Species Heterogeneity of Gly-11 Gramicidin A Incorporated into Sodium Dodecyl Sulfate Micelles

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ABSTRACT Evidence is presented for species heterogeneity of the gly-11 analog of gramicidin A incorporated into sodium dodecyl sulfate (SDS) micelles. The evidence for species heterogeneity has been obtained using one-dimensional (1D) ^{1}H NMR spectroscopy. The 1D spectra of the indole NH moiety of tryptophans 9, 13, and 15 show the presence of more than one species. It has been found that the heterogeneity is dependent upon the gly-11/SDS molar ratio. At high SDS concentration (i.e., gly-11/SDS of 3 mM/700 mM) the heterogeneity almost completely disappears. The temperature dependence of these ^{1}H NMR signals suggests that the two species do not interconvert. The results of nuclear Overhauser effect spectroscopy NMR experiments indicate that one species is embedded within the micelle, while the other is nearer the aqueous interface. The importance of side chain interactions with the membrane environment in producing stable, solubilized species of small peptides in SDS micelles is illustrated.

INTRODUCTION

In a recent two-dimensional (2D) ^{1}H/^{15}N heteronuclear multi-quantum-correlation NMR study of uniformly and selectively ^{15}N-labeled fd and Pf1 coat proteins from filamentous bacteriophages solubilized in sodium dodecyl sulfate (SDS) micelles, resonance doubling (i.e., species heterogeneity) was observed at low molar ratios of SDS to protein (McDonnell and Opella, 1993). The spectra of the fd and Pf1 coat proteins in SDS micelles were found to change dramatically as a function of SDS concentration. The resonance doubling was found to disappear in solutions of high SDS concentration. The doubling of resonances appeared to be restricted to residues that are part of hydrophobic, membrane-spanning helices. Resonance doubling of the M13 coat protein in deoxycholate micelles has been observed using ^{19}F NMR spectroscopy (Wilson and Dahlquist, 1985). The doubling phenomenon was also found to be dependent upon the detergent concentration. We wish to report the results of a similar study with a small peptide, gly-11 gramicidin A, incorporated into SDS micelles. Resonance doubling (i.e., species heterogeneity) was observed at low SDS concentrations for the three tryptophan residues in the peptide. The results obtained for the gly-11 analog will be compared with those obtained with six other gramicidin analogs that do not exhibit resonance doubling. The importance of the presence and location of the tryptophan residues in gramicidin will be discussed.

The gramicidin family of linear polypeptides represents a biologically viable channel system of related peptides in which single amino acid residue replacement can produce changes in cation binding, cation selectivity, and transport (Andersen and Koeppe, 1992, and references therein). Gramicidin A, a naturally occurring gramicidin analog that forms transmembrane channels, is a 15-residue hydrophobic peptide whose amino acid sequence is: HCO-L Val^1-Gly^2-L Ala^3-D Leu^4-L Ala^5-D Val^6-L Val^7-D Val^8-L Trp^9-D Leu^{10}-L Trp^{11}-D Leu^{12}-L Trp^{13}-D Leu^{14}-L Trp^{15}-NHCH$_2$CH$_2$OH (Sarges and Witkop, 1965). When placed into lipid membranes or SDS micelles, gramicidin A forms right-handed, single-stranded $\beta^{6.3}$ helical dimer channels. The two gramicidin A monomers are joined at their NH_2 termini (Urry, 1971; Arseniev et al., 1985; Bystrov et al., 1986; Nicholson et al., 1987; Cornell et al., 1988; Davis, 1988; Nicholson and Cross, 1989; Smith et al., 1989; Arseniev et al., 1986; Prosser et al., 1991; Lomize et al., 1992; Mai et al., 1993; Ketchem et al., 1993). There are two other naturally occurring analogs of gramicidin, B and C, that are identical to gramicidin A with the one exception that gramicidin B has phenylalanine at position 11 and gramicidin C has tyrosine at position 11 (Sarges and Witkop, 1965). Single channel conductance studies have shown that there are differences in channel properties between gramicidin A, B, and C (Bamberg et al., 1976; Sawyer et al., 1990; Heitz et al., 1982). Amino acid substitution and side chain orientation are thought to play an important part in determining the transport properties of the gramicidin channel (Andersen and Koeppe, 1992; Cornell et al., 1988; Koeppe et al., 1991; Andersen et al., 1992; Takeuchi et al., 1990; Etchebest and Pullman, 1985; Becker et al., 1991,1992; Boni et al., 1986; MacDonald and Seelig, 1988; Koeppe et al., 1990; Mazet et al., 1984; Durkin et al., 1990).

