

Molecular mechanism of facilitated transport by carrier ionophores: A study of energetics

N. SREERAMA and SARASWATHI VISHVESHWARA*

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

MS received 4 May 1987

Abstract. The mechanism of ion transport by carrier ionophores is investigated. The electrostatic potential is used as index of the binding energy of a cation with valinomycin and enniatin B. The ion binding capacities of these ionophores are studied as functions of conformation and of distance of an approaching ion-complex. The energetics of dimerisation and the binding energy profile of an ion in dimers of valinomycin and enniatin B are examined. The binding energy profiles and the electrostatic potential surfaces of valinomycin and enniatin B are compared in relation to their biological activities.

Keywords. Ion-transport; valinomycin; enniatin B; binding energy; electrostatic potential.

Introduction

Biological membrane, which separates the interior and exterior parts in a cell, plays an important role in maintaining the gradient of ions. This is a crucial step in bioenergetics and is achieved by the process of active transport (Mitchell, 1961, 1979; Williams, 1974). Even in the process of passive transport, a membrane consisting of only hydrocarbons cannot efficiently transfer ions from one side to the other since the low dielectric constant of the medium leads to a high barrier for ion movement (Parsegian, 1969, 1975; Läuger and Neumcke, 1973). Nature has selectively devised efficiently mechanisms like carriers and channels to overcome the overall hydrophobic barriers arising in both the active and passive transport processes. The transport mechanisms have been dealt in a large number of books and review articles. Recently theoretical considerations have been used at the molecular level to look at the energetics and dynamics of the ion transport in the channels (Eisenman and Horn, 1983a; Warshel, 1979; Pullman and Etchebest, 1984a, b; Nagle and Nagle, 1983; Mackay *et al.*, 1984; Monoi, 1983; Fisher and Brickman, 1983; Läuger, 1982; Lee and Jorden, 1984; Forneli *et al.*, 1984; Kim *et al.*, 1985). The theoretical studies on the energetics of the interaction between carrier ionophores and cations have been carried out mainly by Pullman and co-workers (Gresh *et al.*, 1981, Gresh and Pullman, 1982). Recently we have studied the electrostatic potential of valinomycin in different conformations (Sreerama and Vishveshwara, 1985). Molecular mechanics of valinomycin have also been carried out (Sundaram and Tyagi, 1978; Masut and Kushik, 1984, 1985).

In the present paper an attempt has been made to elucidate the action of carrier ionophores at the molecular level by simulating some of the steps involved during the transport process. The ion binding capacity of the ionophore is investigated under different conditions. The effects of conformational changes, induction due to an approaching ion-complex and dimerisation on the binding energy profiles have

*To whom all correspondence should be addressed.

been studied. These studies are carried out on valinomycin (DVal-LLac-DHyIv-LLac)₃ and enniatin B (LMeVal-DHyV)₃. The mechanism of ion transport is discussed in the light of these studies and a comparison of valinomycin and enniatin B is made.

Methodology

The electrostatic potential is a simple guide to study non-covalent interactions (Kollman, 1981). The interaction energy W_{AB} between two species A and B can be approximated as

$$W_{AB} = V_A(k) q_{kB}(k),$$

where V_A is the electrostatic potential due to A at k and q_{kB} is the charge at k due to B (Scrocco and Tomasi, 1973). The present study deals with the non-covalent binding of cations with the carbonyl ligands of valinomycin and enniatin B, the distance between interacting groups being generally greater than the sum of the van der Waals radii of the concerned atoms. Under these conditions the molecular electrostatic potential of ionophores can be used as an index of its capacity to bind to an ion.

Evaluation of the electrostatic potential is carried out by monopole approximation and the point charges on the ionophore fragments are obtained by the STO-3G method (Hehre *et al.*, 1969). The electrostatic potential values obtained by this method for valinomycin are in very good agreement with those generated by multi-centre-multipole expansion method (Etchebest *et al.*, 1982). The interaction energies of valinomycin dimers and enniatin B dimers are obtained as a sum of the non-bonded and electrostatic energies. The Lennard-Jones potential function with parameters of Momany *et al.* (1975) is used to evaluate the non-bonded interaction energies.

The carrier ionophores valinomycin and enniatin B exist in a non-polar medium as bracelet like structures (Ovchinnikov *et al.*, 1974), in which the liganding groups are pointing inwards to bind the cation and the hydrophobic side chains are exposed to the medium. Since our studies are mainly related to the fate of the ion after it enters the hydrophobic medium, the investigations are carried out only on the bracelet like structures. Such schematic structures for valinomycin and enniatin B are depicted in figure 1. The binding capacity of the bracelet like structure differs depending on the position of the liganding oxygens. Such structures are differentiated by d_1 and d_2 , which is the distance between the plane C_n , a plane passing through the centre of the molecule and the planes formed by the liganding oxygens on either side of the central plane. The different structures are also characterised by the distance from the centre of the molecule to the individual liganding oxygens. These distances and the distances d_1 and d_2 in various bracelet like conformations of valinomycin and enniatin B are given in table 1. Val1 is the conformation corresponding to the crystal structure of potassium complex of valinomycin (Hamilton *et al.*, 1981). Val2 and Val3 are the theoretical conformations, which are structures I and IV, respectively in Sundaram and Tyagi (1978). Distance d_1 is equal to d_2 in Val1 and Val2 whereas, d_1 is greater than d_2 in Val3, due to opening of the carbonyl oxygens on the L-Lac side. In Val1, Val2 and Val3 the plane C_n is almost parallel to

