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Changes in polypeptide conformer populations induced by the solvent environment

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ABSTRACT: Changes in the solvent dielectric properties are correlated with changes in polypeptide conformer populations. Gramicidin A, a peptide native to membrane environments, forms a variety of well defined dimeric conformations in relatively low dielectric organic solvents. However, changes in this environment lead to changes in the conformer populations with the lowest dielectric solvents favoring the conformers with the lowest net dipole moment. Such changes were characterized by cross-peak intensities in GCOSY solution NMR spectra. In studying membrane-bound polypeptides, it is very important to recognize that the structure is not dictated by just the amino acid sequence, but that the environment plays a very significant role in defining the polypeptide conformation.

KEYWORDS: NMR; ^{1}H NMR; GCOSY; gramicidin A; solvent effects; polypeptide structure; conformational change; structural stability; protein folding

INTRODUCTION

Increasingly there are reports of polypeptide structural changes induced by varying solvent conditions. When the surface to volume ratio is high, such as in polypeptides, the solvent environment participates significantly in defining the three-dimensional structure.[1–3] Here we describe a conformational dependent system wherein there exists a close correlation between solvent polarity and peptide global dipole moments. Non-polar solvents favor peptide conformers with low overall peptide dipole moments, while polar environments support conformers with higher dipole moments. For small organic systems, it has long been established that rotamers having a large dipole moment are favored in media of high dielectric constant and likewise rotamers with a small dipole moment are favored in those with low dielectric constants.[4] The analogous observation between organic and biological molecules suggests a general solvent–solute interaction pattern that underlies molecular structural changes in responses to environmental influences.

Gramicidin (gA) is a pentadecapeptide with unique solvent-dependent conformational behavior and hence an ideal system for investigating peptide structural changes under different environmental conditions. Structures of gA in different solvent environments have been studied and extensively reviewed by different authors.[5–8] This 15 amino acid polypeptide has an alternating sequence of D and L stereochemistry: HCO–Val_1–Gly_2–Ala_3–D-Leu_4–Ala_5–D-Val_6–Val_7–D–Val_8–Trp_9–D-Leu_{10}–Trp_{11}–D-Leu_{12}–Trp_{13}–D-Leu_{14}–Trp_{15}–$NHCH_2CH_2OH$. Both termini are blocked so that this polypeptide has no formal charges. In lipid bilayers the monovalent cation-selective channel is formed by a symmetric single-stranded helical dimer. The structure is a β-strand in which all of the side-chains are on one side of the strand, resulting from the alternating D–L stereochemistry. This forces the strand into a helix with parallel β-sheet type hydrogen bonding. In addition, six antiparallel β-sheet hydrogen bonds form across the amino terminus to amino terminus junction at the center of the bilayers.[9] In organic solvents, intertwined dimers either parallel or antiparallel with a left- or right-handed helical sense are formed. These are also β-strand type structures with β-sheet type hydrogen bonding between the strands.[8,10,11]

EXPERIMENTAL

Gramicidin A was prepared by solid-phase peptide synthesis on an ABI Model 430A peptide synthesizer using Fmoc blocking chemistry. Isotopically labeled solvents were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). A peptide concentration of 12 mM was prepared for all samples by dissolving 16 mg of gA in 0.7 ml of solvent. The sample tube was flame-sealed to prevent solvent evaporation. Since the time-scale for the conformational conversions is of the order of a few hours in the presence of protic solvents,[7,11] samples were stored for 72 h before data collection to allow the equilibrium state to be reached.

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Two-dimensional NMR spectra were recorded at a sample temperature of 30 °C on a 500 MHz Varian UnityPlus spectrometer. N-type data were collected for gradient-correlated spectroscopy (GCOSY) experiments and displayed in absolute value mode. GCOSY solution spectra were recorded with four scans (total experimental time 18 min); 4096 data points were collected along the F_2 dimension and 256 data points along F_1 dimension.

