THE PHYSICOCHEMICAL BASIS OF THE FUNCTIONING OF BIOLOGICAL MEMBRANES: CONFORMATIONAL SPECIFICITY OF THE INTERACTION OF CYCLODEPSIPEPTIDES WITH MEMBRANES AND OF THEIR COMPLEXATION WITH ALKALI METAL IONS

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In recent years it was established that certain compounds of depsipeptide and depside nature (valinomycin, enniatins, nactins ets.) are capable of specifically affecting active alkali metal ion transport through biological (mitochondrial) membranes (1,2). A more detailed investigation of this phenomenon with synthetic valinomycin and enniatin analogs we had synthesized showed that the effect of cyclodepsipeptides (CDP) on the ion permeability of the membrane correlates satisfactorily with their antimicrobial activity. On this basis the hypothesis was advanced that the biological action of CDP is associated with their ability to interact complementarily with specific, active cation transport controlling receptors in the cellular membrane (3-5). It was also natural to assume that the effect of CDP on the ion transport through membranes is due not only to their interaction with receptor sites of the membrane, but also with the corresponding cation, and this should be manifested in ability of CDP to form complexes with the alkali metal ions. In principle such

complexes could be formed either before or after the binding of a CDP to the membrane and elucidation of the nature and sequence of the interaction of the CDP with the membrane and ion would be an important step forward to understanding the molecular mechanism of the ionic permeability of biological membranes.

The purpose of the present work was to investigate the capacity of CDP to form complexes with alkali metal ions and to ascertain the extent to which various factors, in particular the conformational states of these compounds, affect this process.

The first objects of study were the enniatin antibiotics and some of their synthetic analogs; the conformational characteristics and biological properties of this class of compounds have long been the subject of our systematic investigation (Fig. 1 and Table 1) (3, 6-9).

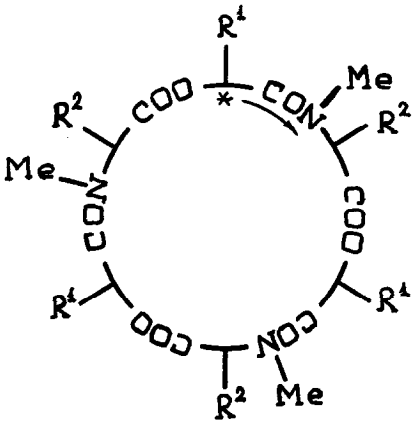

Fig. 1

Structure of the enniatin antibiotics: (1) enniatin A: DLDLDL, R^1=i-Pr, R^2=sec-Bu; (2) enantio-enniatin A: LDLDLD, R^1=i-Pr, R^2= sec-Bu; (3) enniatin B: DLDLDL, $R^1=R^2$=i-Pr; (4) enantio-enniatin B: LDLDLD, $R^1=R^2$=i-Pr; (5) enniatin C: DLDLDL, R^1=i-Pr, R^2=i-Bu; (6) "false" enniatin A: LDLDLD, R^1=sec-Bu, R^2=i-Pr.

Since the only chromophores in the CDP molecules investigated are the amide and ester carbonyls, the shape of the optical rotatory dispersion curves reflecting the spatial arrangements of the chromophores in the molecule, characterizes the conformational states of the systems in question. Comparison of the ORD curves of the biologically active enniatins A and B (1) and (3)

Table 1

Antimicrobial Activity of Cyclodepsipeptides

Compound	Minimal growth inhibiting concentration γ/ml					
	Staph. aureus	Sarcina lutea	Bac. mycoides	Mycob. phlei	Cand. albicans	Sach. cerevis.
Enniatin A*	1.5	2	4.5	1.5-2.5	6	6
Enniatin B*	9	18	25-37	9-12	9-12	9-12
Enniatin C	50	50	50	50	50	50
"False" enniatin A	25-37	-	-	37	-	-

* The enantiomer of this CDP has similar activity.

