THE PHYSICOCHEMICAL BASIS OF THE FUNCTIONING OF BIOLOGICAL MEMBRANES: THE CONFORMATION OF VALINOMYCIN AND ITS K^+ COMPLEX IN SOLUTION

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Earlier we had shown (Shemyakin et al., 1967 and 1968; Ovchinnikov et al., 1968) that the cation transport regulating action of depsipeptides (valinomycin (VM), enniatins, etc.) in artificial and biological membranes is associated with the ability of these compounds to form complexes with cations, the efficiency and high ion selectivity of the complexing reaction being essentially determined by the conformational states of the cyclodepsipeptides under the given conditions (see Shemyakin et al., 1968, and references therein). The important part played by conformational factors in the manifestation of biological activity also follows from the structure-activity relations in VM (Shemyakin et al., 1965 and 1966) and enniatin (Shemyakin et al., 1963a; Mikhaleva et al., 1968) series. The present study had as objective investigation of the conformational states in solution of the main transport inducing substance, VM and its K^+ complex. The study was carried out by the combined use of NMR, IR, ORD and dipole moment techniques which has the advantage over x-ray structural analysis of these compounds (cf. Agtarap and Chamber-

lin, 1967, and Kilbourn et al., 1967) in that it provides information on their conformational equilibria in different media as influenced by various factors.

VM is a 36 membered cyclodepsipeptide (Fig. 1) (Brockmann et al., 1963; Shemyakin et al., 1963b). No experimental data on its conformational states in solution have been reported (cf. Mathieson, 1959, and Warner, 1967).

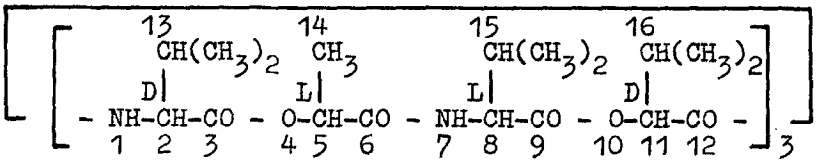

Fig. 1. Valinomycin

The ORD curves of VM (Fig. 2) vary with solvent polarity, which is indicative of equilibrium between conformers of this substance, the point of equilibrium being shifted with change in polarity. More light was shed on this equilibrium by compa-

