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INDUCED FIT AS A DETERMINATIVE OF IONOPHORE SELECTIVITY

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Summary

The conformation of the carboxylic ionophore salinomycin has been examined in a variety of solvents by circular dichroism. The solution conformation of the ionophore has been correlated with Na^+ and K^+ affinity and selectivity. Cation selectivity appears to be a function of both the initial conformation of the free anionic ionophore and the ability of the latter to reorient about the cation. An analogy to the induced fit model of enzyme-substrate interaction is proposed.

Introduction

The ability of ionophores to form lipid soluble cation complexes which act as ion carriers has attracted considerable interest in biology and biophysics (1). One of the most intriguing aspects of these compounds is the extreme variability in complexation affinities which they display within a given cation series. Evidence suggests that the unique cation affinity and selectivity patterns inherent in each ionophore probably arise from the spatial deployment of liganding oxygen atoms determined by molecular conformation (2). Salinomycin (Fig. 1), a highly asymmetric member of the carboxylic subclass of ionophores, lends itself particularly well to conformational studies.

The circular dichroism (C.D.) arising from the $n \rightarrow \pi^*$ transition of the C-11 ketonic carbonyl is particularly suitable for sensing molecular environment and quantitatively reports the chirality around this chromophore. Consequently, the C-11 carbonyl provides a probe for examining the solution conformations of salinomycin. By systematic perturbation of the thermodynamically stable solution conformations and subsequent analysis of complexing capabilities, we were able to ascertain that ion affinity and ion selectivity are variable, conformationally determined properties rather than static molecular properties. These conformational perturbations were achieved by altering the polar and protic properties of the solvent in which the C.D. of salinomycin and its complexes were measured.

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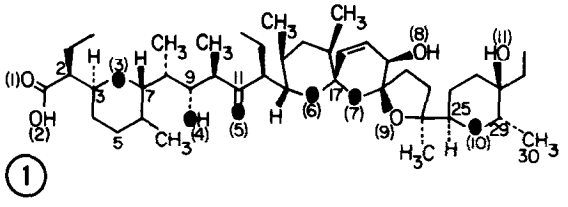

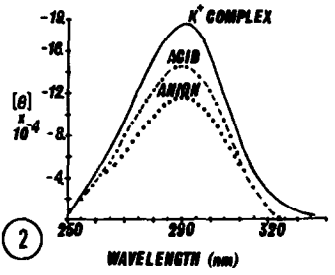

Fig. 1 Structure of salinomycin. Liganding oxygen atoms are filled in.

Fig. 2 C.D. spectra of salinomycin anion (....), acid (-·-·) and K^+ complex (-) in 80% dioxane/water. The band shapes are approximately Gaussian, but are skewed towards shorter wavelength.

Representative C.D. spectra of protonated salinomycin, its K^+ complex, and its uncomplexed anion are presented in Fig. 2. No significant shift of the negative 290 nm peak occurs with solvent change; Beer's law is obeyed from 10^{-4} to 10^{-6} M. The molecular ellipticity [θ] is observed to be a function of the liganding state of the ionophore. The function most suitable for relating C.D. spectra to the conformation of a molecule is the rotational strength (R_o^T) of the observed transition. Since the Gaussian approximation appears to hold for the salinomycin C.D. curves, R_o^T is calculated from [θ] and wavelength in the region of the transition by a standard equation (3).

Methods

Measurements of circular dichroism were performed on a Cary 60 spectropolarimeter with a C.D. attachment. The instrument contained a thermostated cell chamber kept at 22°C. The spectropolarimeter was standardized with an aqueous solution of d-10-camphorsulfonic acid (K and L Laboratories, batch No. 4829), which produced the standard $\varepsilon_L - \varepsilon_R$ of 2.20 ± .05 at 290 nm.

All organic solvents were spectral grade. Water was deionized and twice distilled from glass. Sodium thiocyanate and potassium thiocyanate were obtained from Alfa Chemicals and thoroughly dried in an Abderhalden dryer. Tri-n-butylamine was purified by fractional distillation from powdered zinc. Salinomycin (gift from A.H. Robbins) was purified by column chromatography on EM Silica Gel 60 for column chromatography eluting with 3:7 acetone:hexane. The alkali-free acid was prepared by washing the ionophore in diethyl ether with saturated aqueous citric acid. The organic layer was then washed three times with distilled water and flash evaporated. The product was recrystallized from ethyl acetate and vacuum dried.

The uncomplexed salinomycin anion was generated from the free acid by addition of two equivalents of tri-n-butylamine. The amine itself was not observed to form a complex with the ionophore presumably due to steric hindrance. Titration of the uncomplexed anion to saturation with the appropriate alkali thiocyanate solution (standardized by flame photometry) yielded K_D values.

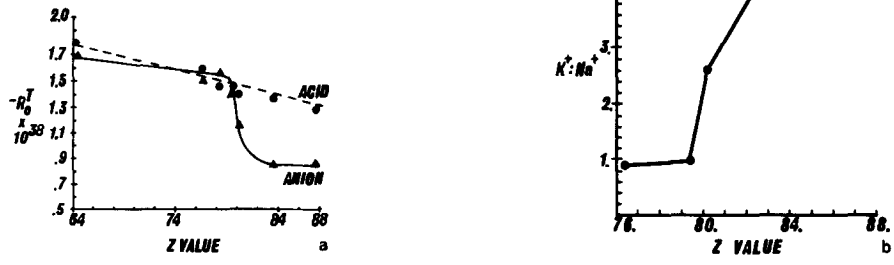

Fig. 3a) R_o^T of the free acid (●) and of the free anion (▲) of salinomycin as a function of solvent Z values. Solvent compositions correspond to those of matching Z values of Table 1. 3b) Cation selectivity (i.e. $K_D K^+/K_D Na^+$) as a function of solvent Z value.

