THE PHYSICOCHEMICAL BASIS OF THE FUNCTIONING OF BIOLOGICAL MEMBRANES: DYNAMIC CONFORMATIONAL PROPERTIES OF ENNIATIN B AND ITS K^+ COMPLEX IN SOLUTION

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Received September 15, 1969

Depsipeptide antibiotics (valinomycin, enniatins, etc.) capable of increasing specifically ion permeability of artificial and biological membranes are presently widely used as effective chemical tools for studying membranes[1-3]. We have shown earlier that functioning of membrane-active cyclodepsipeptides and, particularly, their ability to bind alkali cations in different media are determined to a great extent by conformational factors[4]. In the course of these studies we were able to find the conformations of valinomycin and its K^+ complex in solution, using a wide variety of physicochemical techniques[5]. A similar approach was used in this investigation for conformational studies of enniatin B (I) (Fig. 1), the most popular member of enniatin series, and its K^+ complex.

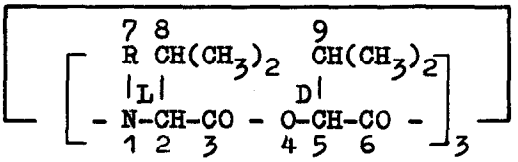

Fig.1. Enniatin B(I,R=CH_3) and (tri-N-desmethyl)-enniatin B (II,R=H)

ORD studies of (I) in several solvents (Fig. 2a) indicate

an existence of conformational equilibrium, its point depending upon the solvent. One conformer (N) with a strong negative Cotton effect at 230 nm is predominant in non-polar solvents (heptane); as the polarity is gradually increased the equilibrium is shifted until a new conformer (P) becomes predominant with a weak positive (~240 nm) and a strong negative (~200 nm) Cotton effects. It is noteworthy that the conformation of the K^+ complex of (I) is of the same type as that of the non-complexed antibiotic in polar solvents (trifluoroethanol) as follows from the similarity of corresponding ORD curves (Fig. 2a).

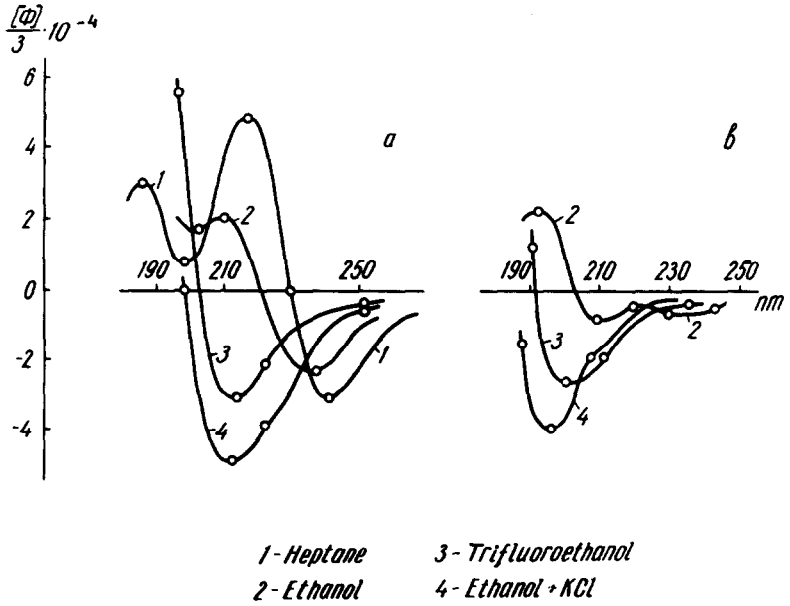

Fig. 2. ORD curves of enniatin B (a), (tri-N-desmethyl)-enniatin B (b) and their K^+ complexes

On cooling a solution of (I) in CS_2 or (2:1) CS_2-$CD_3C_6D_5$ mixture the N-methyl signal in the NMR spectra splits into three singlets of equal intensity; similarly the N-methylvalyl α-proton doublet splits into three equal doublets, the middle one overlapping with the α-proton doublet of the hydroxy acid (Fig.