Tryptophan residues appear to play an important role in modulating both structure and function of proteins and peptides. The hydrophilic/hydrophobic character of the indole side chain of tryptophan permits this residue to partition itself at the aqueous interface of lipid bilayers (Jacobs and White, 1989). It has also been shown that the indole N-H is directed toward the hydrophilic environment in several membrane bound proteins (Chattopadhyay and Mc-

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Namee, 1991; Meers, 1990; Henderson et al., 1990). For gramicidin A it has been suggested that the indole NH moieties may hydrogen bond to the aqueous interface or with the lipid molecules (Lazo et al., 1992; Scarlata, 1991; O'Connell et al., 1990; Meulendijks et al., 1989) and that this interaction may stabilize the gramicidin monomer in the lipid bilayer (O'Connell et al., 1990). The hydrogen bonding of the indole NH moiety to the lipid bilayer surface has also been suggested to play an important role in the transport of cations through the gramicidin channel (Becker et al., 1991). The importance of tryptophan residues in determining the transport properties of the gramicidin A channel was shown in photo-deactivation studies (Jones et al., 1986).

The replacement of tryptophan with phenylalanine or tyrosine in gramicidin has been found to have no effect upon the backbone conformation as determined by solid state ^{15}N NMR spectroscopy (Fields et al., 1989). Recently, we have completed the 3D structure determination of gramicidin A, B, C, Phe-1 gramicidin A, and Phe-1 gramicidin C incorporated into SDS micelles using the combination of 2D NMR spectroscopy and molecular modeling and found that there is little difference in side chain orientation for the tryptophan residues of these analogs. Furthermore, in this study no evidence was found for different stable conformations of the side chains. However, as will be shown, the replacement of tryptophan at position 11 with glycine in gramicidin A produces species heterogeneity in SDS concentrations normally used in the study of other analogs of gramicidin.

MATERIALS AND METHODS

The methods previously described in the literature (Koeppe and Weiss, 1981; Koeppe et al., 1985; Weiss and Koeppe, 1985) were used to obtain gramicidin A from the commercially available gramicidin D, a mixture of A, B, and C (Sigma Chemical Co., St. Louis, MO). The gly-11 gramicidin A analog was synthesized with a peptide synthesizer from Applied Biosystems (431A; Foster City, CA) or Rainin (PS3; Woburn, MA). An amino acid sequence determination was performed for each analog using an Applied Biosystems 473A protein sequencer. The total synthesis of gly-11 gramicidin A was performed twice. Four different samples from the two syntheses were used in the ^1H NMR experiments, each one producing the same result. SDS_{-d25} (98%), $trifluoroethanol_{-d3}$ (TFE, 99%), and deuterium oxide (D_2O, 100%) were obtained from Cambridge Isotopes Laboratories (Cambridge, MA). The SDS was recrystallized from 95% ethanol. A 100 mM pH 6.5 phosphate buffer solution was purchased from PGC Scientifics (Gaithersburg, MD). Approximately 25–50 mM solutions of the gramicidin analogs in TFE were added to 275 mM SDS (89% pH 6.5 buffer/11% D_2O v/v). The samples were sonicated in a Cole-Parmer 8851 sonicator for ~5 min, then 700 μl of the solution was transferred to a 4 mm NMR tube (Ultra High Precision, 535-PP, Wilmad, Buena, NJ). The final concentrations of the samples were ~3 mM in gramicidin analog, 250 mM SDS containing 80% pH 6.5 buffer/10% D_2O/10% TFE v/v/v.

The ^1H NMR spectra were obtained with a Varian (Palo Alto, CA) VXR-500S NMR spectrometer. The first increment of the nuclear Overhauser effect spectroscopy (NOESY) experiment was used to obtain the 1D spectra of each analog. The NOESY spectra were acquired in 1024 t_1 increments and 8192 t_2 data points. A mixing time of 40 ms was used for all experiments. A 6000 Hz spectral width was used in both dimensions in the NOESY experiments. The samples were allowed to equilibrate for 1 h at each temperature before beginning each experiment. Spectra at each temperature were obtained twice, once as the temperature was decreased and then again as the temperature was increased to the starting value. Identical spectra were obtained at each temperature.