NMR data were processed using VNMR 5.3A software. Data were zero-filled to 4K × 1K prior to Fourier transformation. A sine-bell window function of 0.014 and 0.047 s was applied to the F_1 and F_2 dimensions, respectively.

Antiparallel structure GCOSY resonances in the HN–C$^{\alpha}$H region can be easily recognized because the peak intensities are typically higher than those of the other conformers in equilibrium. A definitive sequential assignment was made from a NOESY data set in conjunction with a 2D ^{1}H TOCSY spectrum. Assignments of four conformers in ethanol have been accomplished previously.[10] This combination of assignments permits the estimation of conformer populations present in various solvent systems. The antiparallel/parallel conformer population ratio was estimated based on their respective cross-peak volume ratios.

The dielectric constant (ε) of mixed solvent systems was estimated by a linear sum based on the volume fractions, v_1 and v_2, of the neat solvents:

$$\varepsilon \approx v_1\varepsilon_1 + v_2\varepsilon_2$$

where ε_1 and ε_2 are the dielectric constants of the pure constituent solvents.

The validity of this approach is based on observations that the dielectric constant of many polar–nonpolar binary mixtures can be approximated as a volume fraction weighted sum of the dielectric constants of participating solvents.[12,13] The accurate estimation of polarity for mixtures of solvents with significantly different properties has never been fully achieved in spite of a plethora of theoretical models proposed.[14–16]

RESULTS

The solvent effects on gA conformational equilibrium are demonstrated in Fig. 1, which shows GCOSY HN–C$^{\alpha}$H backbone cross peaks obtained from (A) 100%, (B) 60% and (C) 5% ethanol–benzene solution. In Fig. 1 (A) for 100% ethanol (the bulk dielectric constant $\varepsilon = 24$), a mixture of more than four double-stranded conformers, both parallel and antiparallel, are present in equilibrium. The 15 HN—C$^{\alpha}$H cross peaks corresponding to the backbone of the antiparallel left-handed dimer (APLH), also known as species 3 in the literature,[7,8,10] are labeled. The unlabeled peaks are dominated by parallel dimer structures. These parallel helical conformers are depopulated as the solvent polarity decreases, as shown in Fig. 1(B) and (C). The bulk dielectric constant for the solution in Fig. 1(B) is

