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Conformational characterisation of valinomycin complexation with barium salts—A nuclear magnetic resonance spectroscopic study

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Abstract. Conformations of valinomycin and its complexes with Perchlorate and thiocyanate salts of barium, in a medium polar solvent acetonitrile, were studied using nuclear magnetic resonance spectroscopic techniques. Valinomycin was shown to have a bracelet conformation in acetonitrile. With the doubly charged barium ion, the molecule, at lower concentrations, predominantly formed a 1:1 complex. At higher concentrations, however, apart from the 1:1, peptide as well as ion sandwich complexes were formed in addition to a 'final complex'. Unlike the standard 1:1 potassium complex, where the ion was centrally located in a bracelet conformation, the 1:1 barium complex contained the barium ion at the periphery. The 'final complex' appeared to be an open conformation with no internal hydrogen bonds and has two bound barium ions. This complex was probably made of average of many closely related conformations that were exchanging very fast (on nuclear magnetic resonance time scale) among them. The conformation of the 'final complex' resembled the conformation obtained in the solid state. Unlike the Perchlorate anion, the thiocyanate anion seemed to have a definite role in stabilising the various complexes. While the conformation of the 1:1 complex indicated a mechanism of ion capture at the membrane interface, the sandwich complexes might explain the transport process by a relay mechanism.

Keywords. NMR study; conformation; ionophores; valinomycin-barium complex; trans-membrane ion-transport.

Introduction

Valinomycin is a cyclic dodecadepsipeptide, which has been well studied as a model system for understanding biological transport across membranes in higher organisms (Ovchinnikov *et al.,*1974; Stark, 1978; Ovchinnikov, 1979; Lauger, 1980; Lauger, *et al.,* 1981). Two most important characteristics of the molecule that have come out of these studies are (i) its selective transport of K^+ over Na^+ (by a factor of over 10^4), both in natural (Pressman, 1965) as well as synthetic (Mueller and Rudin, 1967) membranes, which explains its antibiotic activity and (ii) its solvent dependent conformations (Patel and Tonelli, 1973; Ovchinnikov, *et al.,* 1974) that help provide explanations for the mechanism of transport. While it is true that some of the conformations of valinomycin have been characterised to a great extent (Duax *et al.,* 1972; Patel and Tonelli, 1973; Ovchinnikov and Ivanov, 1974; Ovchinnikov *et al.,* 1974; Karle, 1975; Neupert-Laves and Dobler, 1975; Smith *et al.,* 1975; Bystrov *et al.,* 1977), all the conformational

Abbreviations used: NMR, Nuclear magnetic resonance; CD, circular dichroism; ppm, parts per million; TMS, tetramethyl silane; Val, valine; Hylv, hydroxyisovaleric acid; Lac, lactic.

possibilities for the molecule have not been explored efficiently. Providing an exact mechanism of transport by the molecule depends on knowing the various conformational rearrangements the molecule has to undergo within the membrane, and this can possibly be achieved by studying the conformation of the molecule under a variety of solution and salt conditions. To this end, we have characterised the barium complexes of valinomycin in acetonitrile solvent. Ba^{2+} (1·38 A) has almost the same ionic radius as K^+ (1·33 A) but has a double charge and therefore the effect of increased charge on the complex conformation of the molecule can be well studied. Some of the results obtained using circular dichroism (CD), X-ray and nuclear magnetic resonance (NMR) techniques have already been reported (Devarajan *et al.,* 1980; Devarajan and Easwaran, 1981; Devarajan *et al.,* 1983). In this paper, we report a detailed NMR study of barium complexation with valinomycin.

Materials and methods

Valinomycin was obtained from Sigma Chemical Company, St. Louis, Missouri, USA and was used as such. Only Perchlorate and thiocyanate salts were used for complexation studies as they showed low cation-anion interactions. The perchlorate salt was prepared by neutralising barium hydroxide with perchloric acid and then drying. The thiocyanate salt was a gift from Shemyakin Institute of Biorganic Chemistry, Moscow, USSR. The salts were dried in vacuum over phosphorous pentoxide for several days before use.

All the deuterated solvents were purchased from Stohler Isotope Company, Massachusetts, USA and were used as such. The free radical 2,2,6,6 tetramethyl piperiden-1-oxyl was a gift from Prof. K. D. Kopple. The NMR spectra were recorded using Bruker WH 270 FT-NMR spectrometer of the Sophisticated Instrumentation Facility, Indian Institute of Science, Bangalore, under controlled FT mode, at 270 MHz for ^1H and 67·89 MHz for ^{13}C. Five mm and 10 mm diameter NMR tubes from Wilmad Glass Company were used for ^1H and ^{13}C respectively. The ^{13}C NMR spectra were recorded with broad band decoupling of the coupled protons. For ally experiments, deuterium signals from solvents were used for locking the magnetic field of the spectrometer. The chemical shifts were measured in parts per million (ppm) with respect to internally added tetramethyl silane (TMS). The experiments were performed in CD_3CN unless otherwise stated. The stoichiometrics mentioned in this paper correspond to valinomycin: metal salt in that order only.

Results and discussion

Proton NMR studies

Conformation of valinomycin in acetonitrile: The conformation of valinomycin was determined in deuterated acetonitrile (CD_3CN) a solvent used through out this study as valinomycin assumes solvent dependent conformations.