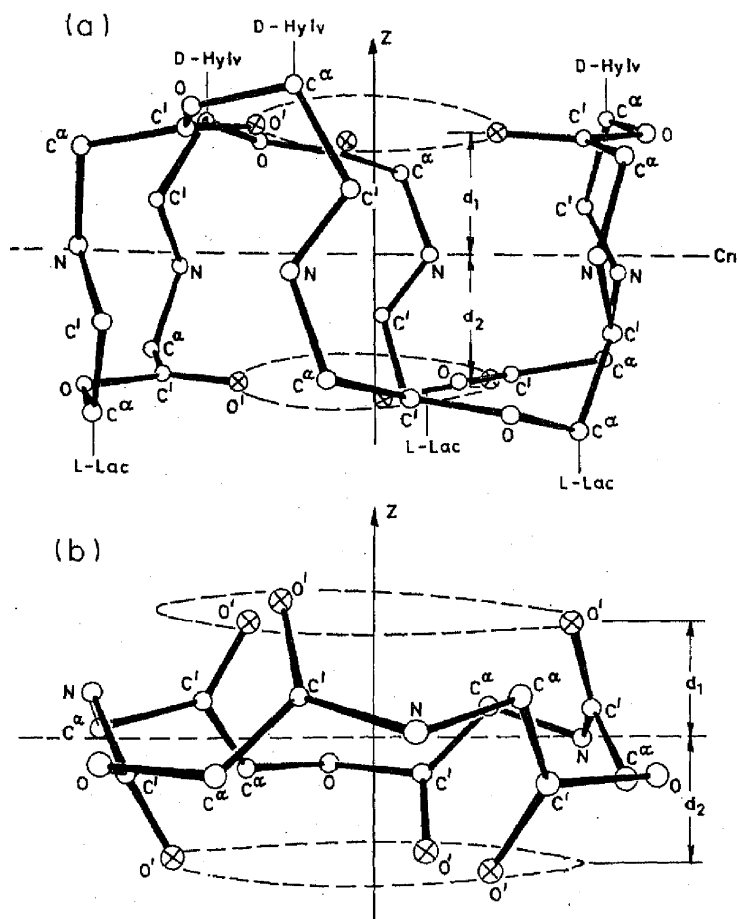


Figure 1. a. An ORTEP drawing of the bracelet like structure of valinomycin. The plane Cn passes through the centre of the molecule. The two planes containing liganding oxygens are at distances d_1 and d_2 , respectively from the plane Cn. Point of intersection of Z-axis and Cn is the origin. b. An ORTEP drawing of disk like structure of enniatin B. The 3 planes are identified similar to 'a'.

the plane containing 3 liganding carbonyl oxygens on a given side. However this is not so in Val4 which corresponds to the uncomplexed crystal structure (Karle, 1975).

Results and discussion

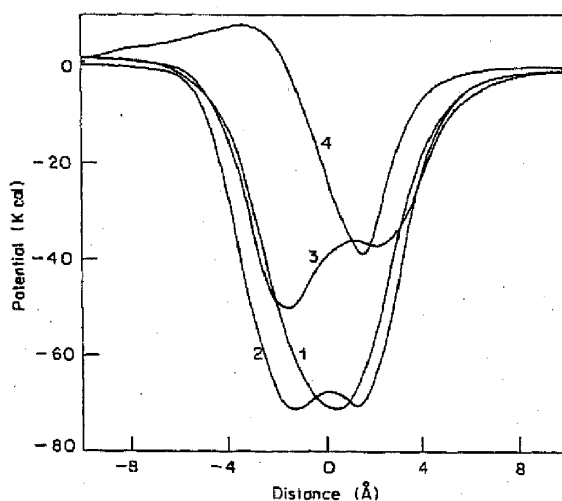
Studies on valinomycin

As mentioned above binding capacity of valinomycin to a cation is investigated. The changes in the potential energy profile of valinomycin-ion are investigated as functions of (i) conformational change, (ii) distance from the approaching ion-complex and (iii) conformation change and induction by an approaching ion-

Table 1. The position of ligands with respect to the centre of the molecule in different conformations of valinomycin and enniatin B.

Structure	Distance from the plane Cn to the plane of lig- ands (as in figure 1, in Å)		Distance from the centre of the molecule to the liganding oxygens (in Å)					
	d1	d2	O1	O2	O3	O4	O5	O6
Val1	1.4	1.4	2.82	2.78	2.73	2.68	2.77	2.71
Val2	1.9	1.9	2.72	2.70	2.70	2.71	2.74	2.69
Val3	2.5	1.9	3.56	3.60	3.52	2.72	2.76	2.68
Val4	—	—	3.54	2.07	4.90	4.84	3.55	2.10
Enn1	1.52	1.52	2.80	2.80	2.80	2.80	2.80	2.80
Enn2	1.18	1.18	2.81	2.81	3.00	2.81	3.00	3.01

complex in valinomycin dimer. The effect of conformational changes on the potential due to valinomycin is presented in figure 2, which shows the potential along the Z-axis. The minimum is deepest (about -70 Kcal as shown in curve 1) for Val1 in which the carbonyl oxygens are oriented towards the centre of the molecule. Distance d1 (same as d2) is smallest in this conformation when compared with the other conformations. Curves 2 and 3 in figure 2 correspond to Val2 and Val3. As the carbonyl oxygens start orienting away from the centre, the depth of the minimum decreases and two minima appear in the planes formed by the carbonyl oxygens. In val2 (d1 = d2 = 1.9 Å), the ion-ligand distances are the same in both the planes and therefore the depth of both the minima are equal. The asymmetry of the side chains has not influenced the binding energy primarily because of the hydrophobic nature of the valinomycin side chains and also they are placed at a considerably longer distance from the Z-axis in the bracelet like structure. However, the asymmetry of the minima is pronounced in val3 since the ion-ligand distances are different in the

**Figure 2.** A plot of electrostatic potential due to valinomycin along Z-axis. Curves 1, 2, 3 and 4 correspond to Val1, Val2, Val3 and Val4, respectively.

upper and the lower plane. The depth of the potential energy minimum is lowest (about -36 Kcal) in Val4 (curve 4, figure 2), which is a crystal structure of uncomplexed valinomycin. Although the general structure is bracelet like with the hydrophobic side chain on the outer side and the carbonyl ligands on the inner side, the structure is more asymmetric than the complexed structure. This is reflected in the binding energy curve. There is a single minimum inside the molecule, which is slightly towards the L-Lac side and the barrier is more at the D-HyIv side.