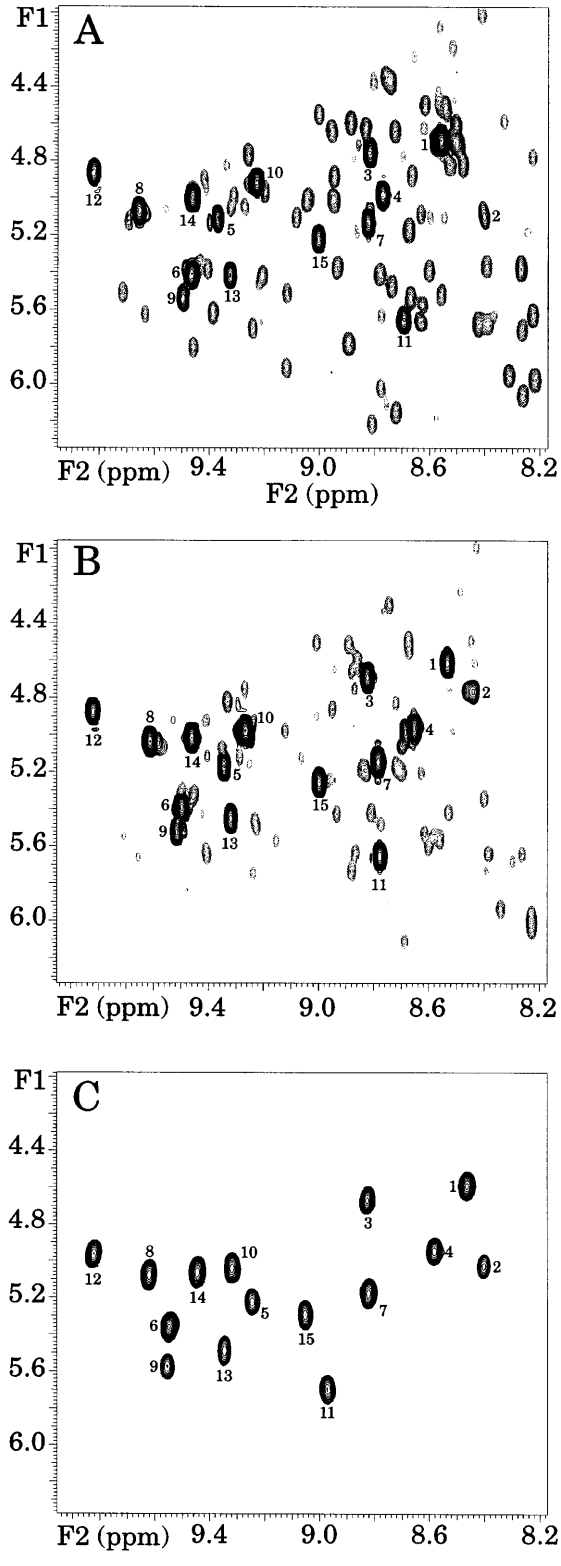

Figure 1. The GCOSY fingerprint regions for gA (12 mM) solutions as a function of solvent content. (A) In ethanol, there are four dimeric helical species present in equilibrium that vary from each other in relative monomeric orientation, helical handedness sense and symmetry. The cross peaks arising from the left-handed antiparallel dimer are assigned and shown in black and the cross peaks from parallel structures are shown in gray. (B) In 60% ethanol–40% benzene the parallel conformers are depopulated. (C) In 5% ethanol–95% benzene the antiparallel conformer becomes predominant (>95%).

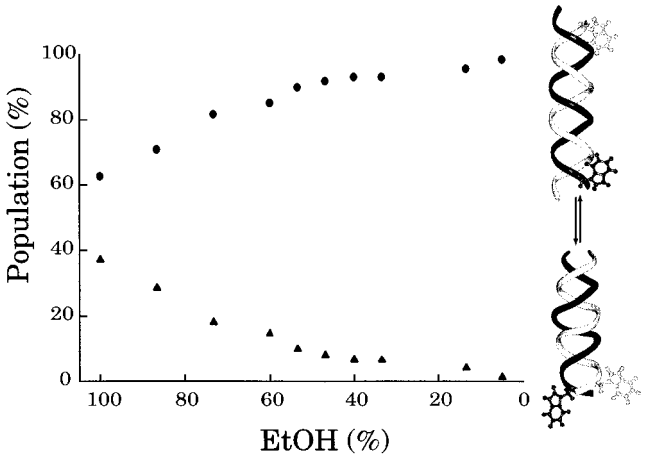

Figure 2. The peptide conformational distribution as a function of ethanol concentration. Peak volumes from the NMR spectra shown in Fig. 1 are compared to estimate the relative population. The inset represents the conformational interconversion between the parallel (▲) and antiparallel structures (●). Trp_{15} at the peptide *C*-terminus is displayed to denote the relative orientation of the two peptide strands.

ca. 17 and APLH conformer population is increased from 58% in Fig. 1(A) to 85%. Note that the chemical shift values change significantly due to solvent effects. In Fig. 1(C), the dielectric constant is *ca.* 5 and the APLH conformer predominates (>95%). Only at very low contours is any trace of the other conformers found.

The correlation between the solvent environment and peptide structure is further illustrated in Fig. 2, where the conformer population is plotted against the percentage of ethanol in benzene–ethanol solutions. A well defined continuous function is shown, leading to a virtually homogeneous conformation at very high benzene mole fractions. Qualitatively, the percentage ethanol scale is proportional to polarity.