Table 1 lists the C^α proton chemical shifts and coupling constants of valinomycin in various conformations. Apart from the similarity of C^α proton chemical shifts, the

Table 1. A comparison of chemical shifts and coupling constants for valinomycin in various states.

	$C^{\alpha}H$ Chemical shifts (ppm)				Coupling constants $^{3}JHNC^{\alpha}H$ (Hz)		
State	L-Lac	D-HyIv	D-Val	L-Val	L-Val	D-Val	References
A	5.31	5.03	4.03	3.91	7.0	8.5	Bystrov *et al.*, 1977
B	5.09	4.82	4.26	4.36	7.5	10.1	Bystrov *et al.*, 1977
CD_3CN	5.25	5.00	4.11	4.14	7.6	7.6	Present studies

coupling constants are also very close for valinomycin in CD_3CN and A conformation. Also, a free radical (2,2,6,6, tetramethyl piperiden-1-oxyl) which is capable of broadening any exposed amide proton (Kopple *et al.,* 1978) on addition to valinomycin and its K^+ complex in CD_3CN under identical valinomycin concentration conditions, does not cause any broadening in both the systems (figure 1). This observation indicated that all the valinomycin amide protons were hydrogen bonded in CD_3CN just as in its K^+ complex. Figure 2 shows the effect of changing the solvent from nonpolar $CDC1_3$ (chloroform) to CD_3CN, on the carbonyl carbons chemical shifts. The amide carbonyl carbons [L-lactic (Lac) and D-hydroxyisovaleric acid (HyIv)] that are involved in hydrogen bonding shift only slightly, indicating that the hydrogen bonding scheme is same in both the solvents. The shifts in the ester carbonyl carbons

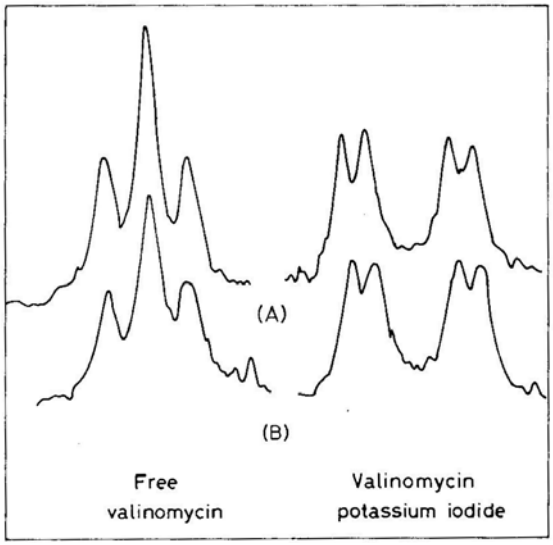

Figure 1. Effect of free radical addition to valinomycin and its K^+ complex both in CD_3CN. Valinomycin concentration is 8·9 mM. **A.** Before addition. **B.** After addition.

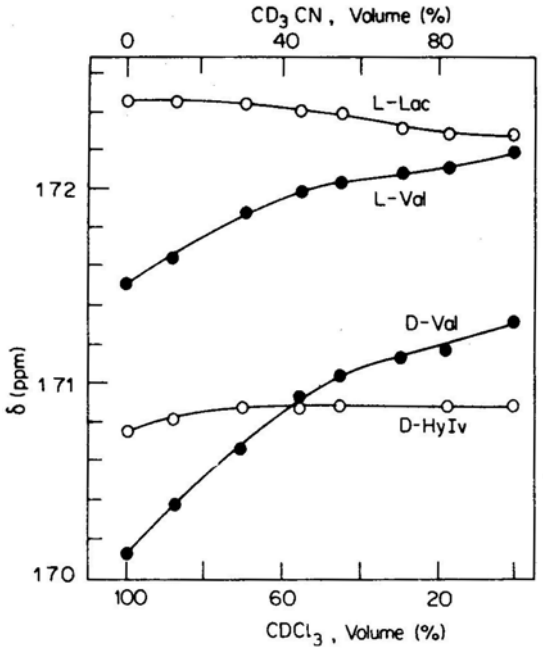

Figure 2. Solvent titration for the assignment of carbonyl signals of free valinomycin in CD_3 CN; X axis: volume per cent of $CDC1_3$ or CD_3CN; Y axis: (ppm) with reference to TMS.

[L- and D-valine (Val)] seen in figure 2, indicated a changed orientation for these groups. Thus valinomycin assumes A conformation in CD_3CN (CH_3CN) with all its hydrogen bonds intact.

Studies on valinomycin-barium complexes: It has been known that chemical shift positions of the C^{α} protons are sensitive to the conformation of the molecule, in particular to the change in orientation of the adjacent carbonyls (Grell and Funck, 1973a). The carbonyls can change their orientation in metal complexation studies to, either ligand effectively with the cation, or accommodate the resultant conformational changes by adjusting the hydrogen bond scheme. Therefore, conformational characterisation of the various complexes can be achieved, by following the chemical shift changes at the various C^{α} protons. The shifts in the amide protons give an idea about hydrogen-bond scheme.