The conformations we have chosen here illustrate the effect on the binding energy profile approximately due to vibrations of the carbonyl groups. As the carbonyl groups open up, the binding capacity of valinomycin to a cation decreases considerably. However the ion can exist in the bound state even in the conformation Val4, corresponding to the uncomplexed crystal structure. The actual presence or absence of the ion in this particular conformation depends perhaps on the energy level in the potential well, the environment and the thermodynamics of the system.

An essential factor for the transport of an ion is the gradient. At the molecular level this can be visualised as induction due to an approaching ion-complex. An analogous situation in channels can arise due to multiple binding sites, where occupation of one site by an ion influences the binding at another site. Such studies on gramicidin channels have been carried out by Pullman and Etchebest (1983, 1984). Their recent studies (Etchebest and Pullman, 1986) include water molecules in the channel. In the following section we have attempted to study the inductive effect by carrier ionophores. Induction in carrier ionophores can manifest itself in two ways. The electric field generated by the ion-complex can either push another ion-complex as a whole or can influence expulsion of ion from the ionophore. We have discussed the second possibility in relation to valinomycin.

The induction studies on Val3 and Val1 are shown in figure 3. The following points emerge from these studies. (i) As expected the overall potential becomes more positive in all regions by the approach of an ion-complex. The increase in potential however is not symmetrical. It is greater on the side of the approaching ion-complex than on the other side. Such an asymmetry introduced in the potential profile favours the movement of the ion in direction of the gradient. Also the potential at the minimum is elevated to a greater extent than at the barrier site on the other side of the complex. This will produce a net effect of decreased binding of the ion, which facilitates its ejection from the binding site. (ii) The distance from the central ion in the inducing complex to the bound complex influences the potential profile to a greater extent. However the angle of the approaching ion-complex has very little effect on the binding energy profile as can be seen from figure 3 a. This leads us to conclude that the interaction between the dipoles of one valinomycin and another is negligible at the distances we have considered (shorter distances will lead to steric repulsion). Because of this result our further studies have dealt with only the head-to-tail approach of the inducing ion-complex.

The transport process described above deals with the binding-unbinding of an ion to a single molecule of valinomycin. However for an ion to move across a membrane of thickness $40\text{-}50$ Å, single carrier and relay mechanism models have been proposed (Simon and Morf, 1973). As mentioned above the two mechanisms differ in that one describes the movement of the ion-complex as a whole while the other describes the movement of a bare ion due to the field of an approaching ion-complex. Depending on factors like the thermodynamics of the system and the molecular dynamics of the

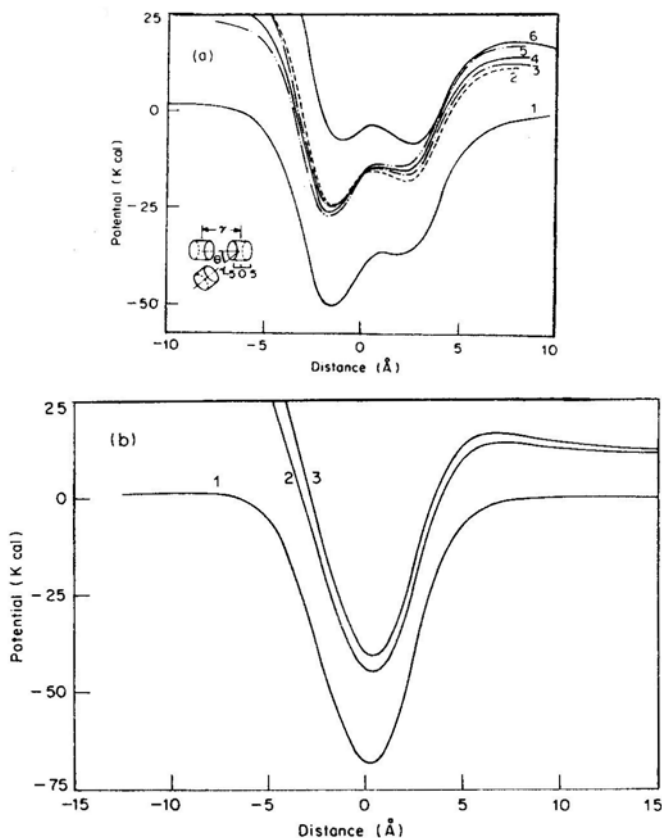


Figure 3. a, A plot of electrostatic potential along Z-axis of Val3 under the influence of an approaching ion-complex. The orientation of two molecules is given in the inset. ' r ' is infinity for curve 1, 15 Å for curves 2-5 and 10 Å for curve 6. ' θ ' is 30, 60, 90 for curves 3,4, 5 respectively and zero for other curves. **b**. A plot similar to 'a' of Val1, ' r ' is infinity, 15 Å and 12 Å for the curves 1, 2 and 3 respectively and ' θ ' is zero for all the curves.

concerned ionophore, the single carrier mechanism or relay mechanism or a combination of both can occur. Even in the case of a single carrier model the interaction between two valinomycins is invoked at the surface of the membrane (Ovchinnikov, 1979), so that the molecule sitting at the surface need not face a drastic change in the polarity of its environment.