Results and Discussion

Fig. 3a illustrates the effect of solvent changes on the R_o^T of the free acid and its anion. Kosower's Z values proved empirically an effective function for describing pertinent solvent properties related to polarity and hydrogen bonding (4). The $|R_o^T|$ of the free acid decreases linearly with a small slope as the Z values rise. In contrast, the $|R_o^T|$ of the uncomplexed anion, *the species active in complexation*, drops sharply between Z values of 80 and 83, remaining stable above and below these values. Thus, the conformation of the salinomycin anion tends toward one of two metastable states depending upon solvent, presumably reflecting some torsional aspect of the conformation of the ionophore's backbone. Fig. 3b indicates that C.D. can be employed to determine complexation K_D's (see below). It is most interesting that the ratio of the K_D's, i.e. K^+:Na^+ selectivity, show a correspondingly sharp shift between Z values of 80 and 83. This indicates that the ability of the complexing form of the ionophore to discriminate between ions is dependent upon environmental influences on conformation.

C.D. was utilized to obtain the solvent dependence of the conformation of the cation-ionophore complexes as well as their K_D's. Saturation isotherms were plotted from linear computer fits of 1/[cation] versus $1/\Delta R_o^T$; the slopes yielded K_D's while extrapolation of R_o^T to infinite [cation] provided the R_o^T of the cation-saturated ionophore (Table 1).

Table 1 Effect of Solvent Z Value on K_D and R_o^T of Na^+ and K^+ Complexes of Salinomycin

SOLVENT	Z	$K_D Na^+$	$K_D K^+$	$\|R_o^T\| Na^+ x10^{38}$	$\|R_o^T\| K^+ x10^{38}$
50% DIOXANE/H_2O	87.6	$1.84x10^{-3}$	$3.87x10^{-4}$	1.17	1.21
MeOH	83.6	$4.89x10^{-4}$	$1.03x10^{-4}$	1.47	1.75
80% DIOXANE/H_2O	80.2	$3.12x10^{-5}$	$1.17x10^{-5}$	1.77	1.80
EtOH*	79.6	$5.69x10^{-5}$	$5.52x10^{-5}$	1.69	1.73
90% DIOXANE/H_2O*	76.7	$5.45x10^{-5}$	$5.48x10^{-5}$	1.71	1.70

* A priori we would expect a progressive drop in K_D's as the solvent Z values decrease since the energies required to desolvate the cations (cf. Ref. 5) and ionophore (6) prior to complexation decrease progressively. The rise in apparent K_D values in solvents of low Z values can be accounted for by progressive increases in ion pairing which reduce the actual cation concentration, i.e. cation activity, available for complexation. Preliminary corrections for ion pairing by means of Bjerrum's equation, however, do not significantly alter the cation selectivity patterns reported here.

Comparison of the $|R_o^T|$ values for the Na^+ and K^+ complexes of salinomycin in Table 1 to the $|R_o^T|$ values for salinomycin anion found in Fig. 3 shows an increase in the magnitude of $|R_o^T|$ upon complexation in all solvents. This corresponds to a change in conformation upon complexation, i.e. the ionophore reorients about the cation. Computer modelling studies in our laboratory and application of the *Octant Rule* (7) to computer generated models of salinomycin anion indicate reorientation to be a constriction of the liganding oxygens about the cation, and predict this reorientation to be accompanied by an increase in $|R_o^T|$. The extent of constriction about the cation as indicated by the $\Delta|R_o^T|$ upon formation of the alkali complex correlates with the stability of the complex as indicated by K_D (see Table 1). The ability of salinomycin to form a stable cation complex thus appears to depend upon its ability to reorient snugly about a given cation.

A considerable body of evidence for *induced fit* (cf. Ref. 8 for analogy with enzyme-substrate complexation) as a determinative of ionophore selectivity exists in the literature. The involvement of conformation has been recognized in the complexation mechanism of the neutral depsipeptides, valinomycin

and enniatin B. ORD and ^{1}H-NMR spectroscopic data have revealed the valinomycin conformation to be highly solvent dependent (9,10). In addition, comparison of the conformations of free valinomycin and of its K^+ complex show that complexing is accompanied by significant conformational rearrangements (11). ORD and ^{1}H-NMR studies of enniatin B revealed the same dependence of complexation on solution conformation (12). Even in the nactin series, the ionophores upon which Eisenman based his "isosteric" mechanism for ionophore selectivity (analogous with the ion selectivity of rigid glass lattices (13)), critical conformational changes occur upon complexation (14). Studies on the carboxylic ionophores lasalocid (15) and monensin (16) in a variety of solvents also indicate profound conformational changes to accompany ionophore complexation. All of this data support the involvement of conformational factors in cation binding by ionophores.

The present study of salinomycin provides a systematic correlation between conformation and the ability to recognize and bind cations. We conclude the ability of ionophores to discriminate between ions must in general be dependent upon environmental influences on conformational stability, and complexation is usually, if not invariably, accompanied by a ligand-induced conformational change. Furthermore, the nature of the conformational change upon complexation is correlatable to the stability of the complex. The stability of the cation-ionophore complex, thus, depends not only on the conformation the ionophore offers to the cation prior to complexation, but also the conformation ultimately assumed by the ionophore following entrapment of its substrate cation. The case for an induced fit component of ionophore-cation interaction appears compelling.

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