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INTERACTION OF MANGANESE(II) WITH VALINOMYCIN: OBSERVATION OF MIXED COMPLEXES

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The interaction of antibiotic valinomycin with manganese (II) has been studied using circular dichroism, electron spin resonance and infrared techniques. Results show that Mn(II) forms complexes with valinomycin in both 2:1 (valinomycin-ion-valinomycin sandwich) and 1:1 (equimolar) stoichiometries. The 1:1 type observed here is very different from the well known K^+-valinomycin bracelet conformation.

INTRODUCTION

Several physicochemical studies have been carried out both in solution (1) and in solid state (2-4) to correlate the conformations of free and cation bound valinomycin with its ionophoric activity and selectivity. So far, only equimolar stoichiometric complexes have been reported and this evidence led to the suggestion that 1:1 complex is responsible for ion transport across model and biological membranes (5). Recently, our laboratory reported a novel conformation for the valinomycin-Ba^{2+} complex (6). In this communication, we report our spectroscopic evidence to show that valinomycin forms mixed complexes with manganese(II) in acetonitrile.

MATERIALS AND METHODS

Valinomycin and manganese perchlorate were from Sigma Chemical Company and Alfa Inorganics respectively. Acetonitrile was distilled over P_2O_5 before use. The Circular Dichroism spectra were run on a JASCO-J 20

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automatic spectropolarimeter calibrated with d-10-camphor sulfonic acid. The ESR spectra were recorded on a Varian E-300 instrument using a quartz cell used for aqueous solutions. The proton NMR spectra were run on a Bruker WH270 instrument.

RESULTS AND DISCUSSION

The circular dichroism (CD) spectra of valinomycin (VM) in acetonitrile showed significant changes on addition of manganese perchlorate. The molar ellipticity value, $[\theta]$, at 217 nm showed a change from a small positive value (2.87×10^3 deg.cm^2.dmol^{-1}) in free valinomycin to a large negative value (-17.64×10^3 deg.cm^2.dmol^{-1}) in the valinomycin-manganese complex. The CD titration graph is shown in Figure 1. A non-linearity in the Scatchard plot (7) of the changes in $[\theta]$ over the concentration range studied indicated that the binding is not of a simple type and suggested the presence of multiple complexes in solution. Using a four parameter computer fit (7), the stability

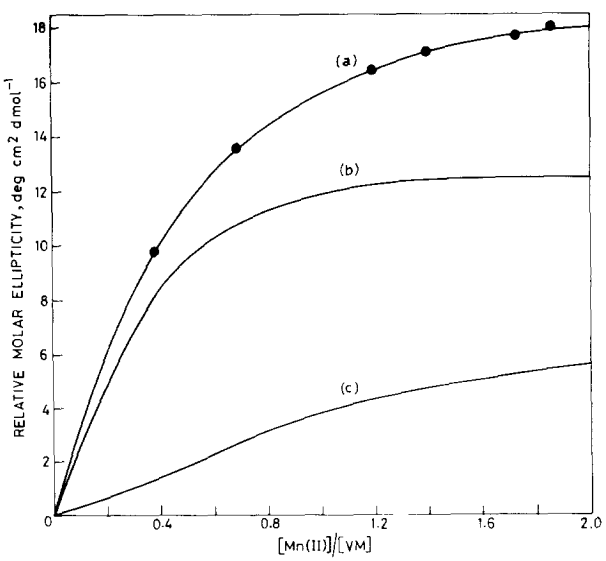

Figure 1 : Circular Dichroism titration graph of valinomycin-manganese perchlorate in acetonitrile. (a) computed four parameter fit with experimental points represented by circles (b) computed contribution of 2:1 complex (c) computed contribution of 1:1 complex ; concentration of valinomycin used is 4.54 mM.

constants K_1 and K_2 obtained corresponding to the following equilibria are 1.6 x 10^2 lit.mole^{-1} and 1.0 x 10^3 lit.mole^{-1} respectively (computed curves for 1:1 and 2:1 species are also shown in Figure 1).

$$VM + Mn(II) \overset{K_1}{\rightleftharpoons} VM\ Mn(II)$$

$$VM\ Mn(II) + VM \overset{K_2}{\rightleftharpoons} VM_2\ Mn(II)$$

The nature of the valinomycin-manganese complex was also studied using electron spin resonance technique. It has been shown (8-12) that the electron spin relaxation of ^6S state ions is governed by the distortions in the symmetry around the metal ion which produce a permanent zero field splitting (Z.F.S) and by fluctuations of the instantaneous Z.F.S produced by molecular vibrations and collisions. The changes in line width and signal intensity of the fourth hyperfine line are indicative of the mobility and environment around the manganese ion. The e.s.r titration graph of the line width and intensity of the fourth hyperfine line is given in Figure 2. As seen in