The energetics of ion transfer is studied by considering the interaction between two valinomycin molecules. The interaction energy curves are given in figure 4. The interaction between two molecules is conformation dependent and is stronger in Val3 than in Val1. Also the two molecules approach closer to each other for maximum stabilisation in Val3. Thus the optimum distance (centre to centre) between the two molecules varies from 9.8 Å to 12.2 Å with stabilisation energies ranging from 3-10 Kcal, depending on the conformation. The presence of an ion in one of the molecules influences the interaction energies (curves 4 and 5 in figure 4) only to a small extent and the optimum distances do not vary much. This indicates the dominance of non-bonded interactions.

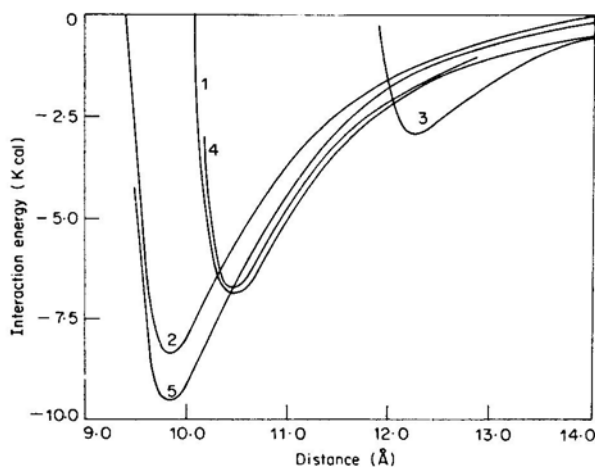


Figure 4. A plot of interaction energy between two valinomycin molecules. Abscissa represents the distance between two molecules. Curves 1, 2 and 3 correspond to Val2, Val3 and Val1, respectively. The interaction between a valinomycin and an ion complex is represented in curves 4 and 5 for Val2 and Val3, respectively.

The potential energy profiles of an ion along the Z-axis in dimers of valinomycin are depicted in figure 5. The dimerisation of Val1 (figure 5a) and Val4 (figure 5c) does not influence the barrier for the movement of the ion from one molecule to the other, whereas the barrier for ion movement is considerably reduced when Val3 dimerises (figure 5b). Further the induction by an approaching ion-complex will result in a positive potential at the barrier site for dimers of Val1 and Val4. On the other hand similar induction not only retains the negative potential in the barrier region for the dimer of Val3, but also reduces the difference between the potential energies at the binding and the barrier sites. These two effects combine to facilitate the transfer of the ion from one molecule to the other. The presence of barrier in the intermolecular region however suggests that ion prefers to exist in the middle of one of the valinomycins and perhaps 1:2 ion-complex is not observed due to this reason.

It is suggested that the action of ionophores like valinomycin can be considered to be that of an enzyme permease, catalysing transmembrane ion transport (Eisenman and Horn, 1983). The present studies suggest that the dimer molecule docked in proper conformation can behave like an activated complex catalysing the action of ion transfer. In this context it is interesting to note that Val4 (the uncomplexed structure) cannot form an activated complex catalysing the process of ion transfer.

Studies on enniatin B and comparison with the valinomycin system

Potential energy curves along Z-axis: Enniatin B is a smaller molecule than valinomycin and its conformational flexibility is limited. The studies similar to those on valinomycin are carried out on the complexed and the uncomplexed crystal structures of enniatin B (Dobbler, 1981), which are referred to as Enn1 and Enn2 in this paper. The potential along the Z-axis due to Enn1 and Enn2 is presented in figure 6. The minimum in Enn1 is -54 Kcal and that in Enn2 is -5.8 Kcal. This

