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ARTICLE

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The effects of calcium ions on double helical forms of gramicidin

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Abstract The effects of binding calcium ions to the double helical forms of gramicidin present in methanol solution were examined using circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopies. It was found that calcium ions principally alter the relative composition of the equilibrium mixture of gramicidin conformers present in the solvent. In the absence of calcium, both parallel and antiparallel double helices are present. However, the addition of small amounts of Ca^{2+} shifts the equilibrium towards the left-handed parallel double helical form. This conformational change prevents monovalent cations (caesiums) from binding to the gramicidin double helix, and even converts the shorter, wider anti-parallel double helical form normally produced in the presence of caesium into the longer, narrower parallel double helical form. Furthermore, a temperature study showed that calcium ions tend to stabilize this form relative to the ion-free forms. The conformation of gramicidin is further changed, becoming a disordered structure, when the concentration of Ca^{2+} is raised. Thus, the binding of divalent calcium ions has a number of dramatic effects on the conformations of gramicidin present in solution.

Key words Ion binding · Circular dichroism spectroscopy · NMR · Divalent cations

1 Introduction

The effects of divalent cations on the structure and conductance of the helical dimer ("channel") form of gramicidin in lipid bilayers have been extensively studied (Bamberg and Lauger 1977; Urry et al. 1982; Olah et al. 1991; He et al. 1993). It has generally been recognized that divalent cations such as Ca^{2+}, Ba^{2+}, Mg^{2+} and Zn^{2+} can destabilize the gramicidin channel and shorten its lifetime. A proposed mechanism is that the divalent cations block the transfer of monovalent cations through the channel by binding to the entrance of the channel (Urry et al. 1982; Olah et al. 1991; Golovanov et al. 1991; He et al. 1993). There is apparently no significant conformational change in the polypeptide backbone of the channel induced by the binding of divalent cations.

In organic solvents such as alcohols and dioxane, Veatch and co-workers (Veatch et al. 1974) have shown that in the absence of ions gramicidin exists as an equilibrium mixture of four distinct double helical structures, each of which consist of two monomers which are intertwined to form β-sheet like hydrogen bonding patterns between their monomers. The four conformers can be separated by thin layer chromatography and are interconvertible over a timescale which depends on the nature of the solvent (Veatch and Blout 1974; Braco et al. 1988 b). Species 1 and 2 are left-handed parallel double helices, which differ from each other in the stagger between the ends of their chains. Species 3 is a left-handed antiparallel double helix, while species 4 is a right-handed parallel double helix. All have 5.6 residues per helical turn (Bystrov and Arseniev 1988). In the presence of the monovalent cation caesium, the molecules convert to a single type of left-handed antiparallel double helix with 6.4 residues per turn (Chen and Wallace 1996).

As in the case of the channel helical dimer form, the interactions of divalent cations with the double helical forms of gramicidin are also of interest. Changes associated with binding calcium ions have been detected (Heitz and Gavach 1983; Braco et al. 1988 a; Doyle 1996), but the molecular nature of those changes has not yet been established. An early infrared spectroscopic study showed that addition of $CaCl_2$ resulted in spectral changes for gramicidin in both ethanol and methanol solvents, with the amide I band shifting from 1637 cm^{-1} for gramicidin alone to 1653 cm^{-1} in the presence of $CaCl_2$ (Heitz and Gavach

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1983). No such conformational change was observed in the presence of $ZnCl_2$. Recently, a circular dichroism (CD) spectroscopic study of the binding of several different multivalent cations to gramicidin has shown a similar selective behavior (Doyle 1996; Doyle and Wallace, submitted): It was demonstrated that only Ca^{2+}, Sr^{2+}, Ba^{2+}, Sm^{3+} and Zr^{2+} induced changes in the CD spectra; no CD spectral changes were observed for Mg^{2+}, Cd^{2+} and Zn^{2+}. A high-performance size-exclusion chromatography (HPSEC) study has also detected changes in an ethanol solution of gramicidin when $CaCl_2$ was added (Braco et al. 1988 a). That study suggested that the calcium form might be a monomer and that calcium was bound in a ratio of one calcium per gramicidin monomer.