Studies on valinomycin-barium Perchlorate complexes

At lower valinomycin concentrations: Figure 3 shows the chemical shift changes for the NH and $C^{\alpha}H$ signals of valinomycin (0·37 mM) with barium Perchlorate addition. A plateau at 1:1 indicated stabilisation of the 1:1 complex. Three points emerged from a comparison of these chemical shifts with those corresponding to K^+ addition (Ovchinnikov and Ivanov, 1974). (1) The amide protons (L- and D-Val N*H*'s) showed a

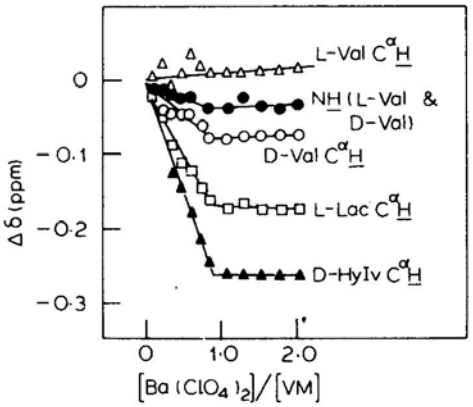

Figure 3. Barium perchlorate addition to valinomycin at 0.37 mM concentration. The stabilisation of the signals at 1:1 is clearly seen

large low-field shift with the K^+ complex formation, but have only a small high-field shift with the Ba^{2+} complex formation. (2) While the L-Val C$^{\alpha}H$ showed a considerable high-field shift (0·28 ppm) with the K^+ complex formation, it almost did not produce shift with the Ba^{2+} complex formation. (3) The shift in D-Val, L-Lac and D-Hylv C$^{\alpha}H$'s was in the same direction in both the complexes. However, the magnitude of shifts for the barium complex was smaller.

The larger low-field shift of the amide protons in the K^+ complex could be attributed to the electron withdrawing effect of the nearby K^+ ion. The doubly charged barium ion (which could have caused a still larger low-field shift, if it were to occupy the K^+ position) could be only at the periphery of the bracelet. The absence of a change in the chemical shift position of the L-Val C$^{\alpha}H$ indicated that the binding was not from the L-Val side definitely, and could be from the D-Val side.

The small high-field shift of the amide protons indicated some weakening of hydrogen bonds. This result suggested that the amide (L-Lac and D-Hylv) were turning away from an hydrogen bonded position, to co-ordinate with the barium ion. The large high-field shifts in L-Lac and D-Hylv C$^{\alpha}H$'s seemed to confirm such a possibility. The shift in the D-Val C$^{\alpha}H$ though high-field as in the K^+ complex, was smaller. This study indicated that this ester carbonyl, though turned inward as in the K^+ complex, did not have to change its orientation markedly.

All these inferences indicated a 1:1 barium complex in which the barium ion was positioned at the periphery of the bracelet, on the D-Val side, complexing with D-Val, L-Lac and D-Hylv carbonyl oxygens (Devarajan and Easwaran, 1981). The ion possibly still retained part of its hydration shell. Preference for the D-Val side for complexation could be attributed to the presence of lesser number of side chains and therefore lesser steric hindrance when compared with the L-Val side. The failure to form 'valinomycin-K^+' like complex was probably due to the larger solution sheath that surrounds Ba^{2+}.

At higher valinomycin concentrations: At concentrations higher than 5 mM, CD studies showed that valinomycin formed, apart from 1:1, 2:1 (peptide sandwich) and 1:2 (ion sandwich) complexes and a 'final complex' (Devarajan and Easwaran, 1981). Figure 4 shows the titration graph obtained by the addition of barium Perchlorate to

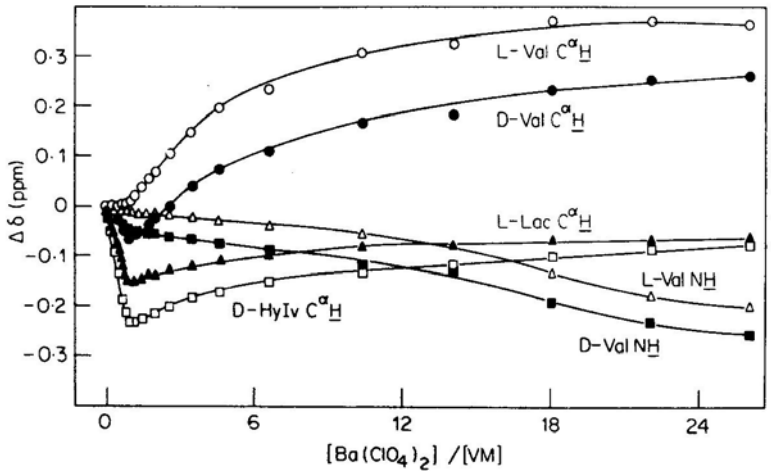

Figure 4. Chemical shift changes at a higher valinomycin concenration, on the addition of barium Perchlorate salt. [VM] ~ 21 mM.

Table 2. Changes in chemical shift values, with barium Perchlorate addition, for various protons upto 1:1, and beyond 1:1 until the end of titration. Valinomycin concentration ~ 21 mM (ppm units).