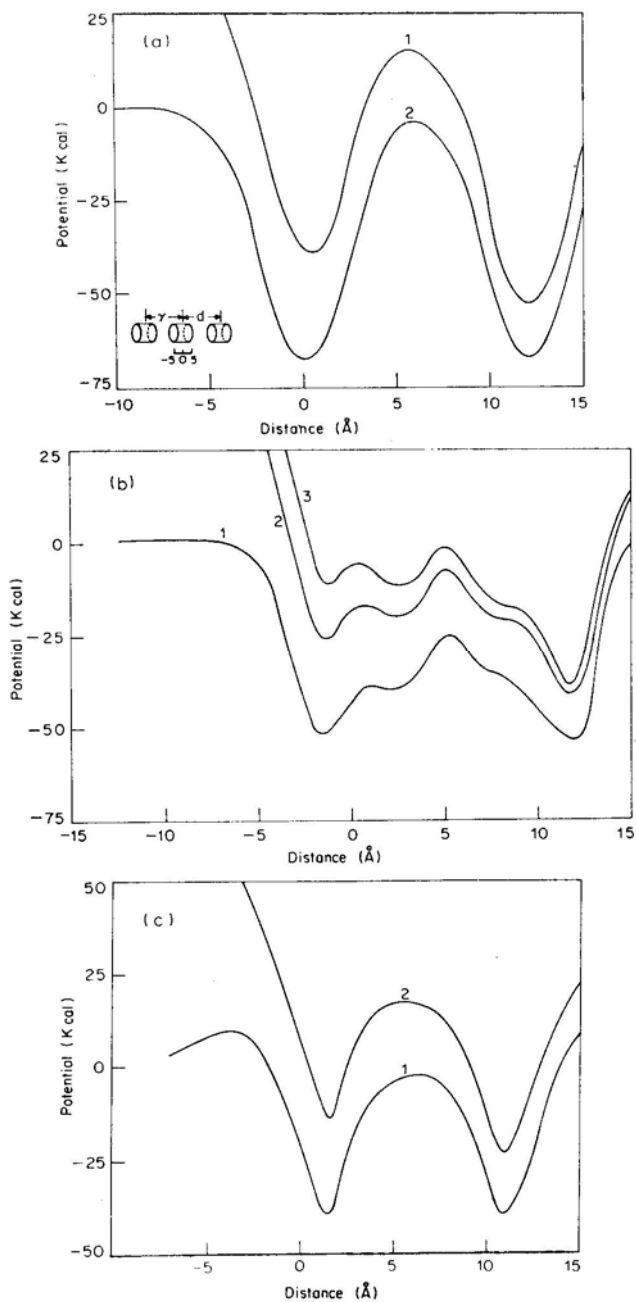


Figure 5. a. A plot of electrostatic potential along Z-axis of dimer of Val1 under the influence of an approaching ion-complex. Dimers are separated by the equilibrium distance d (12.2 Å) and r' is the distance between ion-complex and the nearest valinomycin molecule. r' is 15 Å and infinity for curves 1 and 2, respectively. b. A plot similar to 'a' of Val3. r' is infinity, 15 Å and 10 Å for curves 1, 2 and 3, respectively ($d = 9.8$ Å). c. A plot similar to 'a' of Val4. r' is infinity and 15 Å for curves 1 and 2 respectively ($d = 12.2$ Å).

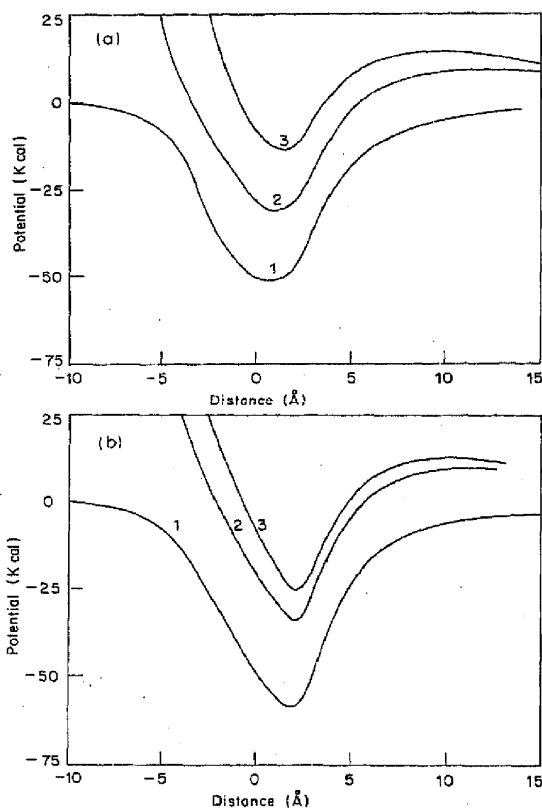


Figure 6. a. A plot similar to figure 3a of Enn 1. Curves 1, 2 and 3 have ' r ' being equal to infinity, 10 Å and 6 Å, respectively. ' θ ' is zero for all the curves. **b.** A plot similar to figure 3a of Enn2. Curves 1, 2 and 3 have ' r ' being equal to infinity, 14 Å and 6 Å respectively. ' θ ' is zero for all the curves.

marks an interesting departure from valinomycin system, where the binding capacity is more in the complexed crystal structure (Val1). Table 1 gives the distances from the centre to the liganding oxygens in valinomycin and enniatin B in several structures. It can be seen that the centre to oxygen distances are approximately of the same order in both conformations of enniatin B, whereas they differ considerably from each other in Val1 and Val4. This factor accounts for the nearly same binding energy for the complexed and the uncomplexed enniatins and the reduced binding energy for the uncomplexed valinomycin in comparison to its complexed structure. These results suggest that the conformational flexibility is operative to a greater extent in valinomycin than in enniatin B to provide the ion with better binding in its complexed structure. In the case of enniatin B the binding is not so specific as in the case of valinomycin, a factor which is probably responsible for the observed low specificity of enniatin B when compared with valinomycin (Dobbler, 1981).

The dimerisation and polymerisation of enniatin B is quite favourable for ion binding. The interaction energy between two enniatin B molecules are shown in figure 7. Curves 1 and 2 correspond to the dimer with an ion and without an ion,

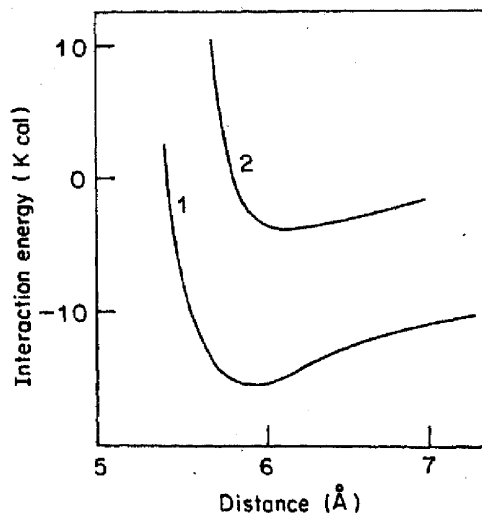


Figure 7. A plot of interaction energy between two molecules of Enn1. Abscissa represents the distance between two molecules. Curve 1 represents interaction energy between Enn1 and an ion-complex of Enn1, whereas curve 2 represents that of two neutral Enn1 molecules.

respectively. The optimum distance for two enniatin B molecules to interact is 6Å. The stability of the dimer is greater when an ion present in one of the molecules. This marks an interesting difference from the valinomycin case where the presence of the ion has very little effect on dimerisation. There is also a difference in the ion-ionophore binding energy profile of the two ionophores. The profiles of Enn1 and Enn2 are given in figure 8 and can be compared with those of ion-valinomycin binding energy (figure 5). The central path along the Z-axis shows a flat potential minimum in the case of enniatin B dimer and has a barrier in between two valinomycin molecules. This factor again is probably responsible for the lower specificity of enniatin B as compared to valinomycin and also, the possibility of 2:1 and 3:2 sandwich complexes in enniatin B (Ivanov *et al.*, 1973) can also be understood by the occurrence of the low barrier between the enniatin B molecules. The potential along the Z-axis of a trimer of Enn 1 is shown in figure 9. It is interesting to note that a flat minimum is present all along the Z-axis of the trimer (curve 1) resembling a channel. The induction by an approaching ion-complex from the negative Z-direction changes the potential profile (curves 2 and 3) in such a way that the ion is smoothly pushed in the positive Z-direction. This suggests that a relay mechanism is more favoured by enniatin B than by valinomycin.