In the present study, the effects of binding calcium ions to both ion-free gramicidin and a caesium complex of gramicidin were examined in detail by circular dichroism and nuclear magnetic resonance (NMR) spectroscopies. It was found that gramicidin undergoes two conformational changes in the presence of calcium ions in methanol. The first occurs at low calcium concentrations, where the CD spectra indicate that the equilibrium is shifted toward a single conformation, similar to species 1 of the ion-free forms. We have recently determined the detailed structure of this form using 2D ^{1}H NMR (Chen et al. 1996) and have shown it to be a left-handed parallel double helix. A comparison of the temperature effects on gramicidin solutions containing calcium ions and on ion-free gramicidin showed that the calcium conformation is more stable over a broader temperature range than are the ion-free conformations.

A second conformational change was detected at higher concentrations of calcium ions. Although the CD spectra of these samples are vaguely similar to those of monovalent cation/gramicidin complexes previous observed, in that they have a single positive peak, the conformation at high concentrations appears, from the ^{1}H NMR spectrum, to be an unordered form.

2 Materials and methods

Materials

Gramicidin D was purchased from ICN Biochemicals (Cleveland, Ohio, USA). Methanol and methyl-d_3-alcohol were purchased from Aldrich Chemical Company (UK) and were spectroscopic grade. Caesium chloride was purchased from Fisher Scientific Company (UK). Anhydrous calcium chloride was purchased from Sigma Chemical Company (St Louis, MO, USA).

Solution preparation

Separate methanolic stock solutions of 15 mg/ml gramicidin and 1.0 M $CaCl_2$ were prepared. The different molar ratios of Ca^{2+}-to-gramicidin (ranging from approx. 1 : 1 to 1300 : 1) were prepared by mixing the appropriate proportions of the $CaCl_2$ stock solution (and methanol) with the gramicidin stock solution to produce a final gramicidin concentration of 0.75 mg/ml in each case. For the thermal studies, the concentration of gramicidin was 0.75 mg/ml in both the ion-free and caesium/gramicidin solutions. The final concentration of $CaCl_2$ was 0.01 M in the thermal studies. The actual concentration of gramicidin in each solution was determined in the same cell used for the CD measurements by UV absorption spectroscopy using an extinction coefficient of 22 000 M^{-1} cm^{-1}.

For NMR measurements, $CaCl_2$ stock solutions were prepared at a concentration of 1.0 M in methyl-d_3-alcohol and sonicated until the salts were completely dissolved. The stock solution of gramicidin was prepared in methyl-d_3-alcohol at a concentration of 50 mg/ml. Solutions of different ratios of gramicidin and calcium chloride were prepared by titration of the gramicidin stock solution with the salt stock solution and dilution with methyl-d_3-alcohol to the final concentration of 20 mg/ml (10 mM) gramicidin. The high concentration samples contained 0.5 M $CaCl_2$, the low concentration samples contained 0.1 M $CaCl_2$.

Circular dichroism spectroscopy

Circular dichroism spectra were recorded using an AVIV 62 DS spectropolarimeter. The optical rotation was calibrated using d-10-camphorsulfonic acid at wavelengths of 192.5 and 290 nm, and the wavelength was calibrated with benzene vapour.

All measurements were made in Suprasil quartz cells (Hellma Kuvetten, Mullheim/Baden, Germany) with pathlengths of either 0.01 or 0.05 cm. In general, data were collected in the wavelength range from 200 to 250 nm at 0.5 nm increments. Every CD spectrum reported is the average obtained from at least three individual samples and three repeated measurements of each sample. The reported circular dichroism spectra were corrected for baseline (using a solvent containing the same concentration of salt), and then smoothed using a Savitsky-Golay filter (Savitsky and Golay 1964). Mean residue ellipticity is reported in each case. All measurements were carried out at 25.0 ± 0.2 °C or at the designated temperature in the temperature study.

Nuclear magnetic resonance spectroscopy

The one dimensional ^{1}H-NMR spectra were obtained using a JEOL XS-500 spectrometer at 300 K. The pulse duration was set at 3.0 μs at a frequency of 6002.4 Hz, and the acquisition time was 2.730 seconds. Spectra were collected at 32 768 points. The digital resolution was set to 32 K points and referenced to tetramethylsilane (TMS). In these spectra, the water signal was suppressed using the presaturation method.

Temperature titrations

In order to understand the stabilising effect of calcium, the temperature dependence of the circular dichroism spectra of 0.01 M Ca^{2+} and ion-free gramicidin samples were measured in the temperature range from –15 °C to 60 °C. The temperature was controlled by a thermal circulator to ±0.2 °C. Samples were equilibrated at the desired experimental temperature for 30 minutes prior to the spectral measurements.

