

A Circular Dichroism Study of Valinomycin Barium Ion Complex

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Synopsis

Complex formation of valinomycin with Ba^{2+} ions was investigated by circular dichroism spectroscopy. The results indicated that Ba^{2+} forms entirely different types of complexes when compared with K^+ . The data with perchlorate salt showed evidence for the formation of less stable V_2C (peptide sandwich), VC (1:1), and VC_2 (ion sandwich) complexes followed by a stable final complex upon gradual addition of salt (V stands for valinomycin and C for the cation). This final complex possibly has a flat structure with no internal hydrogen bonds, similar to that of valinomycin in highly polar solvents. The possible complexation mechanism and the role played by anions and isopropyl side chains are highlighted.

INTRODUCTION

The remarkable ability of the membrane-bound ionophores (ion carriers) such as valinomycin, enniatin, etc., to differentiate selectively between various cations has been the subject of study in recent years by a variety of physicochemical techniques.¹⁻⁹ Valinomycin, so named because of its high valine content and microbial origin, is known to increase the permeability of K^+ over Na^+ in biological membranes, and to do so with a very high selectivity.¹⁰⁻¹² The mechanism involved in the selective transport—with the various conformational rearrangements required by the molecule for binding the cation at the membrane interface, its transport across the membrane, and the subsequent release on the other side—has yet to be resolved.^{6,10,13} This is partly due to the fact that the K^+ ion complex is very stable and is only weakly solvent dependent, whereas the free valinomycin in solution has been shown to have highly solvent-dependent conformations.⁸ Therefore, the mechanism of ion transport can be better understood by studying other cation complexes that possibly form weaker complexes and are thus susceptible to the effects of the surrounding media (i.e., the solvent, anions, etc.). With this in mind, a systematic study of the conformation of valinomycin complexes with a variety of cations of different sizes and charges has been undertaken in our laboratory. In this communication we report our findings on the circular dichroism (CD) studies of the conformation of the valinomycin complex with the divalent cation Ba^{2+} . Ba^{2+} is almost the same size as K^+ (ionic radius of 1.38 Å for Ba^{2+} versus 1.33 Å for K^+) and, according to Mayers and Urry,¹⁴ can form as strong a complex as K^+ . Our data show that it forms entirely distinctive

types of complexes, perhaps due to its double charge, larger solvation sheath, and doubling of the number of anions.

EXPERIMENTAL

Valinomycin was obtained from Sigma Chemical Company and was used without further purification. A lipophilic solvent like acetonitrile that allows a stronger cation-peptide interaction (due to its weaker solvent-cation interaction), as compared with dimethylsulfoxide, methanol, etc., was used.¹⁵ Acetonitrile was made anhydrous just before use by treating it with anhydrous sodium sulfate and refluxing the decanted solvent over P_2O_5 (5 g/500 ml; use of excess P_2O_5 over what is necessary polymerizes acetonitrile into a viscous mass) for 2 hr. The middle fraction collected on distillation was used for the experiment. It was found that valinomycin forms very weak complexes in solvents like methanol and dimethylsulfoxide.

It is imperative that salts like perchlorate and thiocyanate, which have low cation-anion interaction and thus facilitate peptide-cation interaction, be used. However, barium thiocyanate could not be used, due to excessive absorption of the thiocyanate anion in the region of interest ($\epsilon_{233} \approx 6000$ l./mol/cm). Barium perchlorate (prepared by evaporation of a solution obtained by treating barium carbonate with perchloric acid) was kept over P_2O_5 for several days before use.

For the purpose of titration, two stock solutions were prepared with the same valinomycin concentration, and excess salt was added to one of them. Solutions of intermediate concentration ratios were prepared by mixing the two stock solutions in varying amounts. Solutions were prepared well in advance and were kept sealed with Parafilm until just before use. The CD spectra were recorded at $\sim 25^\circ\text{C}$ with a Jasco J-20 spectropolarimeter using 0.1-, 1-, and 5-mm path-length quartz cells; $0.001^\circ/\text{cm}$, $0.002^\circ/\text{cm}$, and $0.005^\circ/\text{cm}$ scale; a time constant of 64 sec; and a chart speed of 4 mm/min.