Electrostatic potential studies: While the above studies focus on the ion binding ability of the ionophores, the overall potential surfaces can give an idea of the interaction between the ionophore and its surroundings. Sreerama and Vishveshwara (1985) and Etchebest *et al.* (1982) have reported some of the potential surfaces of valinomycin. In this section we present a few electrostatic potential surfaces of Val1 which were not reported earlier, as well as those of Enn1. A comparison of the potential surfaces of these two systems is carried out.

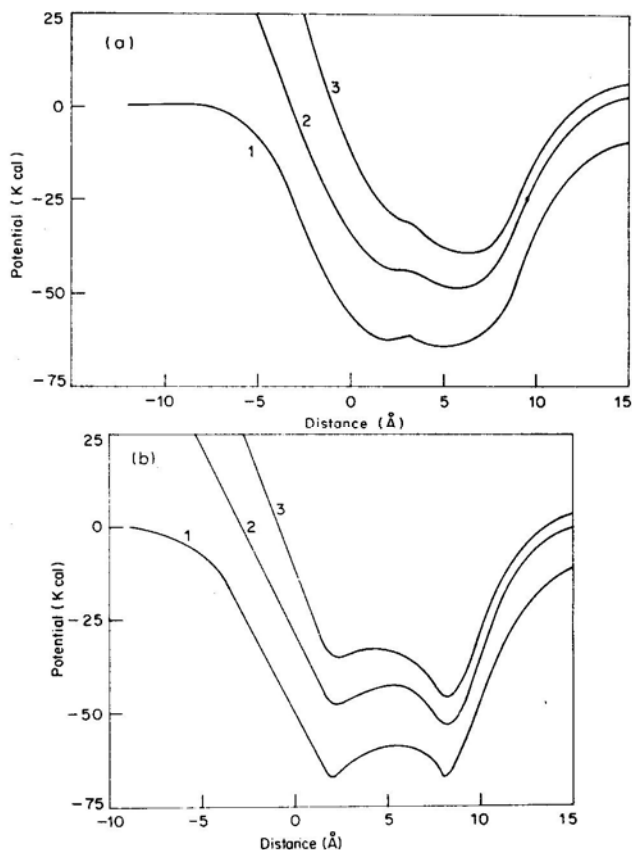


Figure 8. **a.** A plot similar to figure 5a of Enn1. Curves 1, 2 and 3 have ' r' ' being equal to infinity, 14 Å and 6 Å, respectively ($d = 6\text{Å}$). **b.** A plot similar to figure 5a of Enn2. Curves 1, 2 and 3 have ' r' ' being equal to infinity, 14 Å and 6 Å, respectively ($d = 6\text{Å}$).

The potential of Enn1 in the X-Y plane with $Z = 0.0\text{ Å}$ (the plane C_n passing through the centre) and $Z = 1.2\text{ Å}$ (the plane containing the liganding oxygens) are presented in figure 10. A comparison of these surfaces with similar ones on Val1 (figure 2a-d of Sreerama and Vishveshwara, 1985) shows that the qualitative picture is almost the same in both the cases, with the minimum around the centre and the potential outside the molecule in the X-Y direction falling off rapidly to zero. When compared in their crystal structures the potential value at the minimum is greater in the case of valinomycin than in the case of enniatin B. The plots in the X-Z and Y-Z planes of Enn1 and Val1 are shown in figures 11 and 12, respectively. In both cases the potential on the outside of the bracelet falls off to zero immediately after the van der Waals contact region. This feature is favourable for the interaction with the hydrophobic medium. The potential plots along the inner side of the bracelet however are different for valinomycin and enniatin B. The negative potential due to Enn1 extends much beyond the height of the molecule (in the Z-direction), whereas in Val1 the potential reaches a near zero value almost at the edge of the molecule. Such a difference in the potential surface shows that a free molecule of valinomycin

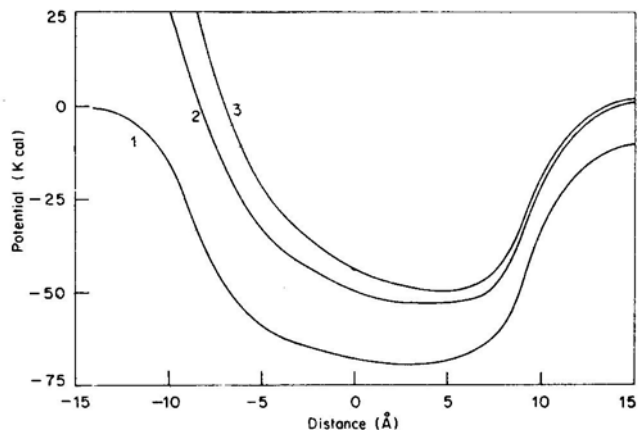


Figure 9. A plot of electrostatic potential of a trimer of Enn1 each molecule separated by 6Å, along Z-axis. Curves 1, 2 and 3 correspond to approaching ion-complex at infinity, 14 Å and 6 Å, respectively.