Conformer analyses

The individual conformers of gramicidin in organic solvents give rise to different CD spectra. Any net spectrum of a mixture of conformers can be represented as a linear combination of the three distinct CD spectra of species 1, 3 and 4 (species 1 and 2 have essentially identical spectra). The spectra of the individual species are virtually identical in a wide range of alcohol solvents (Veatch 1973; Chen and Wallace, in press). By replacement of the data base used in the algorithm for the estimation of secondary structure of standard proteins (Mao and Wallace 1984), the observed ellipticity (θ) of any gramicidin spectrum can be deconvoluted into the individual spectra of the three distinct species, and the fraction of each conformer present determined. The equation is written as follows,

$$\theta_{obs}(\lambda) = f_{1+2}\,\theta_{1+2}(\lambda) + f_3\,\theta_3(\lambda) + f_4\,\theta_4(\lambda)$$

where $\theta_{obs}(\lambda)$ is the observed ellipticity as a function of wavelength, $\theta_{1+2}(\lambda)$, $\theta_3(\lambda)$ and $\theta_4(\lambda)$ are the reference ellipticities for species 1+2, species 3, and species 4 at that wavelength measured in isopropanol by Veatch et al. (1974), and f_{1+2}, f_3 and f_4 are the fractions of species 1+2, 3 and 4, respectively. All data between 200 and 250 nm were used. The function was minimized by a normalized constrained method based on a linear least squares fit (Mao and Wallace 1984; Wallace and Teeters 1987). A normalized root mean standard deviation (NRMSD) was used to indicate the quality of fit for each spectrum such that:

$$NRMSD = [(\theta_{obs}(\lambda) - \theta_{cal}(\lambda))^2/(\theta_{obs}(\lambda))^2]^{1/2}$$

where $\theta_{obs}(\lambda)$ and $\theta_{cal}(\lambda)$ are the observed and calculated ellipticities, respectively. A low value for the NRMSD suggests a good correspondence between the calculated values and the experimental data, and indicates the reference data set includes the types of structures present in the sample analyzed.

3 Results

Changes in the CD spectra as a function of calcium ion concentration

The far ultraviolet circular dichroism spectra obtained with different concentrations of calcium in gramicidin/methanol solutions are shown in Fig. 1 A, B. It is clear that there are distinct spectra produced at high and low calcium ion concentrations. For a gramicidin concentration of ~0.4 mM, at Ca^{2+} concentrations lower than ~0.01 M, the two negative peaks located at 213 and 228 nm appear more pronounced than those of the ion-free form. At Ca^{2+} concentrations above 0.01 M, the two negative peaks gradually decay and convert into a single positive band at around 228 nm. Although the spectra at the highest concentrations superficially appear somewhat similar in shape to the far ultraviolet circular dichroism spectra of the monovalent cation/gramicidin complexes (Fig. 1 B), because they differ in both magnitude and position of the wavelength maximum, they clearly represent distinct conformational species. Figure 2 shows the titration curve for the ellipticity as a function of Ca^{2+} concentration at 228 nm. It appears

