THE MOLECULAR STRUCTURE AND SOME TRANSPORT PROPERTIES OF VALINOMYCIN

by

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SUMMARY

The antibiotic valinomycin is well known to affect the ion transport behavior of mitochondrial systems. We have determined the molecular structure by X-ray crystallography of this cyclododecadepsipeptide as the potassium aurichloride complex, and find it to be wrapped around the potassium ion coordinated to the oxygen atoms of alternate carbonyl groups, held together by hydrogen bonding, and holding the anion less specifically. We have constructed a diffusion apparatus with which the anion-cation transporting properties of valinomycin can be demonstrated. An equation is given for the equilibrium situation.

INTRODUCTION

Valinomycin (Brockmann and Schmidt-Kastner, 1955) is a cyclododecadepsipeptide containing D-valine, D-α-hydroxyvaleric acid, L-valine, and L-lactic acid in the sequence (Shemyakin, et al., 1965) given in Figure 1. We have undertaken the molecular structure determination by X-ray crystallography in an attempt to find an explanation for the remarkable ability of valinomycin to enhance respiration in mitochondria (Moore and Pressman, 1964, and Pressman, 1968), and the strong preference of the antibiotic for potassium ions.

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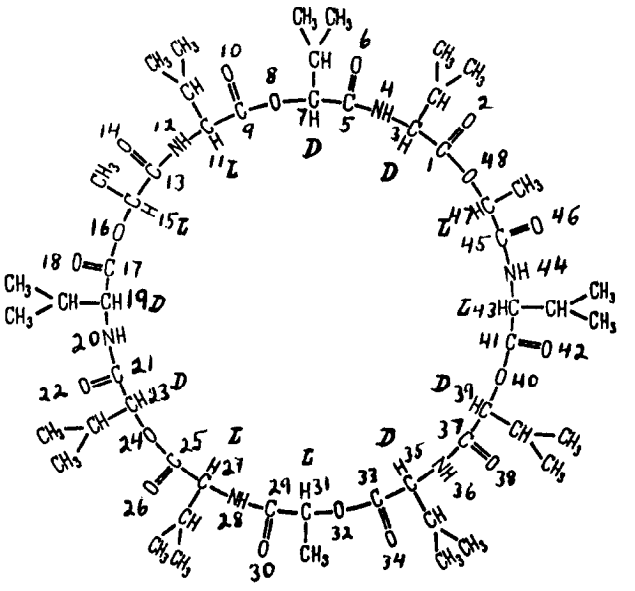

Figure 1. The sequence of valinomycin. Only the ring and carbonyl atoms are numbered.

RESULTS

Crystals of the potassium aurichloride, $KAuCl_4$, complex of valinomycin, were grown from solutions of an equal mixture of chloroform and m-xylene. The complex was made by shaking together a chloroform solution of valinomycin and an excess of an aqueous solution of potassium aurichloride. It could be seen that the color of the aurichloride was immediately taken up by the organic phase.

The small yellow needle shaped crystals were triclinic, space group P1, with one molecule of the $VKAuCl_4$ complex per unit cell. Cell dimensions were found to be $\underline{a}$ = 11.04, $\underline{b}$ = 16.06, c = 13.61 Å, α = 124.0°, β = 97.4°, and $\underline{\alpha}$ = 77.4°. We were able to measure approximately 2,100 independent reflections on the Supper-Pace automatic diffractometer. The structure was solved from an inspection of the Patterson synthesis, and the R value at present stands at 19% with refinement continuing. The potassium ion is coordinated to the carbonyl oxygen of each of the six valine residues.

The potassium to oxygen bond distances range from 2.7 to 2.8 Å. The antibiotic is folded as it circles the potassium, describing what resembles three complete sine waves. The folding is very regular and the antibiotic molecule has very close to three-fold symmetry. Six hydrogen bonds hold the six bends of the polypeptide together; the nitrogen atom of each of the six valines is involved with the carbonyl oxygen three residues away in a hydrogen bond which ranges from 2.8 to 3.0 Å in length. A drawing of the structure is given in Figure 2, in which some accuracy has been sacrificed for clarity. The aurichloride ion resides in a nearly spherical cavity formed by the top of one valinomycin molecule and the bottom of the next. It seems very likely to us that the hydrogen bonds are holding the polypeptide chain in the proper three-dimensional conformation to accept and contain the anion and cation. Indeed, the crystal form of the uncomplexed valinomycin bears a strong resemblance to the $VKAuCl_4$ form reported here.