RESULTS AND DISCUSSION

The indole NH proton NMR signals of the tryptophan residues were found to be particularly useful in this study, because they are well resolved. Fig. 1 shows a comparison of the ^1H NMR spectra of the indole protons of seven gramicidin analogs (~3 mM) incorporated into 150 mM SDS at 55°C. Gramicidin A and Phe-1 gramicidin A have four tryptophan residues; gramicidin B, gramicidin C, Phe-1 gramicidin C, gly-15 gramicidin A, and gly-11 gramicidin A have three tryptophan residues. As one can see in Fig. 1, the number of indole NH peaks corresponds to the number of tryptophan residues in each analog (although only two peaks are observed for gramicidin C at 55°C, at lower temperature three peaks are clearly resolved), except for gly-11 where peak doubling occurs. However, the peak doubling in the spectrum of gly-11 gramicidin A was found to be dependent upon the concentration of SDS in a manner similar to that found with the fd and Pf1 coat proteins (McDonnell and Opella, 1993). Fig. 2 shows the indole NH spectral region for gly-11 gramicidin A as a function of SDS concentration where the peptide/SDS molar ratios are 3/100, 3/150, 3/400, and 3/700. At the molar ratio of 3/700 almost all traces of peak doubling (i.e., species heterogeneity) have disappeared. Changes in the chemical shift and linewidth of each indole NH proton also occur with an increase in SDS concentration in a manner similar to that found with the coat proteins (McDonnell and Opella, 1993). Although it is not quite as obvious, an indication of species heterogeneity can been found in the spectra of the aromatic protons of the tryptophan residues and that of the backbone

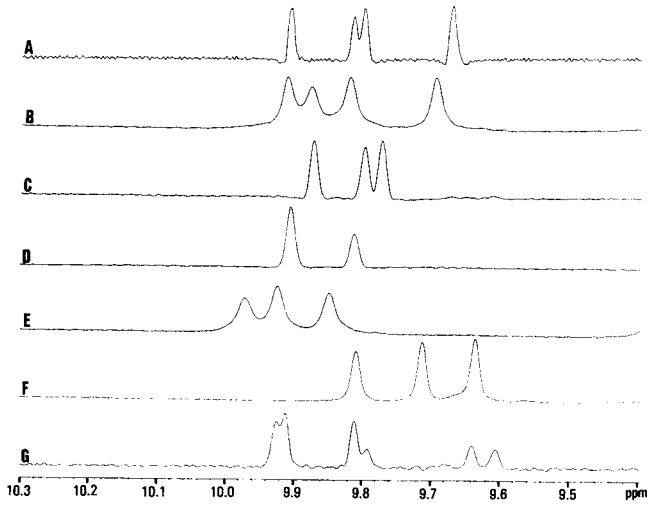

FIGURE 1 Indole NH proton spectra at 55°C for: (*A*) gramicidin A, (*B*) Phe-1 gramicidin A, (*C*) gramicidin B, (*D*) gramicidin C, (*E*) Phe-1 gramicidin C, (*F*) Gly-15 gramicidin A, and (*G*) Gly-11 gramicidin A.

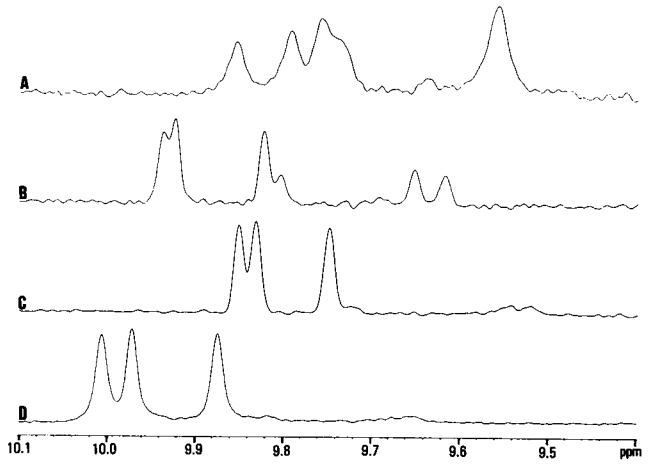

FIGURE 2 Indole NH proton spectra at 55°C of 3 mM gly-11 gramicidin A in: (*A*) 100 mM SDS, (*B*) 150 mM SDS, (*C*) 400 mM SDS, and (*D*) 700 mM SDS.