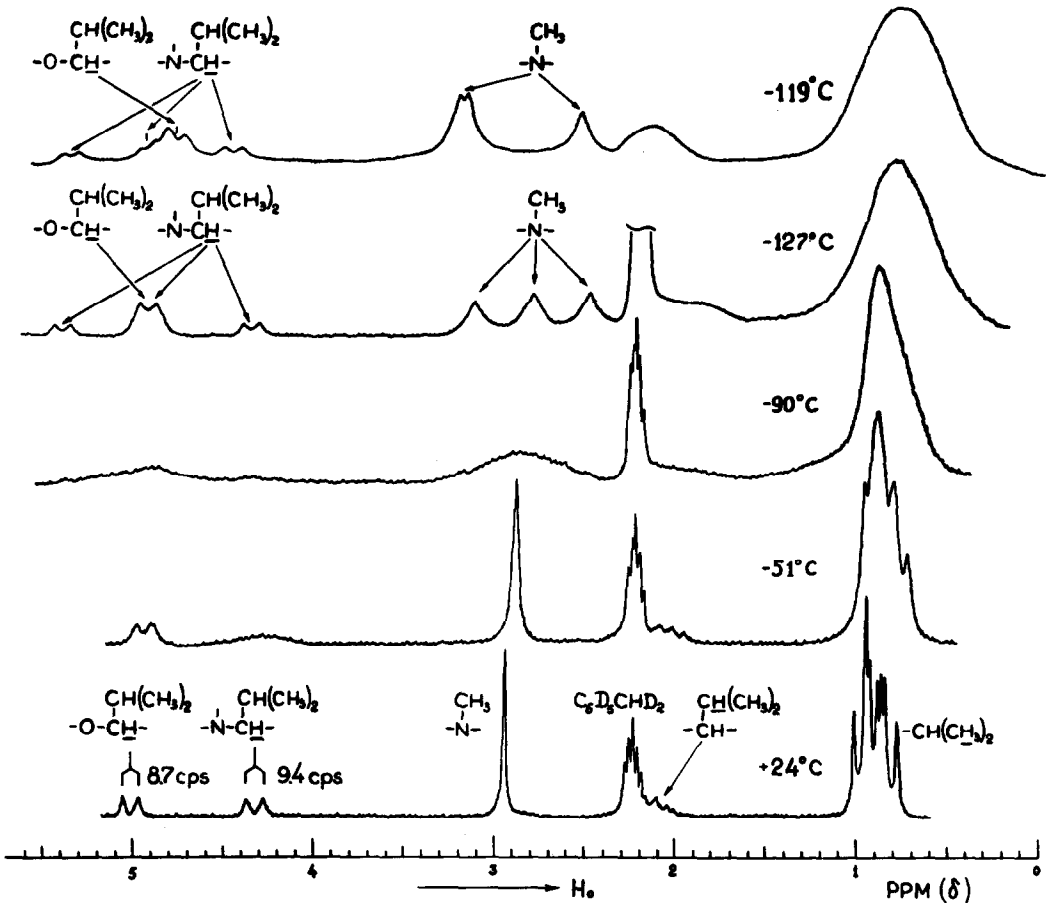

Fig. 3. NMR spectra of enniatin B in CS_2 at -119° (upper spectrum) and in CS_2-$CD_3C_6D_5$ (2:1) at different temperatures (lower spectra)

3)*. From this it follows that although (I) is built up of three structurally identical subunits all three N-methylvaline fragments in the conformer (N) have different spatial structure due to differences in rotation about N-C^{α} and C^{α}-C' single bonds (i. e. in the Φ and Ψ coordinates on the corresponding conformational maps).

*The NMR spectra were obtained on JNM-4H-100 spectrometer operating at 100 Mc/s. Concentrations of solutions were ~0.08 mole/l.