RESULTS

The CD spectra for free valinomycin and its complexes with Ba^{2+} in acetonitrile at different molar concentration ratios are shown in Fig. 1. For comparison, the CD spectrum of valinomycin- K^+ complex corresponding to a 1:1 valinomycin/cation molar concentration ratio is also shown in Fig. 1. (Note: The ratios indicated throughout this text refer to the valinomycin:cation ratio, in that order.) The CD spectra of the Ba^{2+} complexes are essentially characterized by two bands: one with a positive ellipticity around 227.5 nm and the other with a negative ellipticity around 217.5 nm. As seen in Fig. 1, the intensities of the peak and the trough are different at different molar concentration ratios of valinomycin and Ba^{2+} .

The graphs obtained by plotting the molar ellipticity of valinomycin with

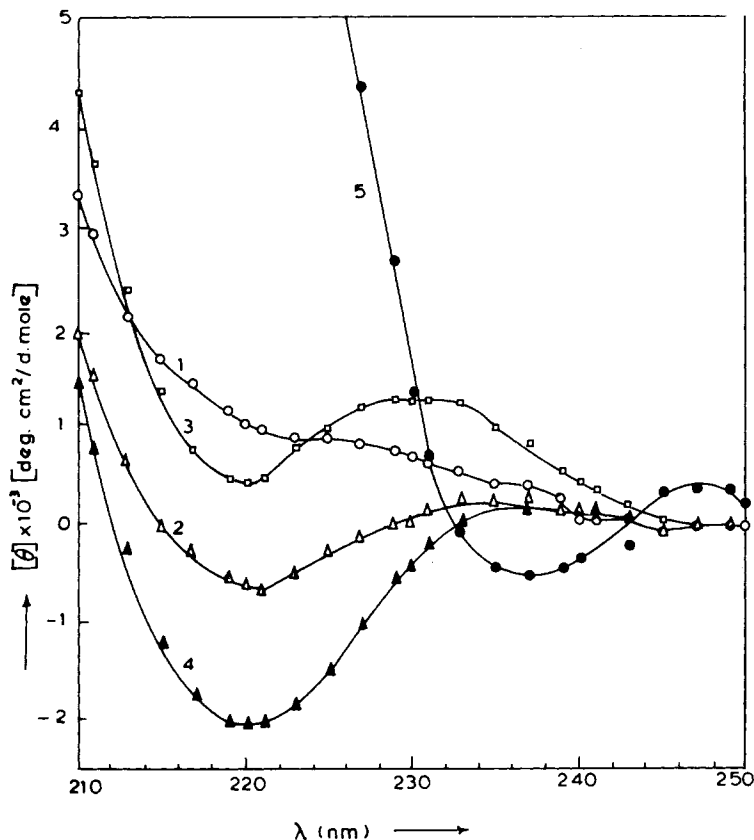


Fig. 1. CD spectra of free valinomycin and its cation complexes in acetonitrile: (1) free valinomycin, (2) valinomycin: Ba^{2+} (1:0.5), (3) valinomycin: Ba^{2+} (1:0.75), (4) valinomycin: Ba^{2+} (1:2), and (5) valinomycin: K^+ (1:1). Valinomycin concentration for Ba^{2+} complexes is 29 mM and that for K^+ complex, 9 mM; temperature, $\sim 25^\circ\text{C}$.

different Ba^{2+} concentrations at wavelengths corresponding to 217.5 and 227.5 nm are shown in Figs. 2 and 3, respectively. The molar ellipticity, $[\theta]$, of the 217.5-nm band decreases on gradual addition of salt up to molar ratio of 2:1, followed by an increase up to $\sim 1:1$. On further addition of Ba^{2+} , the $[\theta]$ value decreases continuously and tends to stabilize with a small plateau region around 1:2. Beyond 1:2, the $[\theta]$ value decreases again. A plateau region indicative of a stable complex is reached only at very high concentration ratios (not shown in the figure). The nature of the titration curve is the same at different valinomycin concentrations, but at high valinomycin concentrations, there is an overall decrease in $[\theta]$. The decrease may be possibly due to molecular association at these high concentrations.