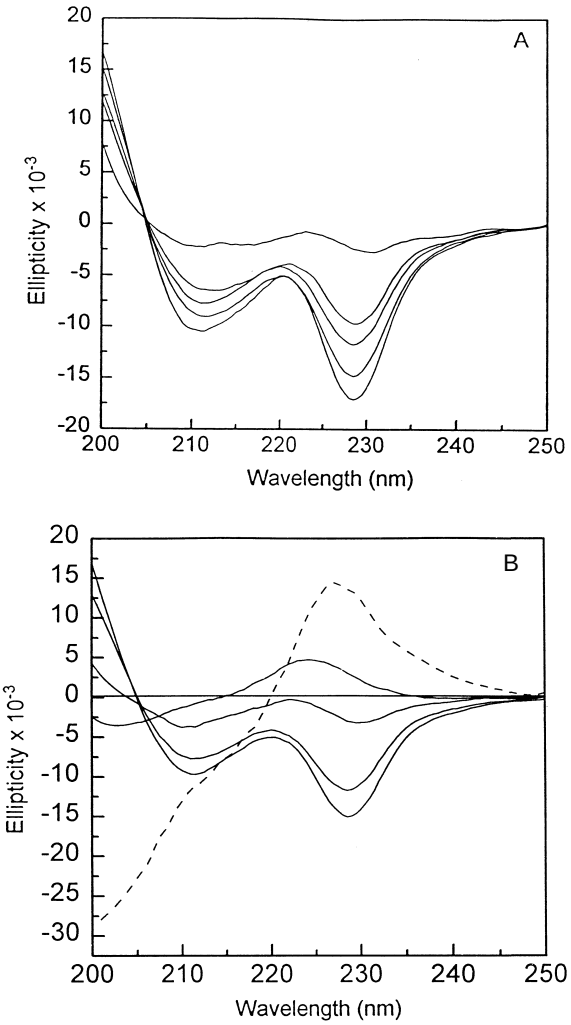

Fig. 1 A, B Far ultraviolet circular dichroism spectra for calcium/gramicidin mixtures at ~0.4 mM gramicidin. **A** low Ca^{2+} concentrations: 0 (*uppermost*), 0.0003, 0.0004, 0.005, and 0.01 M (*lowermost*) and **B** high Ca^{2+} concentrations: 0.5 (*uppermost solid curve*), 0.3, 0.1 and 0.08 M (*lowermost*) (i. e. increasing concentration going up to figure). The *dotted curve* in **B** shows the spectrum of the Cs^+/gramicidin complex for comparison

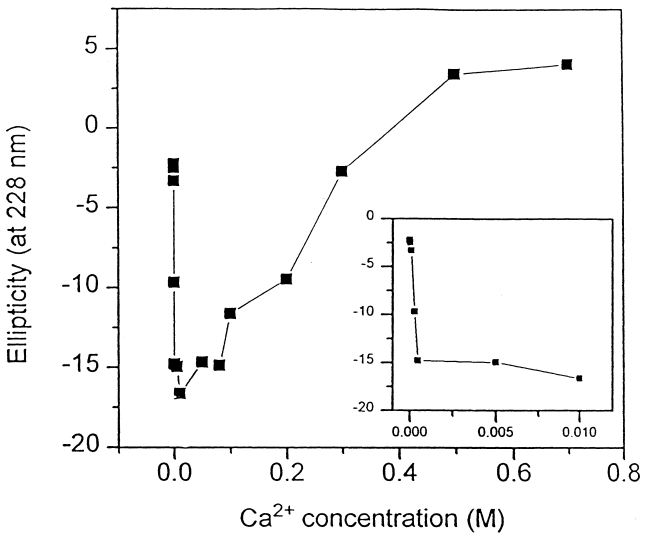

Fig. 2 Plot of Ca^{2+} concentration vs. ellipticity ($\times 10^{-3}$) at 228 nm. The *inset* shows the titration curve of 0.4 mM gramicidin at Ca^{2+} concentrations below 0.01 M

that there are several changes that occur as a function of calcium concentration and/or ratio.

The analysis of conformers

In organic solvents, gramicidin forms a number of different double helical conformations. They are either left-handed parallel or antiparallel, or right-handed parallel double helices. Under the conditions used in this study, in the absence of calcium ions, the spectra indicated a mixture of species 1+2, 3 and 4 in the ratio 65 : 25 : 10 (Table 1). At different concentrations of Ca^{2+}, these ratios change. The conformation is converted almost entirely into a single species which has a spectrum similar to species 1+2, i. e. left-handed parallel double helices at Ca^{2+} concentrations of ~0.005 to 0.01 M. As the Ca^{2+} concentration increases, the NRMSD increases slightly, suggesting the presence of an additional species with a subtle difference in conformation from that of the four reference species. At very high (0.5 M) calcium concentrations, the NRMSD is so large (0.93) it suggests the conformation present does not resemble any of the reference species.

Table 1 Analyses of the proportions of each type of conformer present at different concentrations of calcium ions

Concentration of Ca^{2+} (M)	Species 1+2	Species 3	Species 4	NRMSD
0	65%	25%	10%	0.09
0.0003	74%	18%	8%	0.24
0.005	89%	7%	4%	0.26
0.01	92%	3%	5%	0.20
0.1	80%	15%	5%	0.27