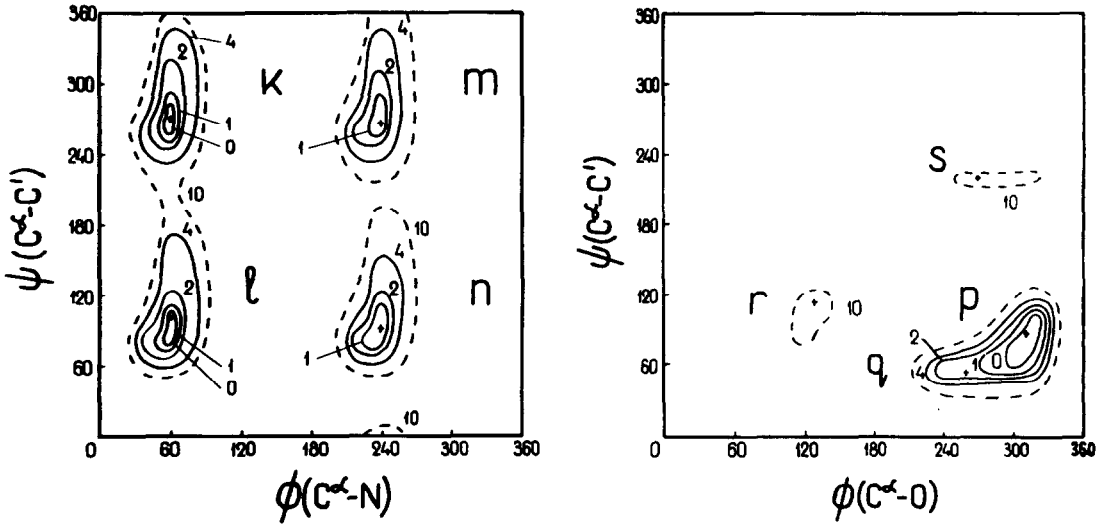

Fig.4. Conformational maps of methyl N-acetyl-N-methyl-L-valinate (left) and O-acetyl-D-α-hydroxyisovaleryl-N-dimethyl amide (right)

In order to determine this unusual structure the conformational maps for MeCO-N(Me)-$\overset{L}{C}$H(CHMe$_2$)-COOMe (A) and MeCO-O-$\overset{D}{C}$H(CHMe$_2$)-CONMe$_2$ (B) were computed by an earlier described method[6], (A) and (B) modelling the amino and hydroxy acid fragments of (I) (Fig. 4). Using the minimization procedure by variation of the angles $\Phi_{C^\alpha-N}$, $\Phi_{C^\alpha-O}$, $\Psi_{C^\alpha-C'}$, $\chi_{C^\alpha-C^\beta}$ and ν_{N-Me} the favourable conformations of the molecules (A) and (B) have been calculated (Table 1)*. As follows from the above results there are four iso-energetic minima (k, l, m and n) an the potential surface of (A), whereas favourable conformations (p and

*Notation of Φ, Ψ and χ angles for amino or hydroxy acid residues (of both, L and D configuration) is used according to the convention[7]; ν refers to rotation about N-Me bond. Conformational maps for (A) and (B) are calculated under the assumption of $\chi = 300^\circ$ and $\nu = 0^\circ$ or 60° (i. e. with cis or gauche conformations about N-Me bonds).

Table 1. Low-energy conformations of models A and B

A						B					
Local minima	Φ	Ψ	χ	ν	E $\frac{\text{kcal}}{\text{mole}}$	Local minima	Φ	Ψ	χ	ν	E $\frac{\text{kcal}}{\text{mole}}$
k	61	270	304	67	0	p	311	90	303	55	0
l	56	104	301	37	0.1	q	260	50	303	64	0.3
m	241	266	301	60	0.2	r	128	120	313	74	3.5
n	238	89	304	60	0.1	s	262	220	315	37	5.3

q) of (B) separated by a barrier of only 0.3 kcal/mole form one low energy area with Φ (260-320)°, Ψ (50-100)°. The further analysis shows that provided D-α-hydroxyisovaleryl residues of (I) have $B_{p,q}$ conformation there exist only two conformations of (I) with the orientations of N-methylvaline residues corresponding to potential minima on the conformational map of (A) (Fig. 5; Φ and Ψ values are approximate).

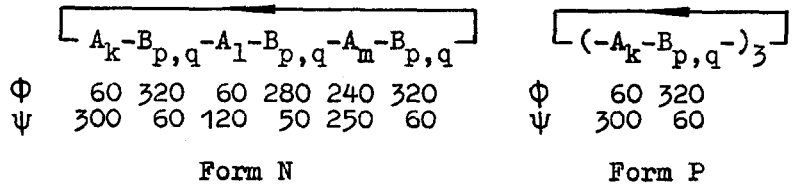

Fig. 5. Preferred conformations of enniatin B

Taking into account the NMR data (Fig. 3) it is natural to assume that first of these two conformations should refer to the form N; an excellent agreement of the calculated (3.5±0.5 D) and measured in CCl_4 (3.35±0.1 D) dipole moments served as a final proof for this structure. Large spin-spin coupling constants of the C^{α}-C^{β} protons in the form N (8.7-9.9 cps in CCl_4, C_6H_6 and CS_2) indicated on their preferable trans orientation, in accord with theoretical analysis (Table 1). This compact structure which has neither a central cavity nor symmetry elements is shown on the Fig. 6. The pseudo-axial orientation of the three adjacent isopropyl groups (fragment A_l-$B_{p,q}$-A_m, at the rear on