The nature of the changes shown in the CD band at 227.5 nm depends on valinomycin concentration (Fig. 3). At low valinomycin concentrations (~ 0.5 mM), there is an increase in the ellipticity value upon addition of salt

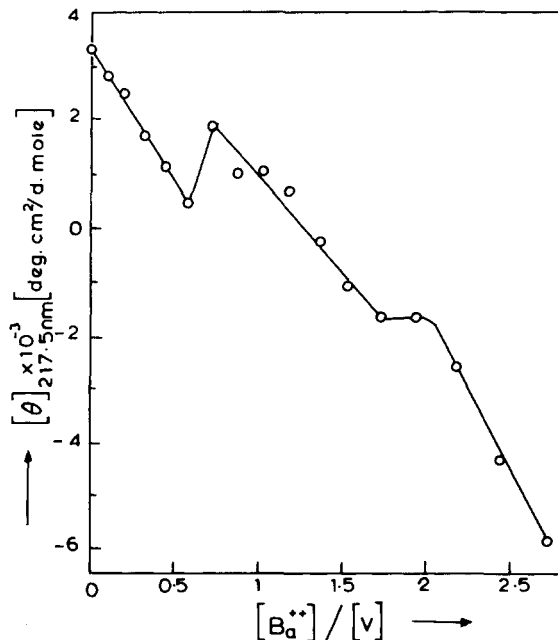


Fig. 2. Barium perchlorate titration of valinomycin in acetonitrile. Molar ellipticity, $[\theta]$, at 217.5 nm is plotted against Ba^{2+}/V (valinomycin) molar ratio. The valinomycin concentration was $\sim 16 \text{ mM}$.

with a plateau region at 1:1. At slightly higher concentrations of valinomycin ($\sim 2 \text{ mM}$), there is an initial trend of decreasing molar ellipticity, followed by an increase up to 1:1, at which point a reversal is noted. At still higher valinomycin concentrations ($\sim 5 \text{ mM}$), upon addition of salt the band behaves more or less as the 217.5-nm band shown in Fig. 2. The band always disappears at high salt:valinomycin values, with a tendency to disappear faster upon increasing the valinomycin concentration. Because of the necessity of decreasing the path length of the solution so as to reduce the noise of the CD signal at 217.5 nm, it was necessary to increase the initial concentration of valinomycin. Therefore, the type of changes that occur at 227.5 nm at low concentration of valinomycin could not be studied at 217.5 nm.

DISCUSSION

Looking at the CD curves for the Ba^{2+} complexes and comparing them with valinomycin- K^+ curve, it is clear that conformations of Ba^{2+} complexes may be quite different from that of the K^+ complex. The type of complex stabilized seems to depend on valinomycin concentration, the 1:1 type predominant at lower valinomycin concentration and the other complexes dominant at higher valinomycin concentrations.

