical scale. Samples for both spectra were originally dissolved and equilibrated in 100% ethanol followed by evaporation of the solvent. (A) Dissolved in 100% dioxane-d_8. (B) Dissolved in 1% ethanol-d_5 and 99% (v/v) dioxane. The assignments for the antiparallel left-handed (APLH) dimer are presented.

In Figure 1 the catalytic effect of ethanol on the conformational interconversion rate is illustrated through TOCSY spectra of gramicidin A in 100% dioxane and in 1% ethanol in dioxane. Both of these samples were prepared initially in 100% ethanol. For Figure 1A the ethanol was evaporated and the peptide dissolved in 100% dioxane. Conformations other than the APLH conformer (assigned in Figure 1) represent trapped conformations from the polypeptide's previous solvent.[3,7] For Figure 1B the sample in a minimal amount of ethanol is diluted to 99% (v/v) dioxane. With this small amount of ethanol, the multiple conformers rapidly convert to a single dominant conformation (APLH). These observations and others[3,4] strongly suggest that the protic solvent has facilitated the rate of conformational interconversion, that it has done so without being consumed, and that it has not significantly modified the equilibrium conformational distribution. Therefore, we suggest that the protic solvent has acted as a catalyst to lower the

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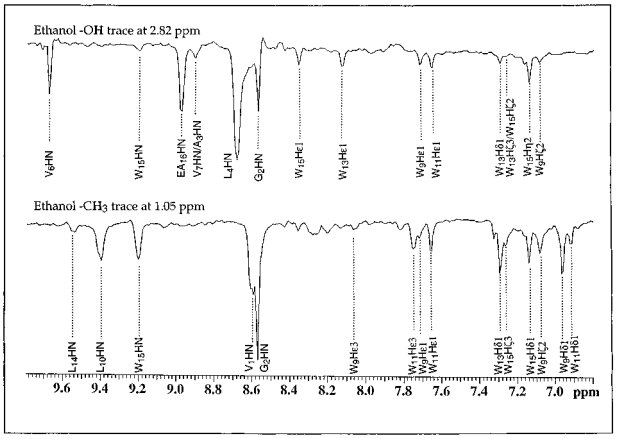

Figure 2. Traces through the homonuclear ROESY spectrum of 19.3 mM gramicidin A in 95% benzene-d_6/5% ethanol-d_5 (v/v) with approximately 20 mM natural abundance ethanol. The ethanol signals were well resolved, but weak (approximately the same intensity as the polypeptide). The solvent methyl ^{1}H resonance did not overlap with the polypeptide resonances. Spectra were acquired at 25 °C using a 200 ms mixing time and a recycle delay of 4 s. A total acquisition time for the 2D spectrum was approximately 36 h. 500 MHz spectra were referenced to benzene at 7.15 ppm, and ^{1}H resonance assignments were made by standard ^{1}H homonuclear means. A trace through the F2 dimension at 2.82 ppm corresponding to the ethanol hydroxyl proton is shown above, and a trace through the F2 dimension at 1.05 ppm corresponding to the ethanol methyl resonance is shown below.

potential energy barrier between conformational states so that the equilibrium population distribution could be achieved more rapidly than in its absence.

While it would be ideal to study the solvent binding sites in the sample used for Figure 1B, the dilute ethanol is dispersed in dioxane, and bound ethanol is below the detection limit. Instead another solvent system, 95% benzene and 5% ethanol, in which gramicidin has the same APLH conformation, was used.[10] Likewise, this solvent system could not be used for the spectra of Figure 1 because a few percent ethanol is necessary to solubilize gramicidin into this nonpolar solvent. Therefore, it was anticipated that ethanol would be closely associated with the polypeptide surface in the benzene/ethanol solution rather than dispersed throughout the benzene environment. Figure 2 shows traces through a ROESY spectrum at the ethanol hydroxyl and methyl resonance frequencies. More than 20 ethanol binding sites have been identified so far, at least 12 involve the gramicidin backbone, and 8 solvate the indoles in the benzene environment. By way of example, two of the ethanols have their hydroxyl protons close to the symmetry-related pair of Val_6 NH sites and the solvent methyl groups close to the Val_6 and Leu_{14} side chain methyls. Figure 3 shows one of these two solvent molecules in a position consistent with the ROESY data and positioned with the ethanol hydroxyl close to the hydrogen bond between the Val_6 NH and Leu_{14} CO. Other ROE cross peaks suggest ethanol−amide interactions along much of the polypeptide backbone. The sign of the ROE intensity (opposite to that of the diagonal peaks) is inconsistent with chemical exchange and spin diffusion and clearly demonstrates specific interactions between gramicidin and ethanol.