The dielectric constant is not a quantitative measure of solvent polarity, and this is especially true of solvent mixtures, since the dielectric constant is a collective parameter that views solvent environment as a non-structured continuum. Many theories and sophisticated multiple-parameter empirical approaches, based on kinetic, equilibrium and spectroscopic measurements, have been proposed to characterize solvent polarity, but there is no readily available method that allows a direct and accurate estimate of polarity for mixed solvents.[17,18] Here we make expedient use of estimated dielectric constants as a qualitative indicator for mixed solvent polarity. Such an assumption is justifiable because there is no intention to treat data in a rigorously quantitative way. It is therefore appropriate to consider that a solvent with high dielectric constant has high polarity, and vice versa.

To understand better such solvent-polarity dependence in a more general context of solvent properties, we investigated a broad variety of solvent mixtures and studied the peptide conformational behavior in these systems. We observed that, regardless of solvent chemical structures, the peptide conformational equilibrium is mainly influenced by the overall dielectric property of the solvent mixtures. Figure 3 shows the free energy change for the antiparallel and parallel conformational states *vs.* the dielectric constant in different solutions. The change in free energy, ΔG° is given by $\Delta G^\circ = -RT \ln K_{eq}$, where R is the gas constant, T is absolute temperature and K_{eq} is the equilibrium constant between parallel and antiparallel conformers. The low dielectric environments increase the free energy difference

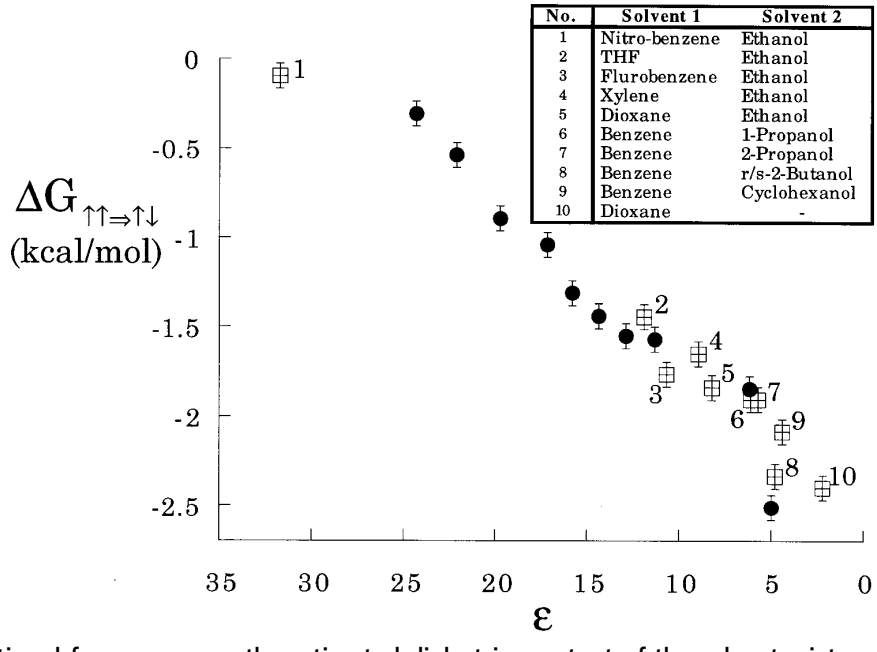

Figure 3. Conformational free energy *vs.* the estimated dielectric constant of the solvent mixtures. $\Delta G_{\uparrow\uparrow\rightarrow\uparrow\downarrow}$ represents the Gibbs free energy difference between parallel and antiparallel conformational states. The data points (●) correspond to the benzene–ethanol solutions shown in Fig. 2, and the other solvent systems (⊞) (solvent 1 : solvent 2 = 20 : 80, v/v) are specified in the table.