The main points to note in comparing Ba^{2+} complexes with the K^+

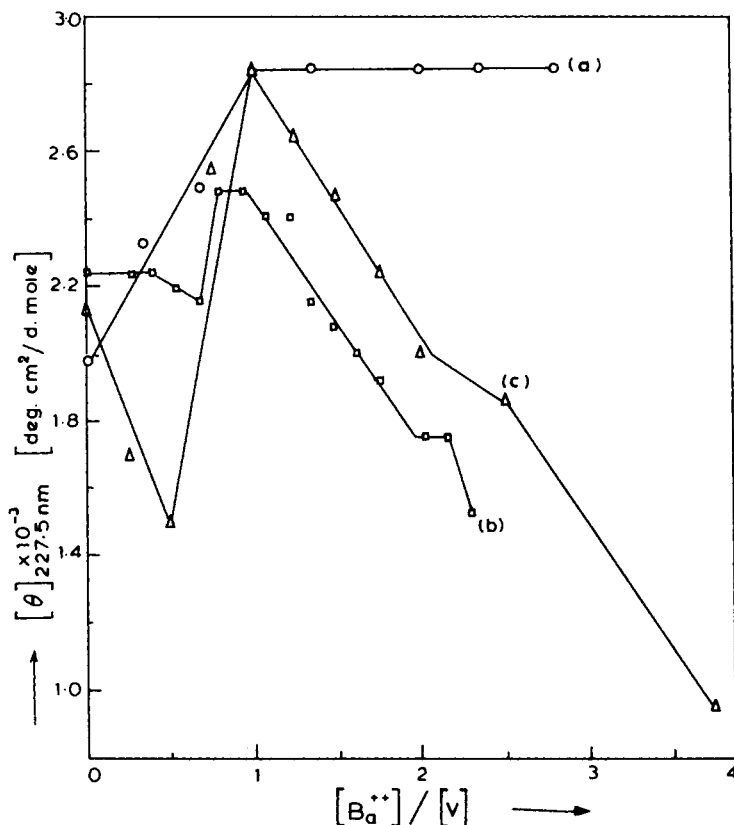


Fig. 3. Barium perchlorate titration of valinomycin in acetonitrile. Molar ellipticity $[\theta]$ at 227.5 nm is plotted against the Ba^{2+}/V molar ratio. Valinomycin concentration was (a) 0.5 mM, (b) 2.5 mM, and (c) 5 mM.

complex are: (1) the K^+ complex, which is a 1:1 type complex (VC type, where V is valinomycin and C any cation) does not have a peak or trough near 217.5 nm; (2) the trends in the changes of band depth for the 217.5-nm band of the Ba^{2+} complex indicate the possibility that this band corresponds to 2:1 (V_2C), 1:2 (VC_2) types of complexes and to a complex forming at values greater than 2 for Ba^{2+}/V ; (3) the Ba^{2+} complex has a positive 227.5-nm band, at least at lower values for Ba^{2+}/V . The maximum ellipticity value observed for this band is around 1:1. Hence this band could be assumed to correspond to a VC-type complex. While the 227.5-nm band of the Ba^{2+} complex disappears at higher Ba^{2+}/V values, the negative 235-nm band of the valinomycin- K^+ complex remains constant from 1:1 at least up to $\sim 1:50$.

The reversal in molar ellipticity of the 217.5-nm band at a 2:1 ratio (Fig. 2) indicates the presence of two distinct complexes, V_2C and VC ,¹⁵ and the reversal around 1:1 indicates the formation of a VC_2 -type complex from VC. The reversal near 1:0.7, rather than at 1:1, in this valinomycin con-

centration of ~ 16 mM is consistently seen at other valinomycin concentration also (~ 29 mM) but not at the lower concentration studied (7.5 mM). This can be explained by the incomplete conversion of the V_2C type to the VC type and direct formation of the VC_2 type from V_2C at valinomycin concentrations higher than ~ 7.5 mM. The 227.5-nm band, which corresponds to VC, is also affected by the formation of various other Ba^{2+} complexes, which form only above a valinomycin concentration of 0.5 mM. At 2.5 mM [Fig. 3(b)], because of the low concentration of valinomycin, V_2C forms more easily only when enough VC is formed (thus causing the initial plateau followed by a decrease in the ellipticity). VC reforms from V_2C , with increased cation availability, causing a rise in ellipticity; but the conversion is incomplete even at a ratio of 1:1, resulting in a lower ellipticity maximum. Beyond this point, with VC concentration remaining steady, the indirect conversion of V_2C to VC_2 does not result in any change in the ellipticity (causing the plateau region). Increased VC_2 formation after this stage lowers the ellipticity value until a molar ratio of 1:2 is reached; a plateau region is then observed. The curve [Fig. 3(c)] at 5 mM indicates facilitation of V_2C formation and the completion of conversion of V_2C to VC by reversing at 1:0.5, and again reversing at 1:1 after reaching maximum ellipticity. The decrease in molar ellipticity beyond a ratio of 1:2 indicates the beginning of the breaking down of the VC_2 -type complex into a new type of complex. When this breakdown of the VC_2 -type complex is complete, the plateau region is reached. (This plateau region is reached at ratios greater than 1:40 for a valinomycin concentration of 16 mM.) Valinomycin begins to precipitate out at still higher salt concentrations ($\sim 1.0M$).