Changes in the NMR spectra as a function of calcium ion concentration

In order to understand further the natures of the different conformational states, nuclear magnetic resonance spectra were obtained at various Ca^{2+} concentrations. Because the gramicidin concentrations necessary to obtain the NMR spectra were much higher than those used in the CD experiments (approx. 25×), the concentrations of calcium were increased so that roughly similar gramicidin/ion ratios were used. Figure 3 shows the 1D ^1H NMR spectra of 10 mM solutions of ion-free gramicidin, gramicidin with 0.1 or 0.5 M calcium chloride, and gramicidin with 0.1 M caesium chloride. It can clearly be seen that the NMR spectra obtained are very different under these different conditions. In the absence of ions, the proton signal of the formyl group at 8–8.2 ppm is a multiple resonance, indicating a mixture of at least four species is present. At a Ca^{2+} concentration of 0.1 M this becomes a single peak, indicating that, in contrast, either only a single conformation is present, or (unlike the ion-free case) any mixture of species present is in rapid exchange. A detailed NMR study showed that only one conformation was present and it has been possible to determine the complete 3-dimensional structure of this low calcium conformer (Chen et al. 1996). Its structure is a left-handed parallel double helix, very similar, but not identical, to species 1 of the ion-free mixture. This correlates well with our CD spectroscopic observations.

At a concentration of 0.5 M Ca^{2+}, the individual peaks throughout the NMR spectrum appear to be well separated and all peaks for a specific type of amino acid tend to coincide rather than being dispersed (the latter being expected for a folded polypeptide). This mono-dispersity suggests a disordered state. The existence of two different conformations at high and low Ca^{2+} ratios shown by the NMR spectra is consistent with the observations from the CD spectra which had indicated two different conformational states.

It is interesting to note that all the NMR spectra in the presence of calcium are very different from those in the presence of caesium. The NH proton signals for the tryptophan indole rings around 10–11 ppm, in which four characteristic peaks appear for the Cs^+/gramicidin complex, are less well-separated in the cases of both ion-free and Ca^{2+}/gramicidins.

The effect of calcium ions on monovalent (caesium) cation binding to gramicidin

It is well known that divalent cations can block the transfer of monovalent cations in the gramicidin helical dimer (channel). In order to determine whether calcium can block the binding of monovalent cations to gramicidin in the double helical forms, or has another effect on gramicidin in organic solvents, two systems were examined: 1) the addition of Cs^+ to a preformed Ca^{2+}/gramicidin complex, and 2) the addition of Ca^{2+} to a preformed Cs^+/gramicidin