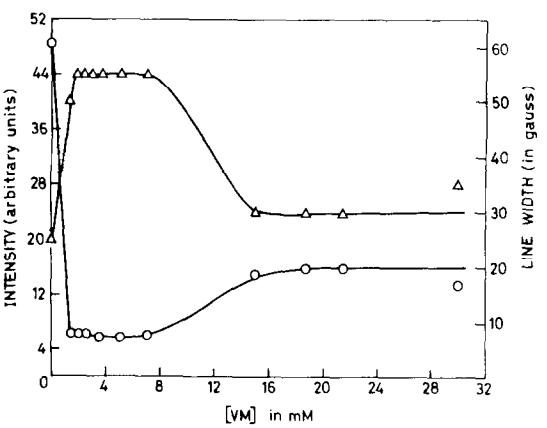

Figure 2 : E.S.R. titration graph of manganese perchlorate with valinomycin in acetonitrile. The linewidth (Δ) and intensity (o) of the fourth hyperfine line are plotted against concentration of valinomycin. Manganese perchlorate concentration = 4.68 mM.

the figure, the intensity of the fourth hyperfine line at first decreases on addition of manganese perchlorate and then increases. The line width changes show a reverse trend. Based on the CD results, the proposed scheme consistent with these data is as follows :

$$\underset{A}{\left[Mn\ (CH_3CN)_6\right]^{2+}} + VM \rightleftharpoons \underset{B}{\left[Mn\ (CH_3CN)_6\right]^{2+}}\ VM$$

$$\underset{D}{\left[Mn\ VM_2\right]^{2+}} \rightleftharpoons \underset{C}{\left[Mn\ (CH_3CN)_3\ VM\right]^{2+}}$$

The loss in e.s.r line intensity is indicative of the existence of unsymmetrical inner sphere complexes (C) in which some solvent molecules are displaced from the first coordination shell (13). Further increase in intensity is due to formation of the symmetrical inner sphere complex D which has a small static Z.F.S. value. The increase in e.s.r linewidth at first is due to the formation of outer sphere complex B which due to its decreased symmetry produces larger Z.F.S. (13). Further decrease in linewidth at higher concentrations of valinomycin is due to the formation of symmetrical inner sphere complex D. It is clear from these results that valinomycin forms both unsymmetrical (C) and symmetrical (D) inner sphere complexes.

The infrared spectra of the valinomycin-manganese complex in acetonitrile showed a new band at 1700 cm^{-1} in addition to the band at 1739 cm^{-1} corresponding to ester carbonyl stretch in free valinomycin (1) whereas the amide I band at 1650 cm^{-1} observed in free valinomycin did not change on complexation. These data indicate that only one set of the six ester carbonyls of the valinomycin

bracelet are involved in complexation. The i.r. spectra in solid state show the ester carbonyl stretch unaffected and the amide I band shifting to lower frequencies by about 30 cm^{-1}. Presumably, the complex in the solid state is similar to the valinomycin-barium complex (6). Proton NMR spectra recorded at -40°C showed two sets of NH proton signals due to free and complexed valinomycin. However, even at this low temperature the lines were excessively broadened that no information on the conformation of the complex based on coupling constant data could be obtained.

Our results show that both 1:1 and 2:1 stoichiometries are possible for the valinomycin-manganese complex. The 1:1 complex observed here has only one set of the six ester carbonyls on the side of the bracelet available for complexation whereas all the six ester carbonyls complex with potassium which is trapped inside the bracelet (1-4). This 1:1 complex is a direct demonstration of the complexation reaction at the membrane-water interface. The 2:1 complex has manganese sandwiched between two valinomycin molecules and its presence indicates the handing over of the metal ion from one valinomycin molecule to another in the lipid bilayer. This is different from the valinomycin-barium complex in that the latter has a completely flat structure (6). Support for the proposal that 1:1 complex is the only species responsible for transport of potassium by valinomycin comes from mainly two sources (5). One is the slope of unity when the logarithm of ionophore concentration is plotted against conductivity and the other is the exclusive observation of 1:1 complex in solution. To our knowledge, there

exist no data in the literature on the concentration dependent conductivity measurements for divalent ion transport. In the light of our results, it is likely that these metal ions are transported by a relay carrier mechanism wherein the metal ion is handed over from one ionophore to another in the lipid bilayer.

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