Complexation Mechanism

Though the sizes of Ba^{2+} and K^+ are comparable, the differences in solvation energy, charge, and increased number of anions surrounding the barium ion probably make it difficult for this cation to take the place of K^+ so as to result in a conformation similar to that of K^+ complex. The possible mechanisms for complex formation of valinomycin with gradually increasing $[Ba^{2+}]$ are as follows:

(1) The initial conformation of valinomycin is a bracelet type with six intramolecular hydrogen bonds, three of them perhaps weakly hydrogen bonded.^{2,8,10}

(2) The initial complex is of the VC type. The conformation of VC may be such that three ester carbonyls ligand with Ba^{2+} from one side and solvent molecules and anions ligand with the ion from other side; the molecule may be in a bracelet conformation. While this type is stable at lower valinomycin concentrations [Fig. 3(a)], it is only a transient intermediate for V_2C at higher valinomycin concentrations [Figs. 3(b) and 3(c)].

(3) Another valinomycin molecule approaches and replaces the liganding solvents and anions with its three ester carbonyls from one side to form the

V₂C type complex. Up to a ratio of 2:1, this V₂C type is increasingly formed.

(4) Beyond a 2:1 ratio, the VC type begins to reform from V₂C with the additional cation.

(5) Beyond 1:1, formation of the VC₂-type complex occurs. Because of their similarity in conformation, V₂C, which is a peptide sandwich, gives a contribution to the 217.5-nm band that is similar to that of VC₂, which is an ion sandwich.

(6) But even the VC₂ type is not stable, possibly due to the fact that at any given time, only one singly charged bulky perchlorate group remains near the doubly charged cation. Because of its bulkiness, this anion does not allow another anion near the cation. Accessibility of the solvent molecules to the cation is also reduced. In addition, the isopropyl side chains stretching out from the periphery of the valinomycin molecule do not allow liganding groups like the perchlorate oxygens and the cyano groups of acetonitrile to approach and ligand effectively with the cations remaining on either side of the ion sandwich.

(7) The breakdown of VC₂-type complex results in yet another complex, which remains uncharacterized. Perhaps in this last complex the valinomycin structure breaks down to a completely stretched conformation having no hydrogen bonds and allowing the isopropyl side chains to move away. This allows the two Ba²⁺ ions inside enough freedom to ligand with anions and solvent molecules. Now that both sides of the cation are accessible to the anion, even two perchlorate groups can approach the Ba²⁺, one on either side of the cation, to neutralize the charge. A schematic drawing indicating the complexation mechanism is shown in Fig. 4.

CONCLUDING REMARKS

Evidence for the kind of role envisaged for isopropyl side chains is found in an analog of valinomycin, cyclo(L-Val-Gly-Gly-L-Pro)₃, where two of the D-residues, D-valine and D-hydroxyisovalerate, are replaced by glycine residues in the repeating unit. This analog has been shown to form a VC-type complex with barium perchlorate salt and to exhibit a conformation similar to that of the valinomycin-K⁺ complex.^{16,17} It is the larger cavity for the analog, without isopropyl side chains in three of the four residues in the repeat unit, that allows the cation to come inside the molecular cavity with its solvation sheath being gradually stripped and replaced by the ester carbonyls to form the final complex.

It is also clear that the choice of anion plays an important role in stabilizing valinomycin-Ba²⁺ complexes. The VC₂-type complex could be stabilized with linear anions with their oriented charge and smaller bulk, which allow more than one of them to come near the cation and also from the same side, thereby neutralizing the charge more effectively. Evidence for this is shown in our preliminary proton nmr results for Ba²⁺ complexes with perchlorate and thiocyanate anions. The plot of the chemical-shift