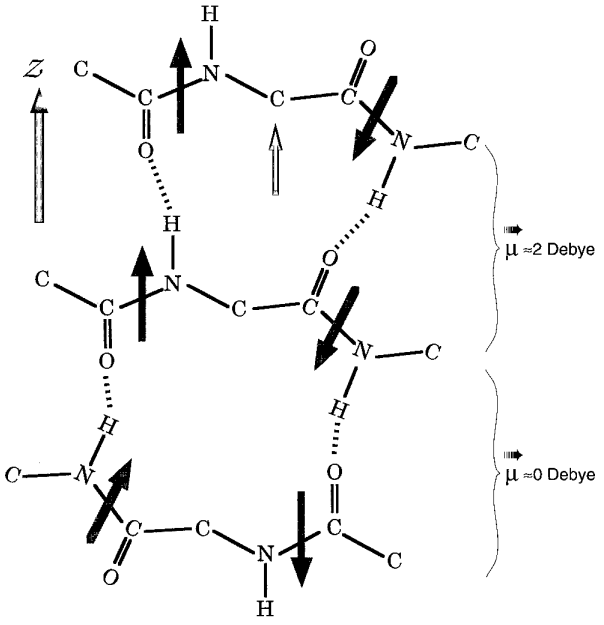

Figure 4. Schematic illustration showing the axial component of the net peptide bond dipole moment for parallel (upper and middle β-strands) and antiparallel (middle and lower β-strands) dipeptide planes. For each dipeptide (the repeat unit in a β-strand) the net dipole is *ca.* 1 D. For a parallel pair of strands the dipoles sum to 2 D and for an antiparallel structure they cancel. The total global dipole moment along the helical axis for the parallel structure is 15 D. In contrast, an antiparallel conformer has a net dipole moment of zero along the helical axis. GRASP[34] was used for the peptide dipole moment calculation.

between two conformational states, i.e. energetically favoring the antiparallel structure by *ca.* 2 kcal mol^{-1} (1 kcal = 4.184 kJ). Polar solvents tend to minimize the free energy difference and promote parallel structures.

Such conformational dependence on solvent polarity can be understood qualitatively in the light of solvent reaction field theory. First proposed by Onsager,[19] this theory has been used by Abraham *et al.*[4] for qualitative studies of organic rotamers in non-protic organic solvents. Based on this theory, a solute is stabilized by the energy of the molecular electric field in its solvent environment. This energy can be estimated on the basis of the classical electrostatic theory of dielectrics. If considering only the dipolar field from the solute and ignoring higher order terms (the quadrupole, octupole fields, etc.), the solvation free energy difference $\Delta\Delta G_{solv}^{\circ}$ for a solute with two different conformational states (A and B) at equilibrium can be described using the following expression:[20]

$$\Delta\Delta G_{solv}^{\circ} = -\frac{1}{4\pi\varepsilon_0}\frac{\varepsilon_r - 1}{2\varepsilon_r + 1}\left(\frac{\mu_A^2}{r^3} - \frac{\mu_B^2}{r^3}\right)$$

where μ_A and μ_B are the dipole moments of conformer A and B, respectively, r is their radius, assuming both conformers have an identical spherical shape, ε_r is the dielectric constant of the solvent and ε_0 the absolute permittivity of a vacuum.

One cannot apply this equation quantitatively to our system, because of the complexity of solute–solvent interactions which involve not only the electrostatic interactions, but also H-bonding, the quadrupoles from aromatic side-chains, the non-spherical shape of peptide conformers, and the preferential solvation by one or the other solvents in mixed solvent systems. It is noteworthy, however, that unlike aqueous solution where H-bonding dominates solute–solvent interactions, here non-specific interactions are much more pronounced. Therefore, one can still use this equation qualitatively to shed light on our data analysis. It follows from the above equation that the isomer with the smaller dipole moment will be preferentially stabilized in less polar media. Moreover,the difference in Gibbs free energy of solvation for two species is a function of both the dielectric constant of solvent and the dipole moment of the isomer.