(Fig. 2) and also their enantiomers (2) and (4) showed that these compounds possess very similar, stable conformations, virtually unchanging on passing from non-polar to polar media. In non-polar media the practically inactive enniatin C (5) has a conformation close to that for enniatins A and B, but in polar

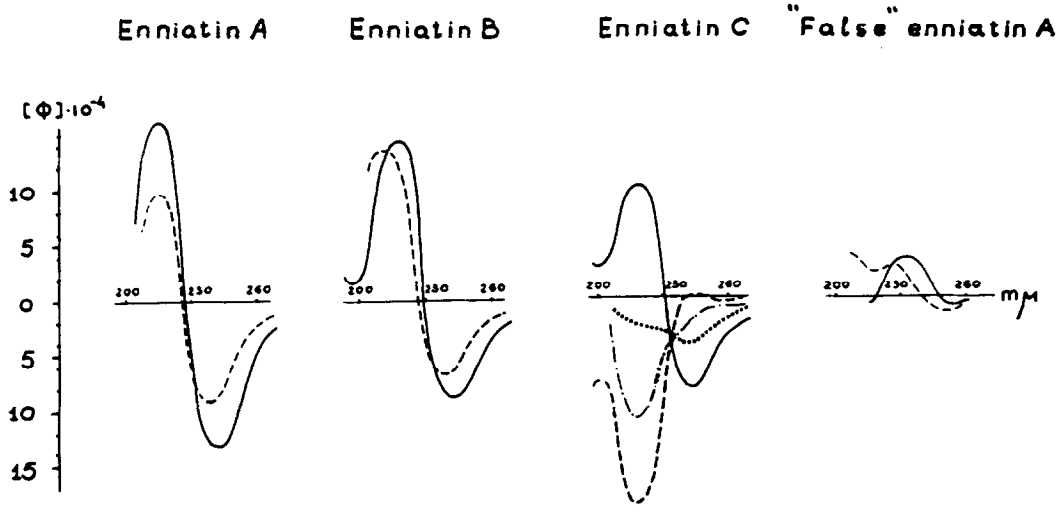

Fig. 2 ORD curves of cyclodepsipeptides (1), (3), (5) and (6)

——— heptane; ····· heptane-ethanol (94:6); -·-·- heptane-ethanol (92:8); ------ heptane-ethanol (50:50).

media (ethanol) its conformation is considerably different. Also the cyclodepsipeptide (6), possessing very low biological activity, has a conformation sharply differing from that of its structurally closely related enniatins A and B, and their enantiomers (Fig. 2). These findings lead to the conclusion that there must be a definite relation between the conformational states of the enniatin cyclodepsipeptides in solution and their antimicrobial activity.

It was also shown that the enniatins and a number of allied CDP are capable of forming complexes with alkali metal ions and a definite relation was found between the complexating properties of these compounds in solution, the specificity of their conformational characteristics under similar conditions and their biological action.

We observed the formation of CDP-cation complexes by three different methods. One was the spectropolarimetric method. We found that the dispersion curves of the initial compound and corresponding complex in many cases differ greatly, as can be seen on the example of the binding of K^+ by enniatin B in alcohol (Fig. 3). Since, enniatin C shows very similar ORD curves for the free and K^+ complexated forms (Fig. 3) it follows that the changes in the ORD curves in enniatin B are apparently due to conformational rather than electronic factors. The spectropolarimetric method not only permitted the complexation to be followed in the solution under varying conditions (temperature, concentration, solvent etc.), but also in a number of cases thermodynamic parameters of the reaction and the stability constant of the complex to be calculated.

Complex formation can be revealed also by the drop in electroconductivity of the 10^{-4} M absolute alcohol solutions of

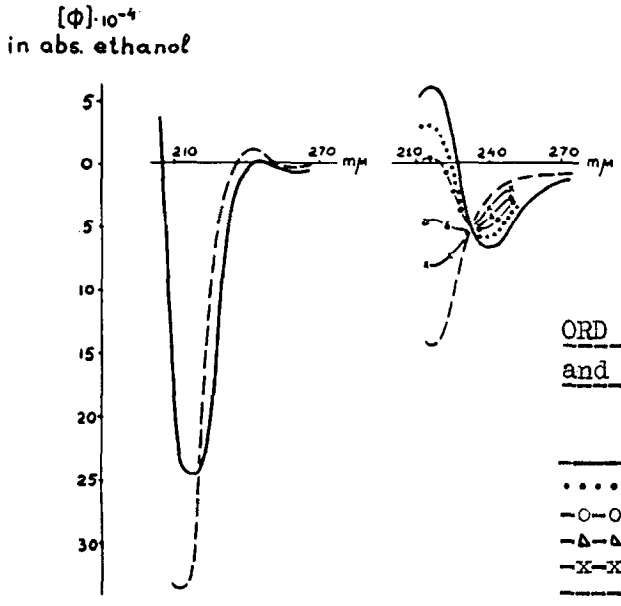

Fig. 3
ORD curves of enniatins C and B and their K^+-complexes

	CDP($M \cdot 10^4$)	KSCN($M \cdot 10^4$)
———	1.5	0
·····	1.5	0.75
-o-o-	1.5	1.50
-Δ-Δ-	1.5	3.25
-x-x-	1.5	7.50
———	1.5	30.00