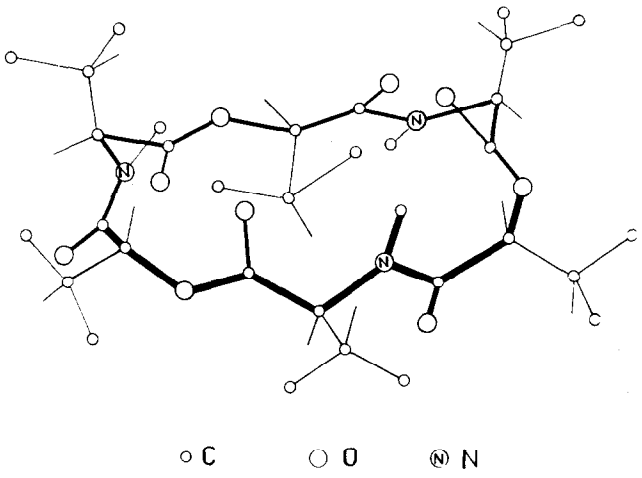

Fig. 6. Conformation of enniatin B in non-polar solvents (form N)

Fig. 6) and pseudo-equatorial orientation of three other isopropyl groups (fragment $B_{p,q}$-A_k-$B_{p,q}$, the front side on Fig. 6) is noteworthy.

The "complexing" conformation of (I) (form P) should be expected to possess, similarly to valinomycin[5], a central cavity capable of accomodating alkali cations, the six carbonyl groups pointing towards the interior of the molecule so as to provide efficient ion-dipole interaction with the central cation. These conditions are met by the second structure found in the course of theoretical analysis (Fig. 5). Therefore such conformation was assigned to the form P of (I), this assumption having been confirmed by NMR studies of the complexes of (tri-N-desmethyl)-enniatin B (II) with various univalent cations*. The use of this

*A similar ("flat disc") conformation has been ascribed to the K^+ complex of (I) by Mueller and Rudin[8] on the basis of general considerations; recently Dobler et. al.[9] were able to demonstrate that this conformation is also characteristic of the crystal (from X-ray analysis).

analog to determine the conformation of (I) itself was justified by the fact that according to the ORD curves (Fig. 2) the two cyclodepsipeptides assume the same conformation on both dissolution in polar solvents and complexation. In a comparison of the complexes of (I) and (II) with cations of varying size one would expect such complexes to differ in sizes of internal cavities formed by the oxygen atoms of the carbonyls participating in the ion-dipole interaction. Obviously the effective size of the cavity for a given ring is determined by orientation of the carbonyl groups which in turn depends upon the cation radius. With small cations the closest distance between them would be with the carbonyls drawn into the center of the molecule. As larger cations are taken all carbonyls are pushed more and more outwards in order to accomodate the cation so that the conformational changes ensuing could be likened to the opening of the flower bud. Analysis of the molecular models shows that for the form P of (II) such change in orientation of the carbonyl groups inevitably leads to simultaneous rotation of the CO-NH plane (Fig. 7), the ultimate result of which would be increase in the dihedral angle between H-N-C^{α} and N-C^{α}-H planes approximately from 130° to 160°, which change should be reflected in the NMR spectra by an increase of $^{3}J_{NH-CH}$ from 3.8 to 9.0 cps[10]. Indeed, the NMR spectra of (II) with Li^{+}, Na^{+}, K^{+} and Cs^{+} displayed monotonous increase in the $^{3}J_{NH-CH}$ constant (5.1, 6.5, 7.9 and 8.4 cps correspondingly), showing that complexes of (II) and, consequently, (I) are preferentially in P conformation. Further, from an analysis of the $^{3}J_{C^{\alpha}H-C^{\beta}H}$ coupling constants of the (I)-K^{+} complex it follows that the $C^{\alpha}H-C^{\beta}H$ protons in the methylvaline residues are <u>trans</u> ($^{3}J_{C_2H-C_8H}$=9.8 cps) whereas in the case of α-hydroxy<u>iso</u>valeryl residues the populations of rotational iso-