From previous studies it is known that the APLH conformer is a symmetrical helical dimer, because only one set of cross peaks is observed for the backbone [Fig. 1(C)]. Furthermore, from solid-state NMR of this helix in lipid bilayers, the orientation of the two monomers is identical with respect to a unique molecular axis.[21] The antiparallel orientation of two monomers would cancel any net dipole moment along the helical axis for this conformer. For parallel structures, the residual dipole moments originating from the dipeptide planes on two monomers will sum to a non-zero value for the axial dipole moment of the overall structure. This rationalization was confirmed by calculating the dipole moment for a parallel structure. The structure of this parallel right-handed conformer has been determined by circular dichroism and solution NMR spectroscopy. A calculation performed using GRASP suggests that this structure has a net dipole moment of 15 D and the dipole direction points from the *N*-terminus to the *C*-terminus. The net dipole originates in this conformer from the summation of the residual dipeptide plane dipoles, estimated to be *ca.* 1 D (Fig. 4) for each pair of peptide planes. The discrepancy in peptide macro dipole moments for antiparallel and parallel β-structures is in agreement with the observations documented by Hol *et al.*[22]

DISCUSSION

Gramicidin A has been studied over the past few decades in a variety of organic solvents.[7,8,10,11,23,24] Four intertwined dimeric structures have been characterized and conformer populations in various solvents reported. Here we describe a reason for changes in conformer population as a result of varied solvent properties, that low dielectric environments favor conformers with a low net dipole moment.

The significance of the protein macro dipole moments in biological systems has been recognized by several authors.[25,26] Hol *et al.*[22,26,27] have theorized upon the roles of dipoles in global protein folding patterns.

According to their proposal, in all-β proteins the β-strands tend to be antiparallel to minimize an otherwise unfavorable interaction between parallel β-dipoles. This reasoning is supported by the predominance of antiparallel over parallel strand pairs in 15 all-β proteins and all-β domains reported. Among 114 β-strand pairs, 109 pairs (96%) orient in an antiparallel fashion. Recently, Ben-Tal and Honig[28] calculated the free energy change associated with two polyalanine α-helices having different lengths and relative orientational angles in membranes. When the helices are completely buried in the low dielectric environment, the antiparallel α-helical pair is more stable than its parallel counterpart by *ca.* 13 kcal mol^{-1}.

The generality of our results here is tempered by much of the work with trifluroethanol (TFE), well known for its ability to promote α-helices from β-sheets or random coils.[3,29–31] Helices have a much greater net dipole moment than β-structures, yet the addition of TFE, which reduces the dielectric of the solvent environment, is widely used for shifting the helix–coil or helix–sheet transition in favor of the α-helix. This apparent paradoxical preference for a structure with a higher dipole moment in a less polar environment is the result of a balance of solute–solvent interactions. Hydrogen bonding forces dominate in polar protic solvents such as water, while electrostatic forces such as those described in this work are more pronounced in relatively low dielectric environments. The mechanism by which TFE promotes helix formation is generally agreed to involve a weakening of H-bonding between the solvent and peptide amide groups, thereby promoting intrapeptide H-bonding and helix formation.[29,30] It is worth noting that when the electrostatic interactions are augmented, TFE may fail to promote a helical structure even for peptides with a high helical propensity. Gierasch *et al.*[1] have reported that *E. coli* OmpA peptide and its mutants did not form stable α-helices when TFE was added to an aqueous environment, because these signal peptides have positive charges at their *N*-terminus which oppose the strong dipole moment of the helix. However, in a micellar environment a much higher α-helical content was induced because the unfavorable monopole–dipole interactions were screened by the negatively charged detergent headgroups.

The environment-specified structural changes reported here are more common than generally recognized. Furthermore, as shown here, these changes can be induced by non-specific interactions between a protein and its environment. The molecular chaperones, such as GroEL/GroEG, provide another example of this by assisting in protein folding. Nascent proteins are encapsulated in the 'folding chamber' of a GroEL heptamer where the unfolded state is stabilized by its largely hydrophobic chamber wall. Upon GroEG binding the hydrophobic environment is switched to hydrophilic and the protein folds to its native state. Such a controlled change of environmental polarity is proposed to increase the efficiency and accuracy of protein folding.[32]

The low dielectric membrane environment represents a structural frontier. Recently, we have shown that non-minimum energy conformational states can be kinetically trapped in this environment.[33] Here we see that changes in a low dielectric environment can lead to different conformer populations. Furthermore, this effect appears to be correlated with the net dipoles of the various conformers, a non-specific intermolecular interaction.

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