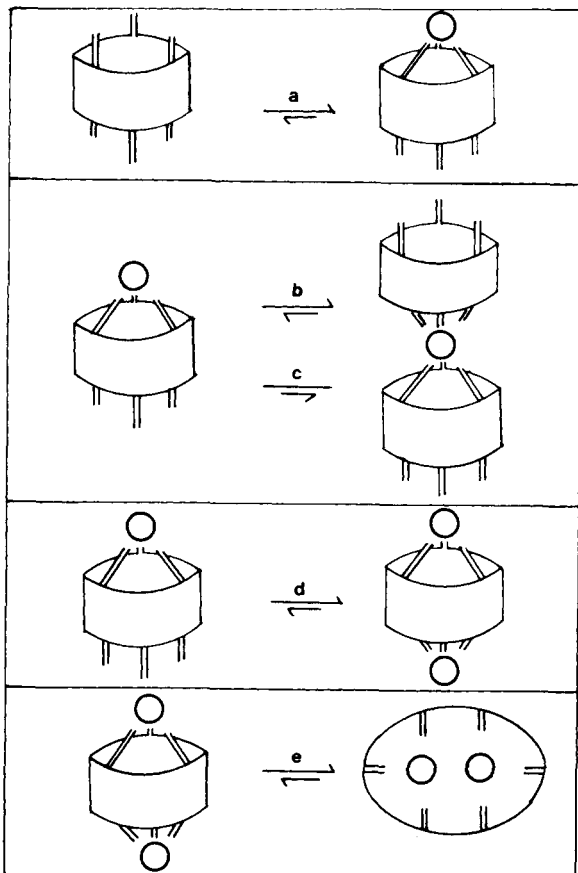


Fig. 4. A schematic diagram showing the various stages of the possible complexation mechanism of valinomycin with Ba^{2+} . (a) From free to 1:1, (b) 2:1 from 1:1, (c) 1:1 from 2:1, (d) 1:2 from 1:1, and (e) breakdown of 1:2 to form a flat structure.

changes with salt concentration indicated a plateau region around a ratio of 1:2 for the thiocyanate salt, whereas these plots indicated a continuously changing chemical shift with increasing *perchlorate* which gave no sign of stabilization even at high concentrations of this salt (Fig. 5). This is because the bulky perchlorate anion does not stabilize VC_2 as linear SCN^- does. VC_2 breaks down to form a flat structure in which perchlorate can approach the barium from both sides. Because of fast exchange at the nmr time scale between these two systems, we get a weighted average position for chemical shift which changes continuously as more and more flat structure forms with the addition of metal. Preliminary crystal-structure analysis¹⁸ for valinomycin- Ba^{2+} perchlorate salt complex at a value of more than 2 for Ba^{2+}/V indicated an open structure (similar to our proposed final complex), devoid of any hydrogen-bond scheme, with two barium ions sitting inside the molecule liganded with three carbonyl oxygens each, perchlorate oxygens, and solvent molecules. X-ray studies of the vali-

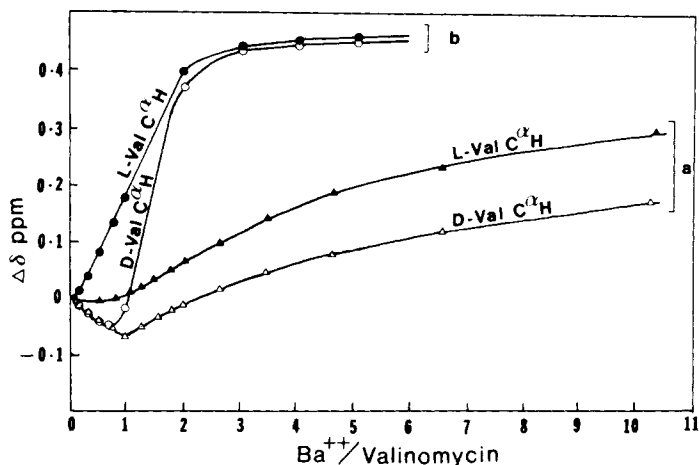


Fig. 5. Barium salt titration curves for the $C^{\alpha}H$ proton nmr chemical shift of L-valine and D-valine residues of valinomycin in deuterated acetonitrile plotted as a function of the Ba^{2+}/V concentration ratio for barium perchlorate (a) and for barium thiocyanate (b).

nomycin- Ba^{2+} thiocyanate complex are also in progress in our laboratory.

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