alkali metal chlorides on addition of CDP due to lower mobility of the complexated cation. By means of this method the quantitative characteristics of the complexes can be easily obtained. Table 2 gives the mean values for the stability constants of the CDP complexes with various monovalent cations*, the free energies of formation calculated from these values and also the limiting mobilities of the complex ions and their effective Stokes' radii.

On comparing the characteristics of the various CDP there comes to the fore the exceptionally high specificity of complexation in the case of valinomycin, a fact which is apparently directly connected with high activity of this compound with respect to mitochondria and microorganisms.

Of considerable interest is the molecular structure of the complexes we have obtained. We were able to isolate in the indi-

* None of the compounds studied affects the electroconductivity of LiCl solutions.

vidual state from ethyl acetate solution the complexes of enniatins B and C with KSCN, which according to analytycal data, proved to be equimolecular. On comparing the IR spectra of these complexes with the spectra of the initial CDP a considerable bathochromic shift (ca. 30 cm^{-1}) is found to occur in the ester carbonyl band, which bears evidence of a strong ion-dipole interaction with the ester group. It may therefore be assumed that complexation occurs with participation of this group and that the complexes formed are similar to the ion complexes of the nactins (10).

Table 2

Characteristics of CDP-Ion Complexes

Compound	Cation*	$K \cdot 10^{-3}$ l/mol	$-\Delta F$ kcal/mol	Λ_{AM^+} cm^2/ohm·mol	r_s, Å
Enniatin B and enantio-enniatin B	Na	1.3	4.30	16.0	4.80
	K	3.7**	4.90	14.5	5.25
	Rb	4.0	4.95	14.5	5.25
	Cs	2.2	4.55	14.5	5.25
Enniatin C	Na	2.5	4.65	15.8	4.75
	K	5.5	5.15	14.4	5.20
	Rb	7.5	5.30	14.4	5.20
	Cs	4.1	4.90	14.4	5.20
"False" enniatin A	Na	0	-	-	-
	K	0	-	-	-
Valinomycin	Na	0	-	-	-
	K	5.2	5.10	***	***

* The stability constants (K) of the complexes were calculated by means of the formula:

$$K = \frac{\varphi}{([M]-\varphi)([A]-\varphi)} \qquad \text{where } \varphi = \frac{\Delta\kappa}{\delta}$$

[A] - concentration of CDP; [M] = [MCl] - concentration of salt; $\Delta\kappa$ - absolute change of specific electroconductivity; $\delta = \Lambda_{M^+} - \Lambda_{AM^+}$ - difference of mobilities of the free and complexated cations.

** This value coincides with that obtained by the spectropolarimetric method.

*** Accurate determination of this quantity is difficult due to very low mobility of the complex ion.

Both spectropolarimetric and conductimetric measurements show complexation to take place in the case of the naturally occuring enniatins A and B and their enantiomers (1) - (4) but not in the biologically little-active CDP (6). On the other hand the stability of the complexes of the practically inactive enniatin C is even somewhat higher than that of the highly active enniatin B. From this it follows that the antimicrobial activity of the CDP and their action on the mitochondria cannot be ascribed only to complexation. In all likelihood the difference in the biological activity of the CDP is due also to differences in their interaction with the receptor sites of the membrane. In this connection it should be stressed that according to our data the complexes display the highest stability in non-polar media, whereas complexation does not occur at all in water. On these grounds it may be assumed that the CDP form complexes only after binding to the membrane. At the same time the question of how the ion transport is carried out, i.e. whether transport occurs of the complexated ion, or whether interaction of the complex with the receptor site of the membrane provides for ion transport at some other site, requires further investigation. From this standpoint highly promising is a study of the effect of CDP on the ionic permeability of various artificial membranes, the first reports of which have appeared in the literature (11, 12). Elucidation of the actual ionic transport mechanism in biological systems can, of course, be achieved only by correlation of the physicochemical and conformational parameters of the CDP and their complexes with their behavior towards artificial and biological membranes and, in particular, their antibiotic activity. It is in this direction that we are now devoting our studies.

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