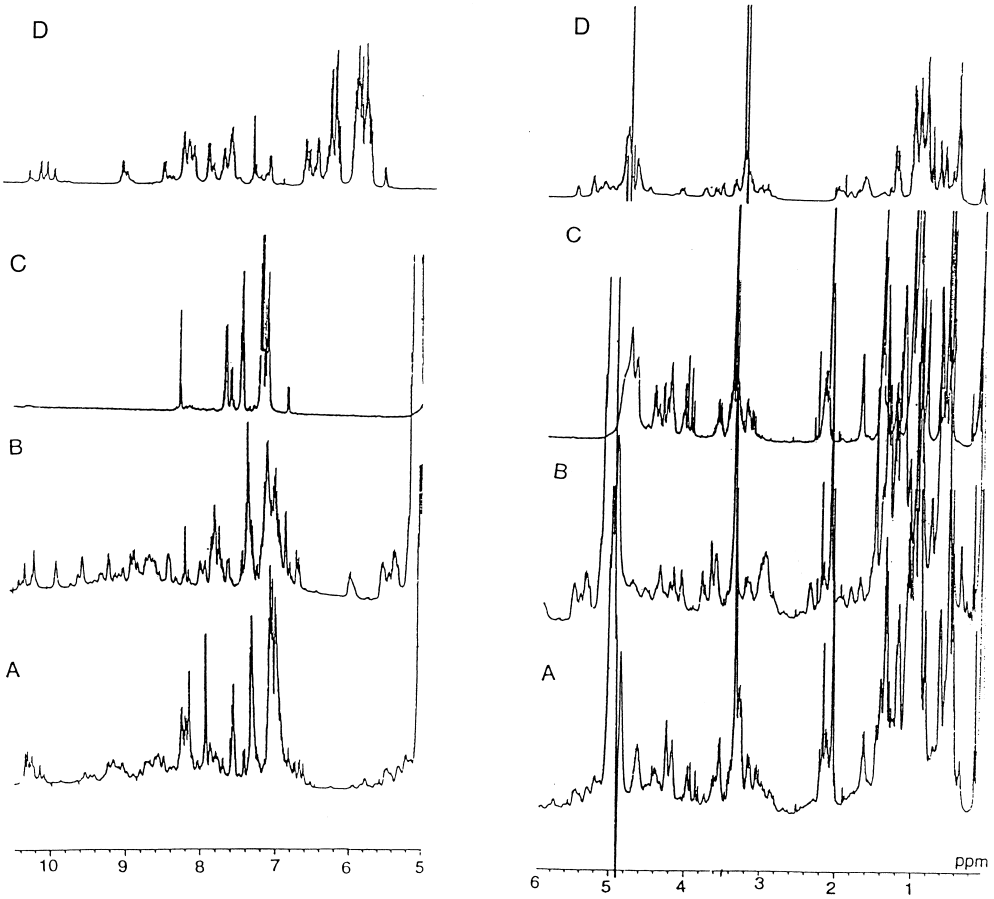

Fig. 3 A–D NMR spectra of 10 mM gramicidin solutions: **A** ion-free gramicidin, **B** low (0.1 M) Ca^{2+}/gramicidin and **C** high (0.5 M) Ca^{2+}/gramicidin, and **D** the Cs^{+}/gramicidin complex (0.05 M Cs^{+})

complex. In the first case, 0.05 M Cs^{+} was added to a 0.4 mM gramicidin solution containing 0.01 M Ca^{2+}. In the second case, a 0.4 mM gramicidin solution containing 0.05 M Cs^{+} had 0.01 M Ca^{2+} added. The concentration of Cs^{+} used represents a saturating concentration for producing the caesium/gramicidin complex (Chen and Wallace 1996).

Figure 4 shows the CD spectra obtained when calcium is added either before or after the addition of caesium. It can be seen that the two negative peaks of the CD spectra in both solutions are nearly identical in both cases and neither CD spectra shows the positive peak at 228 nm which is an important indication that caesium ions have bound (see Fig. 1 B, dotted curve). The two negative CD transition bands in case 1 are slightly less intense than in case 2. This may indicate that there is still some caesium bound to gramicidin at the concentration of Ca^{2+} used in the latter experiment.

The same phenomenon is seen in the NMR spectra (data not shown): The spectra obtained when Cs^{+} is added to a Ca^{2+}/gramicidin solution or when Ca^{2+} is added to a Cs^{+}/gramicidin solution are virtually identical and the most characteristic signals of the Cs^{+}/gramicidin complex, i. e. the tryptophan indole NH protons located around 10–11 ppm, do not appear, no matter whether the Ca^{2+} is added before or after the addition of Cs^{+}. These spectra are very similar to the NMR spectrum obtained with only Ca^{2+} present (Fig. 3 B) and are thus consistent with the CD observations.

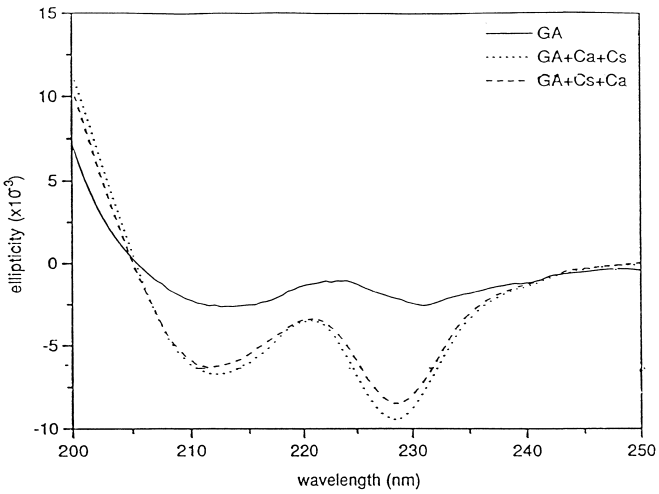

Fig. 4 Far ultraviolet CD spectra for 0.4 mM solutions of ion-free gramicidin (—), the addition of 0.05 M Cs^{+} to a 0.01 M Ca^{2+}/gramicidin solution (•••), and the addition of 0.01 M Ca^{2+} to a 0.05 M Cs^{+}/gramicidin solution (---)

Temperature effects on ion-free gramicidin and Ca^{2+}/gramicidin

Figures 5 A, B show the far ultraviolet circular dichroism spectra for ion-free gramicidin and gramicidin in the presence of 0.01 M Ca^{2+} at different temperatures. It can be seen that the two very small negative peaks in the CD spectra of ion-free gramicidin convert into a single positive peak when the temperature decreases below room temperature and convert into a single more negative peak with a small shoulder when the temperature increases above room temperature. In contrast, in the presence of low Ca^{2+}, the temperature titration of the CD spectra is less dramatic: it shows a slowly but continuously decreasing intensity for the negative peak at 228 nm, but little change in the 213 nm peak. All effects are reversible, suggesting that the changes seen are the consequence of a conformational change rather than self-association.