We had observed while making the $VKAuCl_4$ complex that both the proper anion and cation had to be present, otherwise the complex would not form. This suggested to us that some of the biological properties of valinomycin could be explained on the basis of transport of an anion-cation pair into or through non-aqueous regions of the cell. Pressman (1968) has arrived at similar conclusions independently from considerations based on the biological properties of valinomycin. To investigate this behavior we constructed a device made of two aqueous solutions separated by a non-aqueous barrier, chloroform, by filling a U-tube, 2 cm inside diameter and 10 cm high, with chloroform to just above the bend, and filling the arms with aqueous solutions. The chloroform barrier was stirred gently with a small magnetic stirring bar. When 0.5 mg (5×10^{-7} mole) of valinomycin was dissolved in the chloroform, the system could transport suitable anion-cation pairs in the manner shown in Figure 3. We soon found the picrate anion to be far more satisfactory than aurichloride, and that the picrate was transported in response not only to its own concentration gradient but also in response

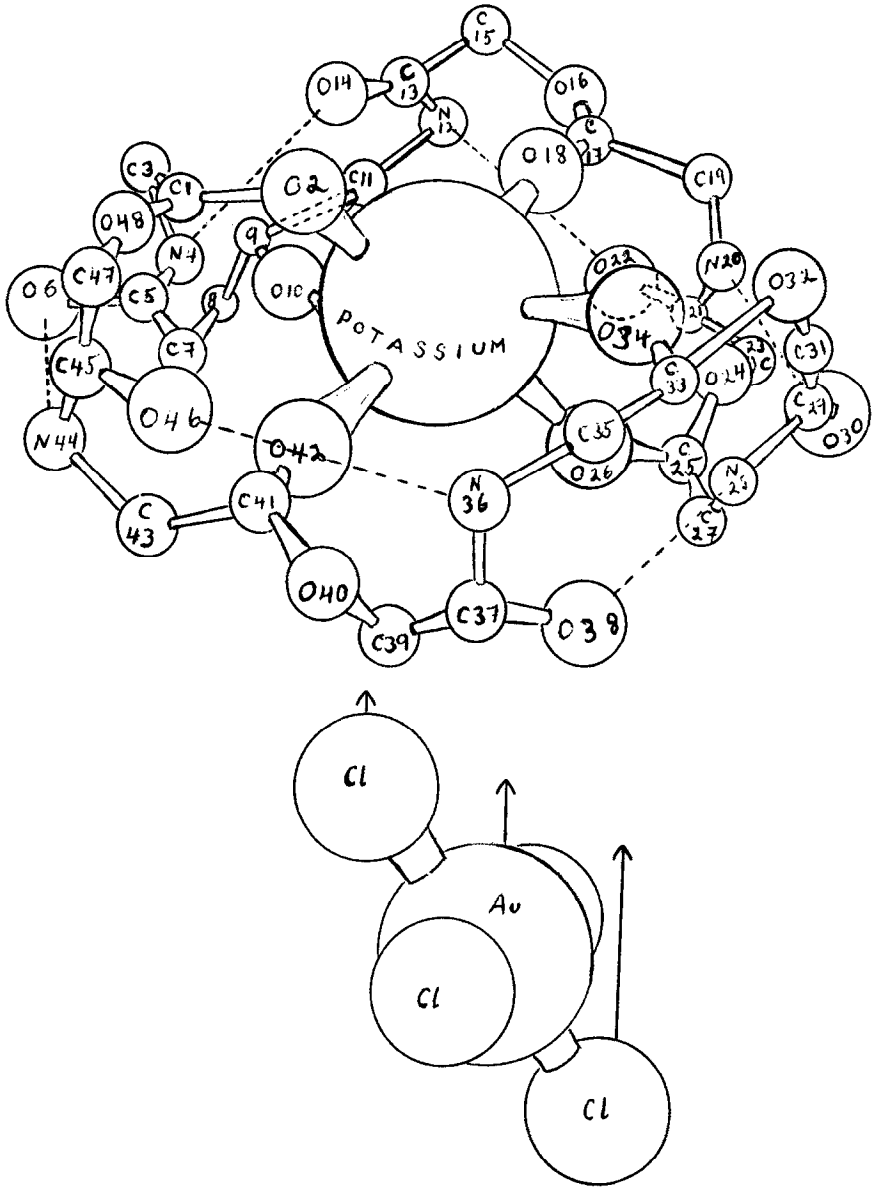

Figure 2. Drawing of the structure of the valinomycin-$KAuCl_4$ complex. Radii of the atoms are greatly exaggerated for emphasis. Dotted lines indicate possible hydrogen bonds. Side chains are not included, but methyl groups would be on atoms 15, 31 and 47, and isopropyl groups on atoms 3, 7, 11, 19, 23, 27, 35, 39 and 43. All side chains would point either straight up or straight down.