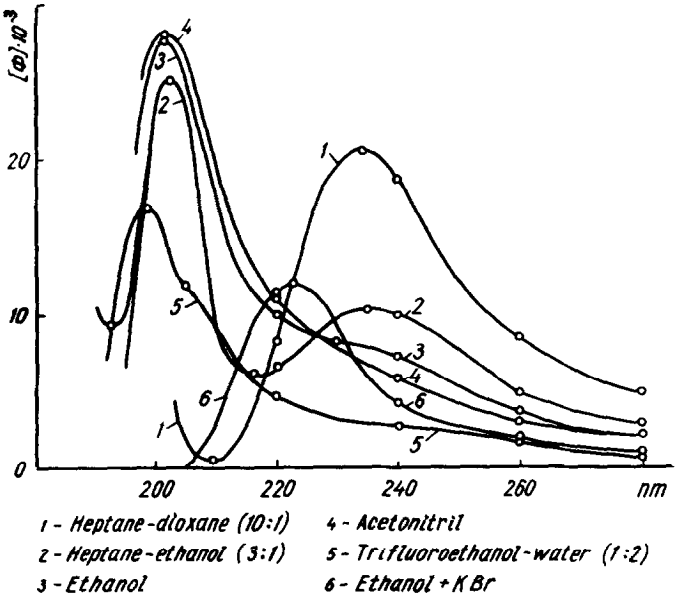

Fig. 2. ORD curves of valinomycin and its K^+ complex

rison of the NMR spectra of VM in CCl_4 and $(CD_3)_2SO$. One can clearly observe two pairs of doublets corresponding to two different types of NH signals ($N_{(1)}H$ and $N_{(7)}H$). In CCl_4 these signals are relatively close to each other (δ=7.90 and 7.76 ppm, respectively) whereas in $(CD_3)_2SO$ the $N_{(7)}H$ signal undergoes a low field shift of 0.6 ppm, $N_{(1)}H$ remaining practically unmoved. This served as basis for the proposal that in CCl_4 the preferred conformation of VM has 6 intramolecular H bonds formed by all NH groups while in $(CD_3)_2SO$ the $N_{(7)}H$ protons form H bonds with the solvent molecules. This proposal is in accord with IR spectral data of VM in CCl_4 and $CHCl_3$ solutions* which display strong band at 3307-3313 cm^{-1} due to intramolecularly H-bonded NH and a much weaker band at 3388-3395 cm^{-1} due to free NH groups. In the CO stretching region there is a symmetric band at 1755-1757 cm^{-1} due to non-hydrogen-bonded ester carbonyls. Amide carbonyls display a band at 1661 cm^{-1} with an inflection at 1678 cm^{-1} and a weaker band at 1540 cm^{-1}, corresponding to amide I and amide II bands. It is noteworthy that the presence of free and H-bonded amide groups are observed to about the same extent in the NH stretching frequency and amide I regions. All this bears witness to the fact that in low polar media VM is in an equilibrium mixture of two forms (Fig. 3), of which one (A) with all amide groups forming 6 intramolecular H bonds is clearly predominant, and the other (B) with only 3 such bonds increases in relative importance with increase in polarity of solvent.

It can be readily shown that for trans configuration of the

*IR spectra of VM in CCl_4 are concentration independent at least in the range $4\cdot10^{-2}$-$4\cdot10^{-4}$ mole/l showing the absence of intermolecular association, also demonstrated by thermoelectrical weight measurements in various solvents.

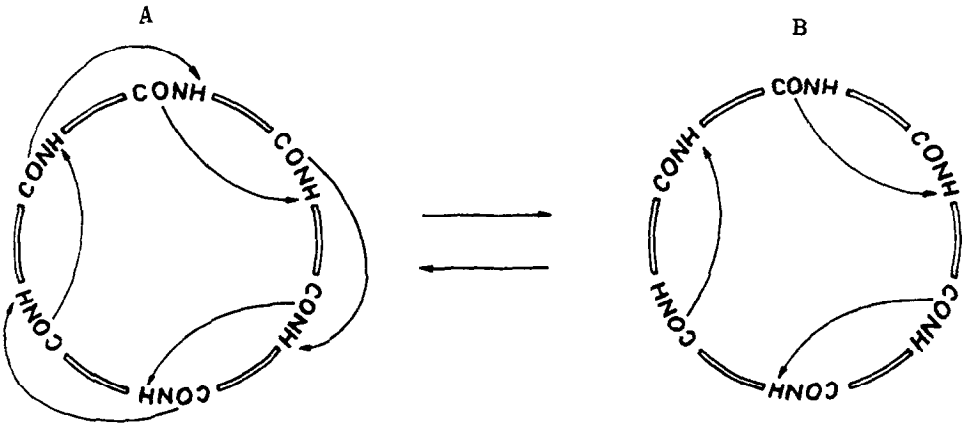

Fig. 3. Schematic representation of A ⇌ B equilibrium

amide and ester groups in VM the A conformation in which each carbonyl is involved in H bonding with the NH of the neighboring amide group in the "direction of acylation" is the only way by which all 6 amide groupings can participate in mutual H bonding. This is a quite rigid framework consisting of 6 condensed 10-membered rings formed by the H bonds, the whole resembling a "bracelet" ca. 8Å in diameter and 4Å high. However, the experimental data described above fit well both the conformation A_1 and its inside-out counterpart A_2 (Fig. 4), the latter differing