Calculations of secondary structure suggest that the ion-free form changes from an equal mixture of species (1+2) and species 4 at 0 °C to almost entirely species 3 at 60 °C, whereas the Ca^{2+} form changes from a 3 : 1 mixture of species (1+2) and species 3 (no species 4) at 0 °C to an approximately equal mixture of species (1+2) and species 3 at 60 °C (again no species 4). Thus, the shift between conformational species appears to be considerably less for the calcium-containing sample than for the ion-free sample.

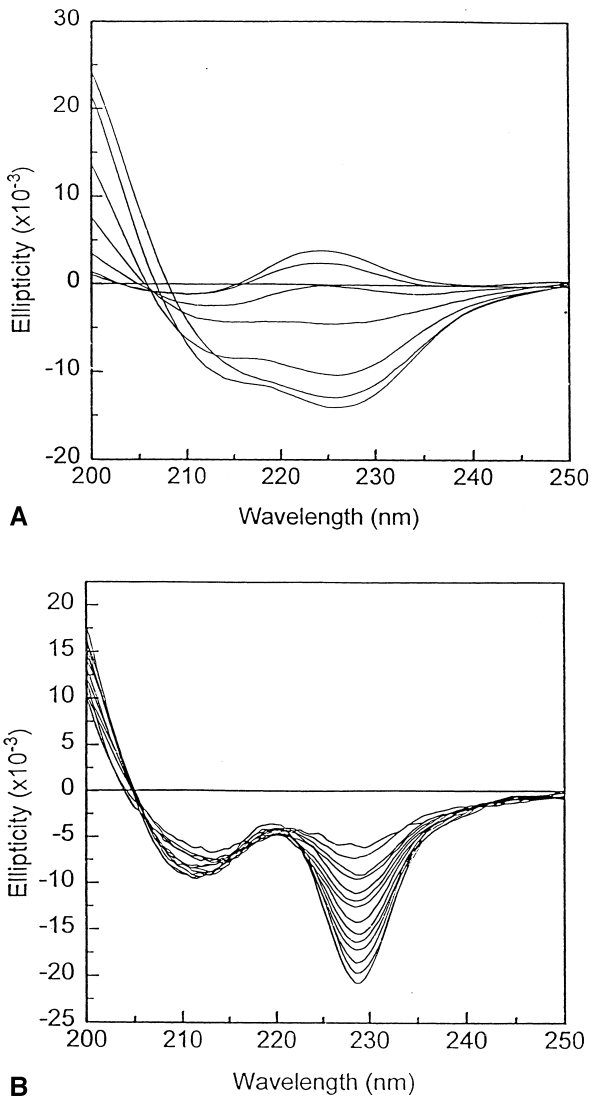

Fig. 5 Far ultraviolet CD spectra (0.4 mM gramicidin) at different temperatures for **A** ion-free gramicidin and **B** gramicidin with 0.01 M of Ca^{2+}. In **A** the temperatures from *top* to *bottom* are 0, 10, 20, 30, 40, 50, 60 °C. In **B** the temperatures from *top* to *bottom* are 60, 50, 40, 35, 30, 35, 20, 15, 10, 5, 0, –5, –10, –15 °C

4 Discussion

The change in conformation induced by Ca^{2+}

In membrane lipid environments, the transfer of alkaline cations through the gramicidin channel can be blocked by the binding of divalent cations at the mouth of the channel (Urry et al. 1982; Olah et al. 1991; He et al. 1993). The binding of divalent cations to gramicidin does not alter the conformation of the helical dimer form of gramicidin (Urry et al. 1982; Olah et al. 1991; He et al. 1993). Although the double helix is not the predominant conducting form of gramicidin in membranes composed of saturated fatty acids, it is present in polyunsaturated lipids (Sychev et al. 1993) and dominates in organic solutions (Veatch 1973). Therefore, it is of interest to examine whether divalent cations have the same type of effect on this form of gramicidin.