Protons	N*H*'s			C$^{\alpha}$*H*'s		
State	L-Val	D-Val	L-Lac	D-HyIv	L-Val	D-Val
Upto 1:1	−0·011	−0·049	−0·148	+0·231	0·012	−0·066
Beyond 1:1 till the end	−0·191	−0·205	+0.083	+0.160	0·353	0·330
Total	−0·202	−0·254	−0·065	−0·071	0·365	0·264

valinomycin at ~ 21 mM concentration. A sharp break at 1:1 and a semblance of plateau region at a large excess addition of the barium salt only were seen. The various complexes that are expected to form (2:1 and 1:1 in the beginning stages, and 1:2 and the 'final complex' in the later stages) seemed to be exchanging at a rate faster than NMR time scale and the chemical shift positions therefore represented an average of the various conformations.

Table 2 lists the changes in chemical shift values for the various protons upto 1:1 and beyond 1:1 until the end of the titration. The changes observed up to 1:1 in this titration were less (though similar), when compared with that carried out at 0·37 mM, indicating formation of complexes other than the 1:1 that have opposing influences on the chemical shift changes. The CD studies have indicated that 2:1 complex forms at this stage of titration and has CD contributions opposite to that of 1:1.

Figure 5 represents the N *H* and the C$^{\alpha}$*H* region for a few of the titration points, in the beginning of the experiment. Except L-Val C$^{\alpha}$*H* all other lines showed considerable

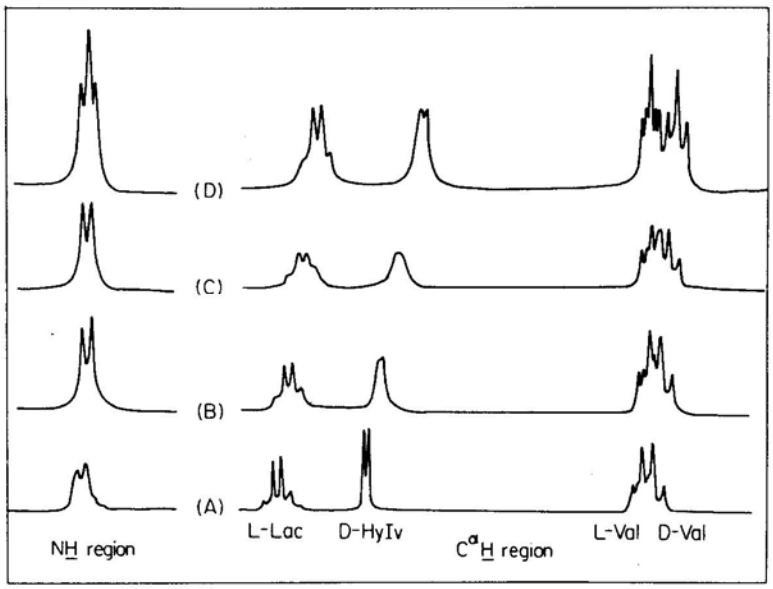

Figure 5. Broadening of the amide and the C$^{\alpha}H$ signals except the L-Val C$^{\alpha}H$, in the initial stages of the Perchlorate salt addition. The data points correspond to the titration represented in figure 4. The ratios for the data points are **(A)** 1:0, **(B)** 1:0·10 **(C)** 1:0·29 and **(D)** 1:0·58.

broadening. The absence of any shift (as indicated earlier) or broadening for the L-Val C$^{\alpha}H$, when the 2:1 complex also formed, indicated that the L-Val side was not involved in metal binding in this complex also probably due to steric reasons. The ion was held by six D-Val carbonyl oxygens (Devarajan and Easwaran, 1981).

Thus, by following subtle changes in NMR parameters at a higher valinomycin concentration (~ 21 mM), it was possible to confirm the formation of the 2:1 complex. Similarly the formation of 1:2 (ion sandwich) is confirmed by following chemical shift changes at a lower temperature (– 40°C), Figure 6 shows that all the lines except that of the L-Val C$^{\alpha}H$ split into two lines, at – 40°C, one representing the free and the other, the complex. The lines nearly remain unchanged in their chemical shift positions till 1:1. The complex lines grew, while the free lines decrease in intensity (not shown). The most important difference to be noted with respect to the room temperature titration (figure 4), was the low-field shift of the N*H* signals beyond 1:1 at low temperature. This observation indicates stabilization of a complex that retained hydrogen bonds—probably the formation of the 1:2 ion sandwich complex, having two barium ions, one on either side of a valinomycin bracelet, held by three ester carbonyls. The other side of the ion would be exposed to interaction with the anions and the solvent molecules.

Determinations of conformations based on NMR parameters was not easy, when these parameters represent an average of many conformations that were exchanging very rapidly among themselves, with no single predominant conformation. Because of this reason, exact conformational characterisation of the various intermediate complexes was not possible. However, at the very end of the titration (as in figure 4), there was an induction of a plateau region, possibly representing the predominant formation