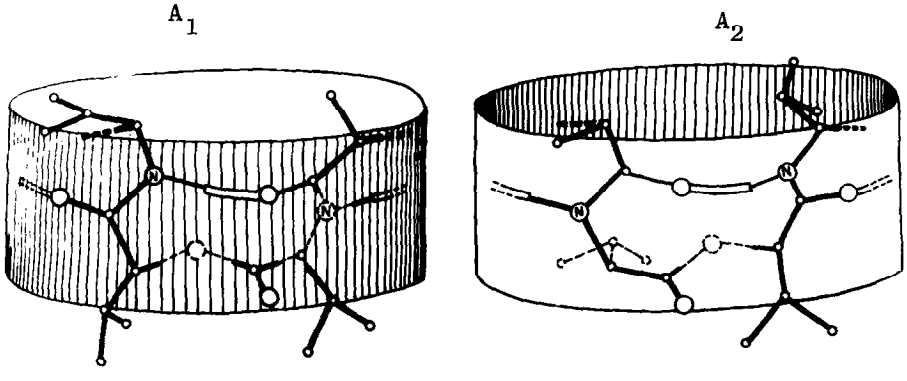

Fig. 4. Schematic representation of forms A_1 and A_2 of valinomycin

from the former in the chirality of the ring system and in orientation of the side chains. The choice between the A_1 and A_2 "bracelet" conformations of VM was made on the basis of NMR data. It can be seen from molecular models that the H-N-C_α and N-C_α-H dihedral angle of the HN-C_αH fragments in the A_2 conformation can vary within the limits 80-120°, which according to our data (Bystrov et al., 1969) should correspond to a spin-coupling constant $^3J_{NH-CH}$=0.5-3.5 cps. However, the experimental $^3J_{NH-CH}$ coupling constants are within the limits of 6.7-9.1 cps (depending on the solvent, see Table 1). This not only excludes the A_2 conformation, but shows that one group of NH-C_αH fragments in the A_1 conformation ($^3J_{N_{(7)}H-C_{(8)}H}$=6.7 cps in CCl_4) are gauche oriented, while the other ($^3J_{N_{(1)}H-C_{(2)}H}$=8.4 cps in CCl_4) are cis oriented. From this the spatial structure of the 6 ester groups in VM could be determined as 3 within and 3 without the ring. Finally from an analysis of the spin-coupling constants in CCl_4 it follows that the L and D valyl side chains have trans

TABLE 1. Spin-spin coupling constants of VM and its K^+ complex*

Fragment \ Solvent	CCl_4	$(CD_3)_2SO$	CD_3OD (CH_3OH)	CD_3OD+KCNS (CH_3OH+KCNS)	CH_3CN	CH_3CN+KCNS
$C_{(5)}H-C_{(14)}H_3$	6.8	6.2	7.0	7.1	7.0	7.0
$C_{(2)}H-C_{(13)}H$	10.0	-	7.1 or 7.6	3.5	8.7	4.8 or 5.1
$C_{(8)}H-C_{(15)}H$	10.0	-	7.6 or 7.1	3.5	9.8	5.1 or 4.8
$C_{(11)}H-C_{(16)}H$	2.9	3.8	4.4	3.8	3.8	3.6
$N_{(1)}H-C_{(2)}H$	8.4	8.5	9.1	5.8	8.3	5.4
$N_{(7)}H-C_{(8)}H$	6.7	8.1	8.8	5.8	7.5	5.4

*Accuracy of measurements ±0.1 cps. The $^3J_{NH-CH}$ constants have been corrected for the electronegativity of the substituents (+0.6 cps, see Bystrov et al., 1969).

oriented $C_{(8)}H$-$C_{(15)}H$ and $C_{(2)}H$-$C_{(13)}H$ protons and the D-α--hydroxyisovaleryl side chains, gauche oriented $C_{(11)}H$-$C_{(16)}H$ protons. We thus arrive at the conclusion that in non-polar solvents VM is preferrably in the "bracelet" conformation A_1, shown in Fig. 5. The dipole moment calculated for this conformation is 2.5±1.5 D, which is in good agreement with the experimental value of 3.5±0.1 D (in CCl_4), especially if one bears in mind