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to the concentration gradient of the cation as well. Thus, a large excess of a nontransported salt of the cation, such as potassium chloride, on one side of the barrier could cause potassium picrate added to that side to be

transported and concentrated on the other side according to the product of the anion and cation activities on the left and right sides.

$$(K^+)^L.(A^-)^L = (K^+)^R.(A^-)^R$$

This equilibrium condition can be derived from purely thermodynamic arguments assuming that electrical potentials are absent. A more complete consideration of selectively permeable barriers is being prepared by us for publication.

TABLE I[a]

$[K^+]^L$	$[P^-]^L$	$[K^+]^R$	$[P^-]^R$	$\frac{[K^+]^R[P^-]^R}{[K^+]^L[P^-]^L}$
.05	14.0	.10	6.8	0.97
.025	22.8	.10	6.2	1.09
.01	72.1	.10	6.8	0.97b
.01	80.8	.10	8.3	1.03
.01	94.7	.02	51.9	1.09
.005	85.5	.01	46.1	1.07

a. Equilibrium concentrations of potassium and picrate ions in moles per liter (picrate x 10^{-6}). Valinomycin was 5 x 10^{-7} mole. No detectable transport when either valinomycin or picrate was omitted.

b. Here picrate was added to the left side; all others to the right side only.

We offer the data in Table I to show that the above relationship is reasonable rather than as proof of its correctness. Experimental errors are about the magnitude expected due to the use of concentrations rather than activities. Equilibrium usually required about 5 days, but at sig-

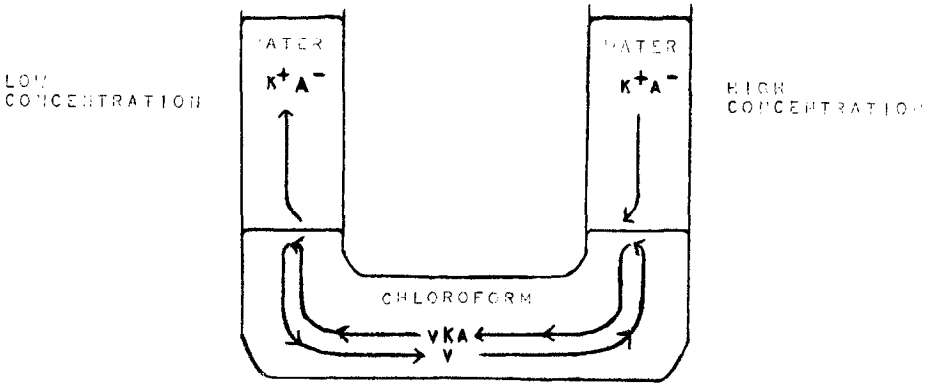

Figure 3. Drawing of the U-tube for demonstrating transport of anion cation pairs by valinomycin through a barrier of nonaqueous solvent. Potassium chloride, a nontransportable salt, is in the right arm. As soon as a suitable anion such as picrate is added, transport can take place as indicated.

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nificantly higher or lower concentrations was so slow as to prevent satisfactory results from being obtained. Longer equilibration times at low concentrations are not unexpected, but at high concentrations the reason is perhaps not obvious. However, from a consideration of Figure 3, it is obvious that the carrier, valinomycin, must return empty if the cycle is to be complete. High concentrations of the transported ions on both sides of the barrier would prevent discharge of the loaded carrier, and so there would be no empty carrier for the return. Therefore, the maximum rate of transport should be at half saturation of the carrier. Perhaps shuttle is a better word to describe the transporting molecule. Such a control mechanism may possibly exist in biological systems. Certainly there is an obvious utility for such a device which would limit the upper concentration permitted for a species.

CONCLUSIONS

We have found the molecular structure of valinomycin to be highly specific for the cation and to a lesser extent for the anion, and to be held in the proper conformation by hydrogen bonding.

We have demonstrated that valinomycin can interact with the suitable anion-cation pairs and transport them into and through barriers of non-aqueous solvent.

Using valinomycin as a carrier, we have constructed a device for coupled ion transport which will concentrate one ion across a barrier using the concentration gradient of a second ion as the driving source.

Valinomycin can be described as a catalyst which lowers the activation energy for an anion-cation pair to cross a barrier of nonaqueous solvent.

The rate of transport will depend on the amount of loaded carrier and the amount of returning empty carrier, so that the maximum rate of transport occurs at half saturation of the total carrier.

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