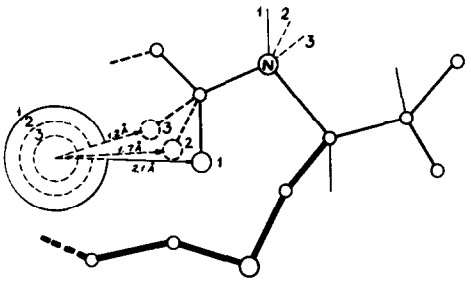

Fig. 7. Effect of the size of the cation on $^3J_{NH-CH}$ coupling constants of (tri-N-desmethyl)-enniatin B complexes

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mers about $C^{\alpha}-C^{\beta}$ bonds are essentially averaged ($^3J_{C_5H-C_9H}$=6.5 cps). The above data lead to the conformation of the K^+ complex of (I) as depicted on Fig. 8. Characteristic features of this conformation are the compact arrangement of the functional groups around the central cation, pseudo-equatorial orientation of all isopropyl groups and presence of symmetry axis; the exact shape of the "complexing" conformation is strongly dependent upon the radius of the cation.

These findings can serve as a basis for detailed analysis of the behaviour of enniatin cyclodepsipeptides in the complexa-

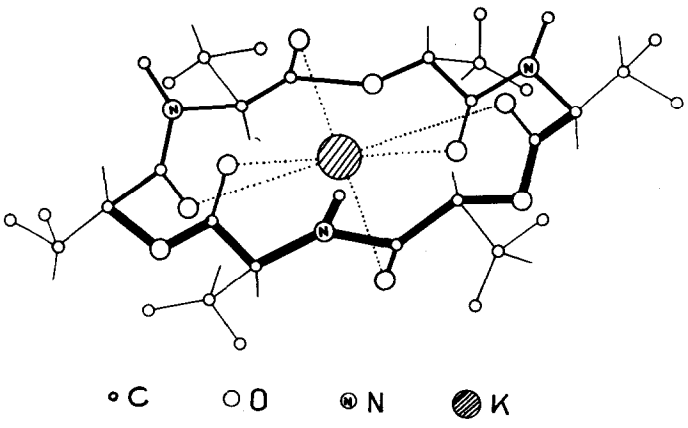

Fig. 8. Conformation of the K^+ complex of enniatin B

tion reaction as well as their functioning on the artificial and biological membranes (see [3]).

REFERENCES

1. B.C.Pressman, Proc. Natl. Acad. Sci. USA, 53, 1076 (1965)
2. D.C.Tosteson, P.Cook, M.Tiefenberg, J. Gen. Physiol., 50, 1949 (1967)
3. M.M.Shemyakin, Yu.A.Ovchinnikov, V.T.Ivanov, V.K.Antonov, E.I. Vinogradova, A.M.Shkrob, G.G.Malenkov, A.V.Evstratov, I.A. Laine, E.I.Melnik, I.D.Ryabova, J. Membr. Biol., in press
4. M.M.Shemyakin, Yu.A.Ovchinnikov, V.T.Ivanov, V.K.Antonov, A.M. Shkrob, I.I.Mikhaleva, A.V.Evstratov, G.G.Malenkov, Biochem. Biophys. Res. Commun., 29, 834 (1967)
5. V.T.Ivanov, I.A.Laine, N.D.Abdullaev, L.B.Senyavina, E.M.Popov, Yu.A.Ovchinnikov, M.M.Shemyakin, Biochem. Biophys. Res. Commun., 34, 803 (1969)
6. E.M.Popov, G.M.Lipkind, S.F.Arkhipova, V.T.Dashevskiy, Mol. Biol. (USSR), 2, 622 (1968)
7. J.T.Edsall, P.J.Flory, J.C.Kendrew, A.M.Liquori, G.N.Ramachandran, H.A.Scheraga, Biopolymers, 4, 121 (1966)
8. P.Mueller, D.O.Rudin, Biochem. Biophys. Res. Commun., 26, 398 (1967)
9. M.Dobler, J.D.Dunitz, F.Krajewski, J.Mol.Biol., 42, 603 (1969)
10. V.F.Bystrov, S.L.Portnova, V.I.Tsetlin, V.T.Ivanov, Yu.A.Ovchinnikov, Tetrahedron, 25, 493 (1969)