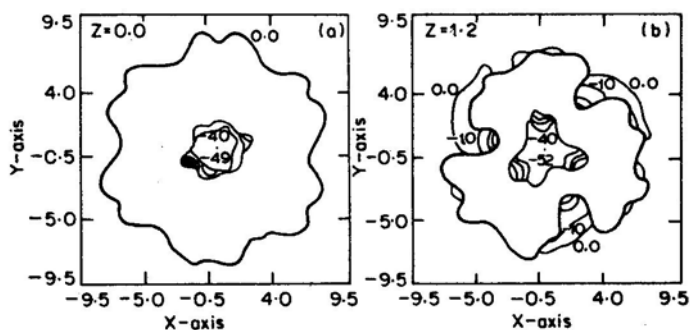


Figure 10. The electrostatic potential maps of Enn1 in (i) the C_n plane (with $Z = 0.0$) and (ii) the plane containing liganding oxygens (with $Z = 1.2$). X-axis and Y-axis are according to figure 1b. The contours are drawn at an interval of 10 Kcal and the large blank areas represent the van der Waals contact area ($< 1.2 \text{ \AA}$) in this figure and in figures 11 to 14.

can exist more comfortably in the hydrophobic medium than an enniatin B molecule. This may perhaps be responsible for the efficient action of valinomycin over enniatin B as an ion carrier across the membrane (Dobbler, 1981). As expected the potential surfaces of the ion-complexes are positive all around (figures 13 and 14).

In light of the above studies on the potential surfaces, the process of dimerisation of ionophores can be examined. When two molecules approach each other, they can do so until the van der Waals contact limit is reached. In the case of valinomycin, since the potential reaches zero around the edge of the molecule (figure 11), the approach of another valinomycin or ion-valinomycin complex is dominated mainly by the van der Waals forces. Therefore the process of dimerisation is affected to a very small extent by the presence of an ion in one of the molecules as shown in figure 4. On the other hand, negative potential due to enniatin B extends beyond the edge of the molecule (figure 12) and this influences the process of dimerisation. When two

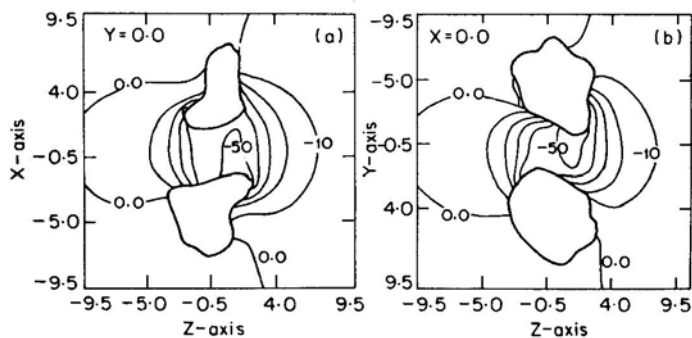


Figure 11. The electrostatic potential maps of Enn1 in (i) Y-Z ($X = 0.0$) and (ii) X-Z ($Y = 0.0$) planes.

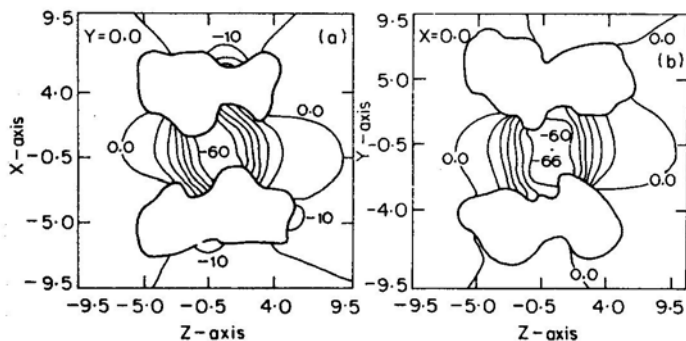
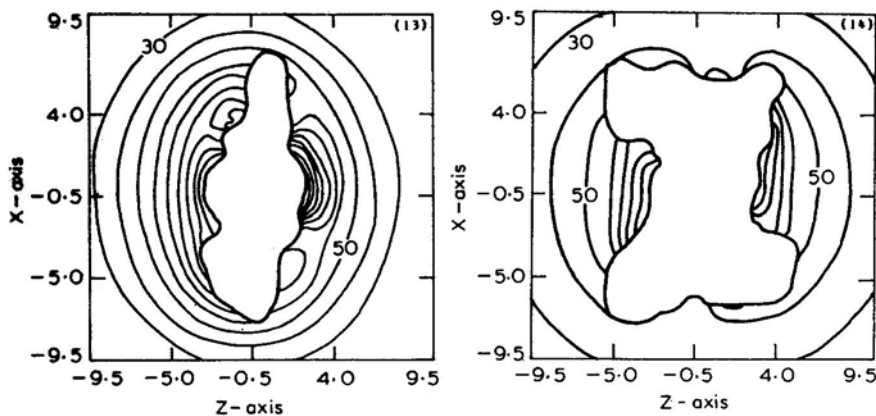


Figure 12. The electrostatic potential maps of Vall in (i) Y-Z ($X = 0.0$) and (ii) X-Z ($Y = 0.0$) planes.



Figures 13 and 14. 13. The electrostatic potential map of ion-complex of Enn1 in X-Z ($Y = 0.0$) plane. 14. The electrostatic potential map of ion-complex of Vall in X-Z ($Y = 0.0$) plane.

neutral molecules of enniatin B interact with each other the negative potentials due to carbonyl oxygens overlap at distances allowed by van der Waals contact limit and therefore the stability of enniatin B dimer is not as high as that of the valinomycin dimer (curve 2 in figure 7). However, when a neutral molecule and a cation-complex of enniatin B interact with each other, the negative potential due to the neutral molecule overlaps with the positive potential generated by the ion-complex and hence the dimer is considerably stabilised (curve 1 in figure 7). Thus the presence of an ion is crucial for the dimerisation of enniatin B and has very little effect on the approach of two valinomycin molecules.