For gramicidin to interconvert between double-helical structures, hydrogen bonds must be broken and reformed. Such a process would be easily accomplished in an aqueous environment, but not in a hydrophobic environment where an abundant pool of dipoles is not available. The association of the ethanol with the polypeptide backbone suggests that ethanol may help to destabilize the backbone interpeptide hydrogen bonds and/or stabilize the transition state conformation where the interpeptide hydrogen bond has been broken and not yet reformed. By both mechanisms the ΔG of activation is reduced thus increasing the rate of reaction. For gramicidin this reaction or

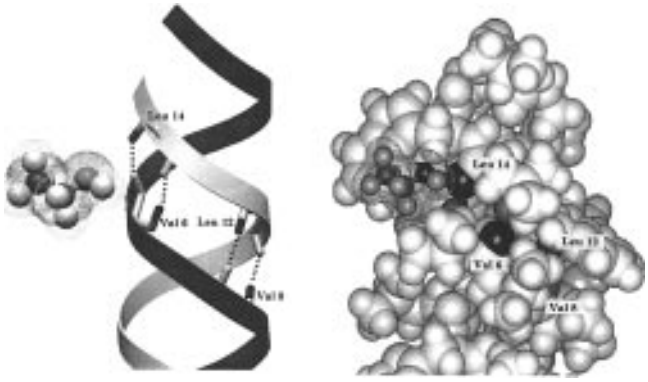

Figure 3. Models illustrating the interaction of gramicidin A with one of the ethanol molecules. Due to the alternating stereochemistry of the amino acids in this sequence, the β-strand structure has all of the side chains on one side of the strand forcing the formation of a helix, known as a β-helix. (A, left) The interactions of this ethanol with the Val_6 NH−Leu_{14} CO hydrogen bond is an example of how the solvent destabilizes the dimeric structure. Furthermore, in the structural transition of one monomer sliding by a dipeptide unit with respect to the other monomer, the solvent may help to stabilize the partial charges of the carbonyl during the transition state. (B, right) The molecular structure of gramicidin obtained by solution NMR in an 80% benzene-d_6/20% ethanol-d_6 mixed solvent system[8] and confirmed by identification of the essential NOEs in this 95% benzene-d_6/5% ethanol-d_5 solvent system. This view of the molecular surface shows that the hydrogen bonds of the amide backbone are exposed and accessible to the solvent. The ethanol is positioned manually with respect to the Val_6 NH hydrogen bond in such a way as to satisfy most of the observed ROE cross peaks that occur in the vicinity of this site.

interconversion between double-helical structures has been reasoned by several groups to be the untwisting of two monomers from each other.[10,12] For example, the Leu_{14} CO hydrogen bond with the Val_6 NH would break and then reform with the Val_8 NH in a coherent process with the other hydrogen bonds as one polypeptide chain slides with respect to the other in increments of a dipeptide unit.

While acid and base catalysis leading to the breaking and formation of covalent bonds is well recognized, here hydrogen bond breakage and formation is catalyzed by hydrogen bond donating and accepting interactions between the protic solvent and the polypeptide. The role of water in destabilizing hydrogen bonds on a protein's surface, inducing structural fluctuations and hydrogen exchange, is also well-known.[13] Here such solvent activity in a hydrophobic environment leads to a kinetic rate enhancement for conformational rearrangement. The structural transitions observed here for gramicidin in a hydrophobic environment may be representative of structural rearrangements occurring in the hydrophobic core of the lipid membrane or of protein folding in hydrophobic cores. In such environs the scarcity of protic solvent molecules may lead to a considerable reduction in the rate at which conformational space is searched.

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