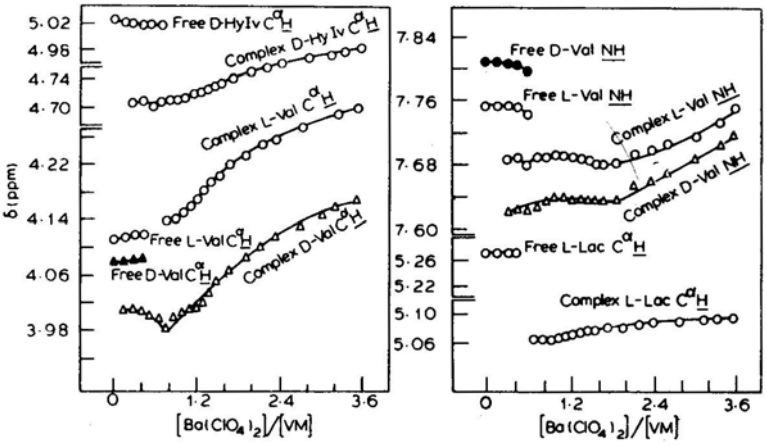

Figure 6. A low temperature (—40°C) titration with barium Perchlorate salt. [VM] ~ 12 mM. Splitting of all lines except that of L-Val C$^{\alpha}H$ can be seen. Free lines disappear at 1:1.

of the 'final complex' as reported previously (Devarajan *et al.,* 1980; Devarajan and Easwaran, 1981). Conformational characterisation of this complex will be reported in the latter part of this paper.

Studies on valinomycin-barium thiocyanate complexes

At lower valinomycin concentration: With barium thiocyanate salt, valinomycin at low concentrations (0.37 mM) formed a 1:1 complex involving the sterically less hindered D-Val side, as with the Perchlorate salt. However, unlike in the Perchlorate case, the complex was not stable even at this low valinomycin concentration as indicated by the lack of plateau region for the L- and D-Val C$^{\alpha}H$ signals beyond 1:1.

At higher valinomycin concentration: At a higher valinomycin concentration (~3 mM), even the L-Val C$^{\alpha}H$ begins to show changes in the chemical shift position, just from the beginning of barium thiocyanate addition (figure 7). This was in contrast to the titration corresponding to barium Perchlorate addition (figure 4). Also, a plateau region beyond 1:2, which was not obtained unless a large excess of the Perchlorate salt was added, was easily discerned just beyond 1:2 in the thiocyanate case. While the changes in the L-Val C$^{\alpha}H$ from the beginning of the thiocyanate salt addition indicated the formation of a L-Val side involved complex, the faster approach of the plateau region beyond 1:2 indicates a facilitated formation of the 'final complex' in the presence of the thiocyanate anion.

It can be seen below that the L-Val side involved valinomycin barium thiocyanate complex, that forms in the beginning of the thiocyanate salt addition, has to be a 1:1 complex and definitely not a 2:1 complex. This complex was not formed upon the addition of the Perchlorate salt and therefore must involve the linear thiocyanate anions for its formation. The formation of the 2:1 complex does not involve anions or

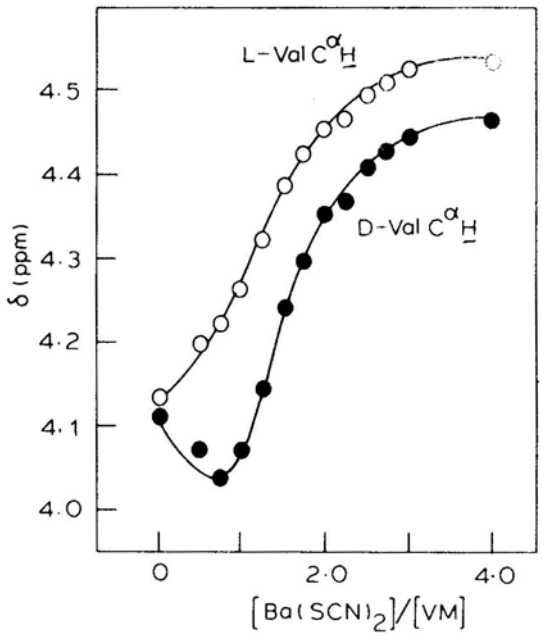

Figure 7. Chemical shift changes at a valinomycin concentration of ~ 3 mM, with barium thiocyanate salt addition, at L- and D-Val $C^{\alpha}H$ region.

solvent molecules, while the formation of the 1:1 complex is definitely aided by anions and solvent molecules. Because of the involvement of the sterically crowded L-Val side for the complex formation, the superior liganding capacity of the linear anion (thiocyanate) might be needed to stabilise this complex.

Carbon-13 *studies*

Because of the lower natural abundance (1·1 %) and the lesser inherent sensitivity of carbon-13 with respect to proton (Deslauriers and Smith, 1980), there is always a necessity to use a larger concentration of the sample to obtain a good ^{13}C spectrum. Therefore, the interesting observations made in proton NMR at lower valinomycin concentrations (like at ~ 0.4 mM), could not be confirmed by carbon-13 studies. The low field shift of carbonyl carbons in metal binding (Bystrov *et al.,* 1977), was exploited by following the chemical shift changes of these carbons to supplement the conformational characterisations of the complexes using proton NMR studies.