Conclusion

The mechanism of facilitated transport by carrier ionophores is investigated at the molecular level. The electrostatic potentials and the ion-binding capacities of valinomycin and enniatin B are studied. The studies on the effects of conformational change, induction from an approaching ion-complex and dimerisation of the ionophore on its ion binding capacity have led to the following conclusions.

A suitable conformation and induction by the approaching ion is necessary for facilitated transport. Because of greater conformational flexibility, the binding-unbinding of ion to valinomycin is more selective than to enniatin B in accordance with the experimental results.

A comparison of the electrostatic potential profiles of the two molecules shows that an uncomplexed valinomycin can exist more comfortably in a hydrophobic medium compared to an uncomplexed enniatin B. This is in agreement with the higher biological activity of valinomycin. Also enniatin B can dimerise effectively in the presence of an ion which supports the existence of 1:2 ion-complex.

The presence of an intermolecular barrier for ion movement in valinomycin and its absence in enniatin B suggests that a relay mechanism is more favoured by the latter than by the former.

Acknowledgement

This work was supported in parts by the Council of Scientific and Industrial Research (New Delhi) grant for the theoretical studies on ion binding (Scheme no. 9(178)/84-EMR-II, feb 84).

References

- Dobblar, M. (1981) *Ionophores and their structures* (New York: Wiley).
- Eisenman, G. and Horn, R. (1983a) *J. Membr. Biol.*, **76**, 195.
- Eisenman, G. and Horn, R. (1983b) *J. Membr. Biol.*, **76**, 197.
- Etchebest, C., Lavery, R. and Pullman, A. (1982) *Stud. Biophys.*, **90**, 7.
- Etchebest, C. and Pullman, A. (1986) *J. Biomol. Struct. Dyn.*, **3**, 805.
- Fisher, W. and Brickman, J. (1983) *Biophys. Chem.*, **18**, 323. (References therein).
- Forneli, S. L., Vercauteren, D. P. and Clementi, E. (1984) *J. Biomol. Struct. Dyn.*, **1**, 1281.
- Gresh, N., Etchebest, C., De la Luz, Rozas, O. and Pullman, A. (1981) *Int J. Quantum Chem. Quantum Biol. Symp.*, **8**, 109.
- Gresh, N. and Pullman, A. (1982) *Int. J. Quantum Chem.*, **12**, 709.
- Hamilton, J. A., Sabesan, M. N. and Steinrauf, L. K. (1981) *J. Am. Chem. Soc.*, **103**, 5880.

- Hehre, W. J., Stewart, R. F. and Pople, J. A. (1969) *J. Chem. Phys.*, **51**, 2657.
- Ivanov, V. T., Eystratov, A. V., Sumskaya, L. V., Melnik, E. J., Chumburidze, T. S., Portnova, S. L., Balashova, T. A. and Ovchinnikov, Yu. A. (1973) *FEBS Lett.*, **36**, 65.
- Karle, I. L. (1975) *J. Am. Chem. Soc.*, **97**, 4379.
- Kim, K. S., Nguyen, H. L., Swaminathan, P. K. and Clementi, E. (1985) *J. Phys. Chem.*, **89**, 2870
- Kollman, P. A. (1981) in *Chemical Applications of Atomic and Molecular Potentials* (eds P. Politzer and D. G. Truhler) (New York, London: Plenum Press) p. 243.
- Laüger, P. (1982) *Biophys. Chem.*, **15**, 89. (References therein).
- Laüger, P. and Neumcke, B. (1973) in *Membranes* (ed. G. Eisenman) (New York: Marcel Deker) vol. 2, p. 1.
- Lee, W. K. and Jorden, P. C. (1984) *Biophys. J.*, **46**, 805.
- Mackay, D. H. J., Berens, P. H., Wilson, K. R. and Hagler, A. T. (1984) *Biophys. J.*, **46**, 229.
- Masut, R. A. and Kushik, J. N. (1984) *J. Comput. Chem.*, **5**, 336.
- Masut, R. A. and Kushik, J. N. (1985) *J. Comput. Chem.*, **6**, 148.
- Mitchell, P. (1961) *Nature (London)*, **191**, 144.
- Mitchell, P. (1979) *Eur. J. Biochem.*, **95**, 1.
- Momany, F. A., McGuire, R. F., Burgess, A. W. and Scheraga, H. A. (1975) *J. Phys. Chem.*, **79**, 2361.
- Monoi, H. (1983) *J. Theor. Biol.*, **102**, 69.
- Nagle, J. F. and Nagle, S. T. (1983) *J. Membr. Biol.*, **74**, 1.
- Ovchinnikov, Yu. A. (1979) *Int. J. Biochem.*, **94**, 321.
- Ovchinnikov, Yu. A., Ivanov, V. T. and Shkrob, A. M. (1974) *Membrane Active Complexones* (Amsterdam: Elsevier).
- Parsegian, A. (1969) *Nature (London)*, **221**, 844.
- Parsegian, A. (1975) *Ann. N.Y. Acad. Sci.*, **264**, 161.
- Pullman, A. and Etchebest, C. (1983) *FEBS Lett.*, **163**, 199.
- Pullman, A. and Etchebest, C. (1984) *FEBS Lett.*, **170**, 191.
- Scrocco, E. and Tomasi, J. (1973) *Top. Curr. Chem.*, **42**, 95.
- Simon, W. and Morf, W. E. (1973) in *Membrane* (ed. G. Eisenman) (New York: Marcel Decker) vol. 2, p. 329.
- Sreerama, N. and Vishveshwara, S. (1985) *J. Biosci.*, **8**, 315.
- Sundaram, K. and Tyagi, K. S. (1978) *Int. J. Quantum Chem.*, **13**, 17.
- Warshel, A. (1979) *Photochem. Photobiol.*, **30**, 285.
- Williams, R. J. P. (1974) *Ann. N. Y. Acad. Sci.*, **227**, 98.