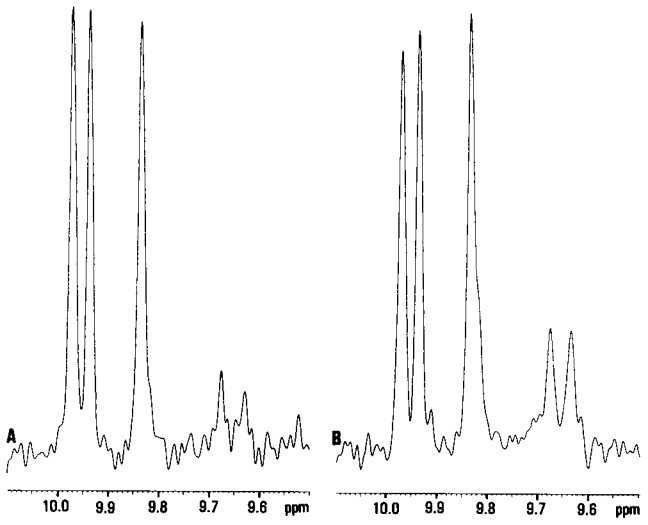

FIGURE 3 Indole NH proton spectra of 3 mM gly-11 gramicidin A in 150 mM SDS obtained using (*A*) NOESY with water presaturation and (*B*) NOESY11 with no water presaturation.

amide protons for gly-11 gramicidin A in the low SDS concentrations.

The apparent existence of more than one species of gly-11 gramicidin A in low concentrations of SDS suggests the possibility of interconversion of one species into the other or perhaps the existence of this analog in two different environments. The tryptophans could be embedded in the micelles or located at the aqueous interface. To determine whether the two species interconvert, the effect of temperature upon the spectrum of the indole NH protons was studied. No evidence was found to suggest interconversion of one species into the other. This is unlike that found for the M13 coat protein where the peaks representing the two conformers merged at high temperature, indicating conformational interconversion (Wilson and Dahlquist, 1985).

To determine whether the two species are the result of being in different environments, two NOESY experiments were performed. The first increment of each NOESY experiment was used to obtain the 1D ^{1}H NMR spectra. One NOESY experiment used water presaturation with higher than normal radio frequency (RF) power. The other one was the NOESY11 (Sklenar and Bax, 1987) pulse sequence, which does not involve presaturation to remove the water signal. The rationale behind using these two experiments was the assumption that one of the species of gly-11 gramicidin A in low SDS concentration has the tryptophan resides embedded in the micelles, while in the other the tryptophan residues are at the aqueous interface. Those indole NH protons at the aqueous interface should exchange more readily with water protons than those embedded in the micelles. Consequently, in the NOESY experiment using high RF power to presaturate the water protons, one might expect to observe a decrease in the indole NH proton signals for those tryptophan residues at the aqueous interface. No such decrease in the indole NH proton signals would be expected to occur in the NOESY11 experiment where no water presaturation is used. Fig. 3 contains the results of these two experiments. Indeed, a comparison of the spectra suggests that the smaller, high field indole NH proton signals are diminished in the spectrum obtained in the NOESY experiment using water presaturation compared to that obtained without water presaturation. This is also the species (i.e., the one producing the smaller, high-field signals) that disappears at high SDS concentration.

Compared with large proteins embedded into membranes, where there are very many interactions of side chains with the membrane, relatively small peptides (e.g., gramicidin) must maximize the effect of side chain interactions with the membrane environment to form a stable, solubilized species. With the small peptides the removal of one residue that strongly interacts with the membrane environment can dramatically change the solubilization and form of the peptide within the membrane. For gramicidin it is not only the removal of a tryptophan residue but the position of removal that determines the stability, degree of solubilization, and form of the species in SDS micelles. Gly-15 gramicidin A behaves like other analogs of gramicidin in low SDS concentration. However, the substitution of glycine for tryptophan at position 11 instead of position 15 produces an analog that requires approximately a five-fold increase in SDS concentration to produce a single channel species of this analog that is like that of the other gramicidin analogs.

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