On valinomycin-barium Perchlorate complexes: Figure 8 represents the chemical shift changes at the carbonyl carbon region of valinomycin, upon the addition of barium perchlorate. The titration graph clearly showed the break at 1:1, and stabilisation at 1:2. The high-field shift in the L-Val $^{13}\mathbf{C}'$ signal, in the initial stages of salt addition,

$$\mathbf{C}'=\mathbf{O}$$

confirmed the non-participation of L-Val side in metal binding probably due to steric

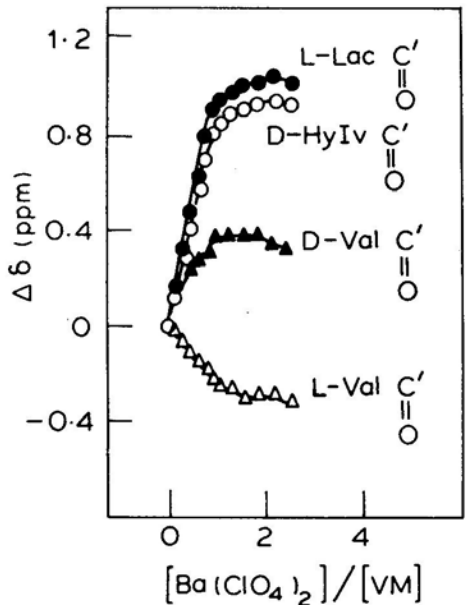

Figure 8. A ^{13}C NMR titration, representing chemical shift changes at the carbonyl region of valinomycin, with the addition of barium perchlorate salt

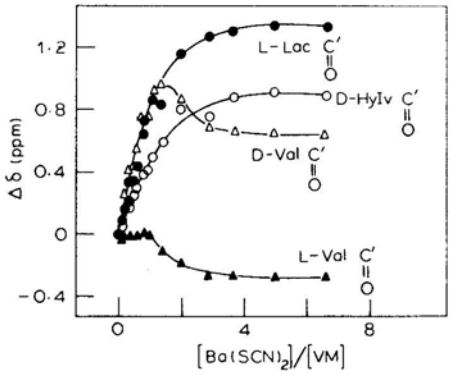

Figure 9. A ^{13}C NMR titration, representing chemical shift changes at the carbonyl region of valinomycin, with the addition of barium thiocyanate salt

reasons. The low-field shifting of D-Val L-Lac and D-HyIv $^{13}C'$s confirmed the

participation of all these carbonyls from the D-Val side in metal binding and provided a possible explanation for the weakening of hydrogen bonding scheme. When a large excess addition of the Perchlorate was added (1:13, not shown in figure 8, *i.e.* the 'final complex' is expected to form), L-Lac and D-Hylv $^{13}C'$s remained at low field

($\Delta\delta$ = 1·29 and 1·37 ppm respectively) indicating disengagement of the D-Val $^{13}C'$

from metal binding. Therefore, the 'final complex' seemed to involve only amide carbonyls for metal binding unlike all other valinomycin-metal complexes so far reported in the literature (Ovchinnikov *et al.,* 1974; Bystrov *et al.,* 1977).

On valinomycin-barium thiocyanate complexes: Figure 9 represents the chemical shift changes at the carbonyl carbon region of valinomycin, on the addition of barium thiocyanate salt The break shown at 1:1 by the L-Val $^{13}C'$ and the stabilisation of the

chemical shift changes of all the signals just beyond 1:2 confirmed the formation of 1:1 and 1:2 respectively.

The proton studies indicated that an L-Val side involved 1:1 complex was also formed in the presence of the thiocyanate anion. However, the L-Val $^{13}C'$ did not shift low field even to a limited extent contrary to expectations. It could be seen from literature (Bystrov *et al.,* 1977) that when there was opening of the bracelet conformation, the L- and D-Val $^{13}C'$s shifted high-field. Therefore, it is possible that in the initial stages of the thiocyanate salt addition, the low-field shift of the L-Val $^{13}C'$ expected with the formation of the L-Val side involved 1:1 complex was compensated with the high-field shift of the above signal caused by the formation of a small amount of the 'final complex' with its open conformation. It is interesting to note at this point that in the corresponding ^{13}C titration performed with the Perchlorate, the L-Val $^{13}C'$ moves high-field from the beginning of the salt addition. The chemical shift changes in the carbonyl region beyond 1:1 in the present titration (figure 9) are similar to those in the Perchlorate case (figure 8), confirming the formation of a 'final complex' with 1:2 stoichiometry.

Conformation of the final complex

From solution studies: In our earlier paper dealing with CD studies (Devarajan and Easwaran, 1981), the complex that was formed near the end of the barium salt addition was termed as the 'final complex'. From ^{13}CNMR studies (see above), it was clear that this complex had 1:2 stoichiometry and the amide carbonyls were involved in metal binding. The inference from the CD studies that this complex may have an open conformation was confirmed by the shift of N*H* to highfield near the end of barium salt addition to valinomycin (figure 4) and also from a free radical experiment as shown in figure 10. This experiment was conducted at identical concentrations for both valinomycin and the free radical as in the case of uncomplexed valinomycin and its K^+ complex (see figure 1). While there was no broadening of the amide protons in the case of the both free valinomycin and its K^+ complex (figure 1), noticeable broadening for these protons was observed, in the case of the 'final complex' (figure 10).