A conformational change in gramicidin induced by Ca^{2+} cations was previously reported by IR spectroscopic (Heitz and Gavach 1983) and HPSEC (Braco et al. 1988 a) studies, but the molecular nature of the change was not determined. In the present study, we have shown that the net mixture of conformers of gramicidin in methanol changes dramatically in the presence of Ca^{2+}, and exhibits two distinct conformational transitions. It is not simply a concentration effect, but also depends on the ratio of calcium-to-gramicidin present. At low Ca^{2+}, the two negative peaks in the CD spectra become more pronounced with increasing Ca^{2+} concentrations, and the peak at 228 nm becomes more intense than the peak at 213 nm. On the other hand, at high Ca^{2+}, the two negative peaks of the CD spectra disappear and a single positive peak appears.

The NMR spectra further illuminate the molecular nature of the changes detected in the CD data. The NMR spectra in the presence of Ca^{2+} are very different from those of Cs^{+}/gramicidin complexes and other gramicidin double helices such the left-handed antiparallel double helix (Pascal and Cross 1993; Zhang et al. 1992) and the helical dimer (Arseniev et al. 1985). Combining the results from our CD and NMR spectroscopic studies, we find that the conformation of gramicidin in low Ca^{2+} concentrations is a left-handed parallel double helix, while the conformation in high Ca^{2+} concentrations is an unstructured form. The

nature of the left-handed parallel double helix has been confirmed by a detailed structure study using 2D ^{1}H NMR (Chen et al. 1996). This structure is similar to the ion-free species 1 of Veatch and coworkers (Veatch et al. 1974), but the details of its structure at the N-terminal end (where the calcium ion binds) are somewhat different. The unstructured conformation at high concentrations is not a double helix. In dimethyl sulphoxide, gramicidin switches between a structured monomer and an unstructured form (Hawkes et al. 1987; Roux et al. 1990). The high Ca^{2+} form may also possibly be a mixture of unstructured and structured monomers.

It appears that the function of calcium cations at low concentrations is to drive the equilibrium from a mixture of conformers into a single left-handed parallel double helical conformation. Electrostatic calculations (Chen et al. 1996) show this form has a negatively charged pocket at the N-terminal end where the Ca^{2+} can bind, which may ultimately stabilize this species relative to the other species found in the ion-free mixture. When even more calcium ions are added to the solution, the double helical structures apparently cannot be maintained. The conformation changes into an unstructured form. A similar observation, that gramicidin switches to an unstructured monomer, has been observed when more polar solvents such as water are added to organic solutions of gramicidin (Braco et al. 1992).

The effect of Ca^{2+} on the binding of alkaline cations to gramicidin

Both CD spectra of gramicidin in the presence of Cs^+ plus Ca^{2+} shown in Fig. 4 appear to be almost identical to the gramicidin spectrum produced in the presence of calcium but in absence of caesium ions. The characteristic positive peak at 228 nm, which is indicative of the binding of monovalent cations to gramicidin, is not observed. This directly suggests that under these conditions the caesium cations are ineffective at binding to, or producing the conformational change in, gramicidin. Furthermore, whether the addition of Cs^+ is before or after the addition of Ca^{2+} has little effect on the final spectrum produced. Since a previous study showed that the binding constants for the two caesium ions to gramicidin are 390 and 700 M^{-1} (Chen and Wallace 1996) (binding to the first site produces the conformational change), this indicates that the interaction between the gramicidin and calcium must be even tighter than the tightest caesium binding constants.

Under these circumstances, the equilibrium is shifted to the left-handed parallel double helix, which cannot convert to the left-handed antiparallel double helix form and thus cannot provide a binding environment for the caesium ions.

Stabilization of the double helix by calcium ions

The temperature study of ion-free gramicidin and Ca^{2+}/gramicidin suggests that the presence of Ca^{2+} in solution stabilizes the parallel double helical form of gramicidin relative to the mixture of forms present in the absence of ions. The species 1-like spectrum of Ca^{2+}/gramicidin persists over a wider range of temperatures, and the calculated species mixture is relatively invariant over a large temperature range. The ion-free form, however, changes from entirely antiparallel to entirely parallel species over the same temperature range. These results would tend to indicate that calcium ions stabilize the secondary structure of double helical gramicidin.

In summary, calcium produces a number of effects on the conformations of the double helical forms of gramicidin present in solution and changes the stabilities of the different conformers present relative to each other. These effects are very different from what occurs when calcium binds to the helical dimer (channel) form of gramicidin, and suggest a totally different type of interaction of divalent cations with the two types of gramicidin conformational motifs.

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