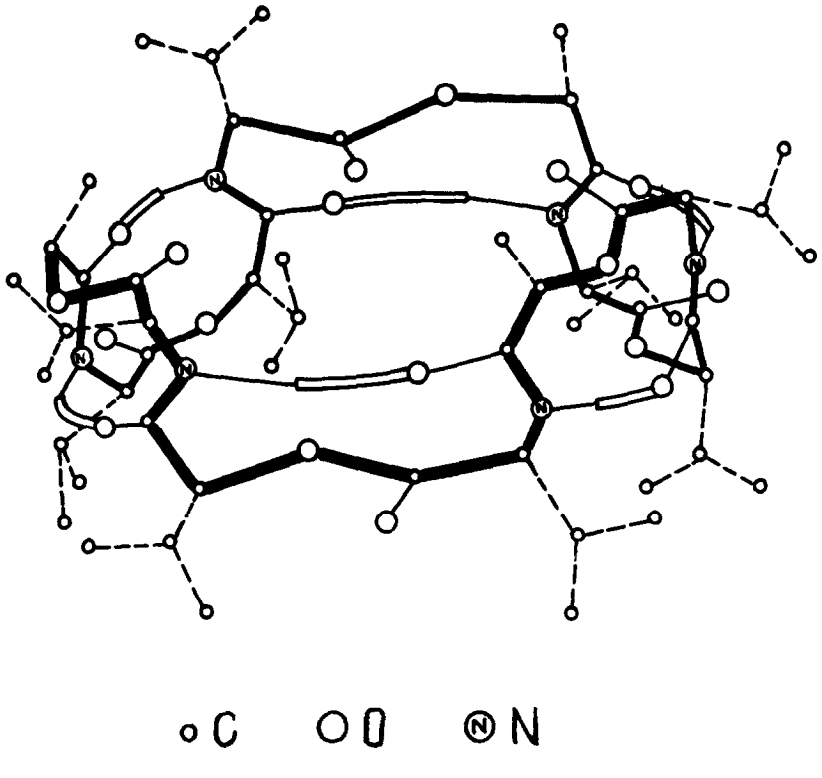

Fig. 5. Conformation of valinomycin in nonpolar solvents

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that VM solutions also contain a certain amount of the less symmetric B form (Fig.3). The exact conformation of the latter cannot be determined so unequivocally as A_1. However, from analysis of the corresponding coupling constants in polar solvents it follows that all NH-$C_\alpha H$ fragments are apparently cis oriented ($^3J_{NH-CH}$=

=7.5-9.1 cps), whereas the valine isopropyl groups are relatively free rotating (for instance, $^{3}J_{C_{\alpha}H-C_{\beta}H}$=7.1-7.6 cps in CD_3OD).

Analogous physicochemical studies led to the conformation of the VM K^+ complex in solution. The IR spectra of this complex (in $CHCl_3$ or CCl_4-CH_3CN, 9:1) display no free NH band, whereas the ester CO stretching frequency is both narrowed and shifted by ca. 20 cm^{-1} in the direction of longer wavelengths (in comparison with VM under similar conditions). This indicates that the H-bonded framework of the A_1 conformation of VM is retained in the complex, but all ester carbonyls are now involved in ion-dipole interaction with K^+. Complexation is accompanied by conformational changes in the VM molecule such that the 3 formerly outlooking ester carbonyls are now oriented towards the middle of the ring, which contains the cation, to form a hexagonal system of oxygens around the latter. The ORD curves of the K^+ complex differ sharply from those of both A (curve 1) and B (curves 3, 4) forms of VM (Fig. 2); changes in the polarity of the solvent (from ethanol to a 1:3 ethanol-heptane mixture) do not affect the shape of the curve, evidence of the rigidity of the system. The NMR spectra of the complex indicate a gauche orientation for all six NH-CH fragments ($^{3}J_{NH-CH}$ values ~5.6 cps) which (as could be seen from molecular models) must have been a consequence of the reorientation of the ester groups. All these results correspond to a rigid symmetric conformation of the K^+ complex of VM as shown on Fig. 6. A dramatic feature of this conformation is that the K^+ ion and the system of H bonds are effectively shielded from solvent action by the hydrophobic branched side chains on the molecular periphery.

These findings lead one to regard in a new light the interaction of VM with membranes and the high ion selectivity of its transmembrane cation transport. They allow for a new approach to

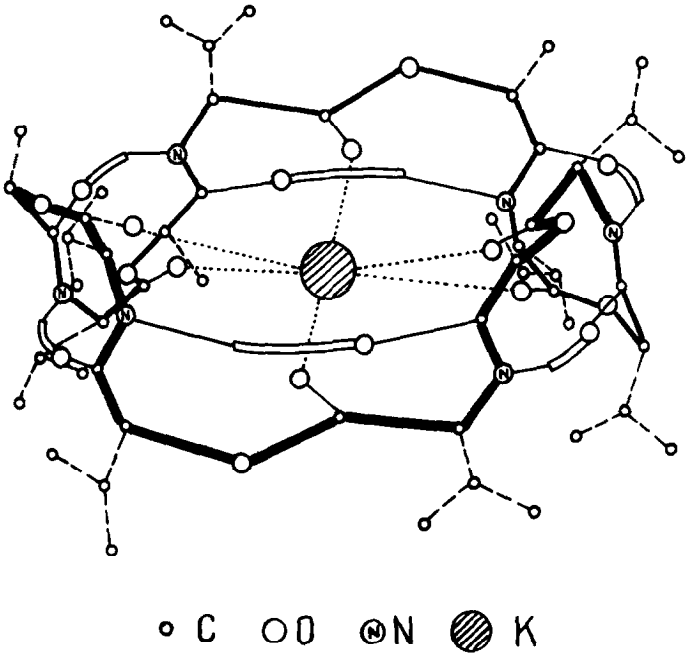

Fig. 6. Conformation of the K^+ complex of valinomycin

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the molecular mechanism of ion transport and to the problem of the structure-activity relation in depsipeptides.

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