The coupling constants that are measured for the 'final complex' and the probable ø values corresponding to these constants are listed in table 3. Model building studies clearly ruled out conformations with ø values +40° and +80° for L-Val (–40° and –80° for D-Val), but allowed the other two conformational angles. It can be seen from energy maps corresponding to the various residues present in the valinomycin molecule (Ovchinnikov and Ivanov, 1974; figure 11) that, although at one Ψ value or the other, each of the four ø values mentioned above become allowed, it is only around ø = – 80° for L-Val (+ 80° for D-Val), the global minimum energy is found. Also, only at this region of the energy map, large changes in the conformational angles are allowed with only a small increase in energy. For example, the ø values can be changed by ± 50° with

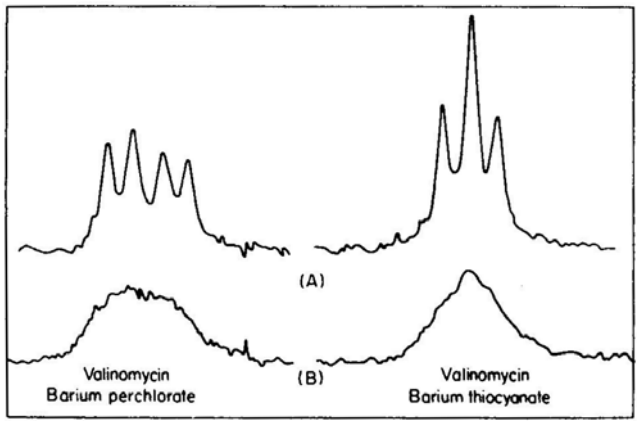

Figure 10. Effect of free radical addition on the 'finale complexes' of the barium salts, with valinomycin. Valinomycin concentration is ~ 8·9 mM in both the systems. **A.** Before addition. **B.** After addition.

Table 3. Coupling constants measured and the corresponding dihedral angles for the 'finale complex'.

Residue	$^{3J}HNC^{\alpha}H$	ϕ values
L-Val	6·3 Hz	−160°, −80°, +40°, +80°
D-Val	6·3 Hz	160°, 80°, −40°, −80°

only 1 or 2 kcal increase in energy. Since the 'final complex' has an open conformation, it may have a lot of flexibility and ø values — 80° for L-Val and + 80° for D-Val seem to be ideal for accommodating this flexibility.

Comparison with the conformation in the solid state: In the single crystal studies of valinomycin barium Perchlorate complex (Devarajan *et al.*, 1980), it has been found that valinomycin molecule was in an open conformation (with no hydrogen bonds) and held two cations by its amide carbonyls (figure 12). Thus in all major aspects, the conformation observed in the solid state was very similar to that in the 'final complex'. However, while the NMR studies on the 'final complex' have constantly shown a conformer that is C_3 symmetric in NMR time scale, the solid state conformer is non C_3 symmetric. Possibly if there are a good number of near equi-energy conformational states in solution that are non C_3 symmetric in nature, a fast exchange (in NMR time scale), among these conformers may result in the C_3 symmetry observed in NMR.

Exchange among near equi-energy states

Figure 11 shows the conformational energy maps of the four individual residues L-Val, D-HyIv, DVal and L-Lac. The conformational angles observed in the solid state and their mean values are shown in figure 4. Table 4 lists all the mean values and the maximum deviations of the observed values from these mean values on either side, for

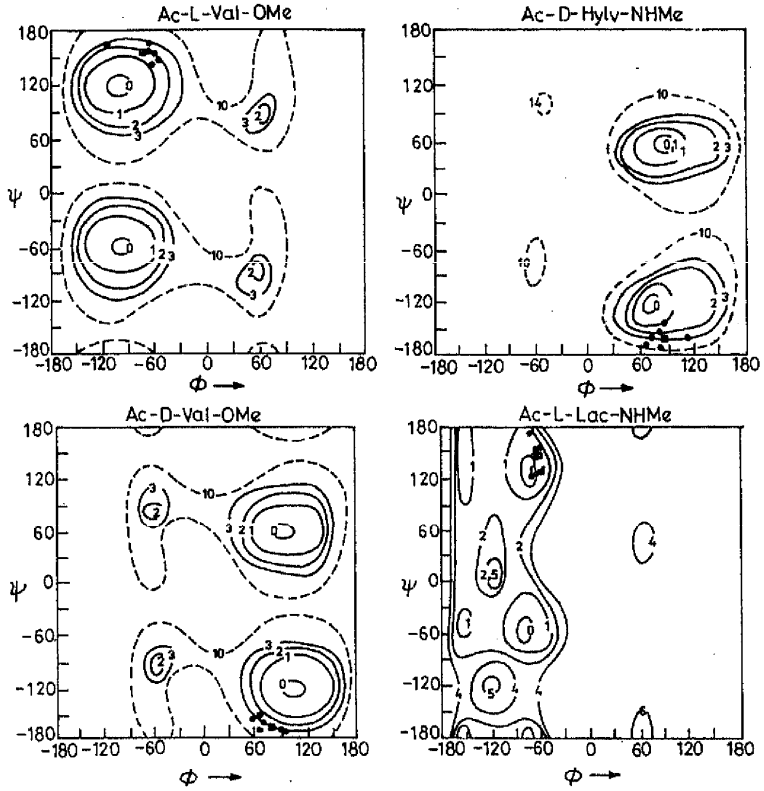

Figure 11. Conformational energy maps for the various residues present in valinomycin (taken from Ovchinnikov and Ivanov, 1974).

all the four individual residues. The deviations from the mean values were less than 30° except for the ø value of one L-Val residue which deviated by about 44°. It can be seen from figure 11 that the ø value assumed by this residue (–121°) falls on an energy contour that shows the same energy at the ø value (– 77°) that is mean for all the L-Val residues. Therefore there will not be any increase in conformational energy despite such a large deviation from the mean.

It can be seen from figure 11 that, for a residue, the conformational changes on either side of the mean (± 30°) can be achieved by 3 or 4 kcals gain or loss of energy. In the molecule, conformational changes can occur such that the gain in energy at one residue can compensate for the loss at another residue. Apart from such equi-energy states, there can be many other conformational states which differ just by 1 or 2 kcal change in energy. Assuming that the mean conformational angles represented in table 4 correspond to an average conformation in solution, all the conformational angles that are observed for a residue (Devarajan, 1982) on either side of this calculated mean value can be achieved with a minimum change in the conformational energy. As the energy separation among these various conformational states is not high, the populations that occupy each individual conformational state may be very close, and the exchange among these also may be fast, leaving the molecule to achieve C_3 symmetry on an

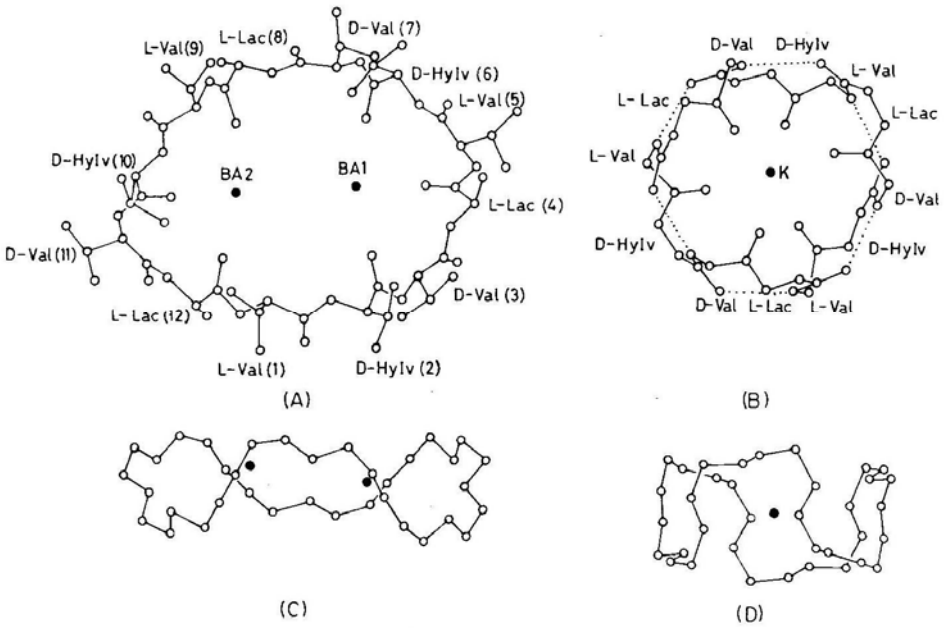

Figure 12. 'Top' view of the valinomycin molecule in its **(A)** barium Perchlorate complex (Devarajan *el al.,* 1983) and **(B)** Potassium iodide complex (Neupert-Laves and Dobler, 1975). **C** and **D.** Represent the corresponding 'side' views, with the side chain atoms and carbonyl oxygens omitted for clarity. The residue numbering is indicated in **(A)**.

Table 4. Mean values and the deviations on either side from the mean values for the various conformational angles (degrees) observed in the two valinomycin-barium structures solved in the solid state (Max = maximum value, Min = minimum value; Devarajan, 1982).

Residue	L-Val			D-HyIv			D-Val			L-Lac		
Angle	ϕ	ψ	ω	ϕ	ψ	ω	ϕ	ψ	ω	ϕ	ψ	ω
Mean	−77	157	−179	82	−153	−177	73	−162	−178	−67	149	174
(Max-Mean)	17	10	3	28	17	5	19	16	6	7	22	7
(Mean-Min)	44	15	6	16	19	6	16	13	12	8	21	9

average, from many near equi-energy non C_3 symmetric conformational states. Thus the 'final complex' obtained in solution on an average may resemble the conformation observed in the solid state.

Conclusions

It is known that barium ion interferes with physiological functions involving specific binding of K^+ ion, because of its similar size (Armstrong and Taylor, 1980). Due to its

higher charge density and the resultant larger solvation sheath, the double charged barium ion shows a tendency to retain part of its solvation sheath in almost all the complex structures (Johnson *et al.,* 1970; Metz *et al.,* 1971; McClelland, 1974; Raston and White, 1976; Hughes *et al.,* 1978). This tendency was found even in situations where K^+ did not retain any solvent molecule under identical conditions (Metz *et al.,* 1971). This could be one of the reasons why the 'final complex' and the two barium complexes solved in the solid state have a flat conformation probably to allow the alkaline earth metal to be accessible to anions and solvent molecules from both sides.

The 'final complex' of valinomycin-barium system has been unambiguously characterized as an open conformation. This can serve as a model for valinomycin conformation in polar solvents. We have also confirmed the formation of both peptide as well as ion sandwich complexes apart from two peripherally-ion-bound-1:1 complexes. While the open conformation may not serve any useful role to explain the transport process, the peripherally-ion-bound 1:1 complexes definitely aid in explaining the complexation mechanism at the membrane interface and the sandwich complexes help in understanding a modified carrier system involving more than one carrier (a relay mechanism) and indicate the possibility of ion-transport by a channel mechanism involving a column of stacked carriers.

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