ISOSTERIC METAL COMPLEXES OF IONOPHORE A23187

A basis for cation selectivity

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1. Introduction

Ionophore A23187 (A) transports predominantly divalent cations M^{2+} in exchange for H^+ through formation of the charge-neutral complex A_2M. Its activity as a Ca^{2+} ionophore and transport selectivity for divalent over monovalent cations is unique among the carboxylic acid ionophores [1]. In [2] a structure was proposed for the A_2Ca complex of A23187 (in chloroform solution) which is believed to be responsible for its activity as a Ca^{2+} ionophore. Further studies have examined the crystal structure of the A_2Ca complex [3,4]. Although the structures derived from X-ray data differ in detail from that deduced from NMR data [2], the same cation liganding groups and intracomplex hydrogen bonding scheme were found in both the solution and in the crystalline forms: i.e., Ca^{2+} is complexed by the benzoxazole carboxylate and ketopyrrole oxygen atoms, and the benzoxazole ring nitrogen atoms from each of the two ionophore molecules (see fig.1), forming a complex with nearly octahedral geometry. Both ionophore molecules are bound equivalently, resulting in a pseudo two-fold symmetry axis which passes through the cation. The dimeric structure is stabilized by two equivalent intramolecular hydrogen bonds between the hydrogen of the ketopyrrole and the non-binding carboxylate oxygen from the opposite ionophore molecule.

Many ionophores which display good cation selectivity are induced to take up 'unfavorable' conformations when binding cations of other than optimal size (see [5]); the resulting complex is thus destabilized relative to that with the preferred cation. By analogy, one might predict a wide range of conformations for A23187 in complexes with divalent cations of radius 0.66 Å (Mg^{2+}) to 1.34 Å (Ba^{2+}). However, ultraviolet absorbance and fluorescence spectra of the ionophore in complexes with several divalent cations were similar [6]. Similarly, infrared spectra recorded in chloroform solution for a series of A23187 complexes (A_2Sr, A_2Ca, A_2Zn, A_2Co, A_2Ni, A_2Mg, and A_2Mn) revealed no significant variation in the N–H stretching regions or carbonyl regions [7]. In order to clarify the relationships between the cation selectivity of A23187 and its conformational responses to complexation as a function of cation types, we have examined some of its complexes by NMR and circular dichroism spectroscopy – complimentary techniques known to be sensitive to structural and conformational features.

2. Materials and methods

Ionophore A23187 was a gift from investigators at Eli Lilly and Co. Chloroform-d was purchased from Merck and Co., and spectral grade chloroform from Aldrich Chem. Co. Divalent cation chlorides or, in some cases, nitrates were obtained from commercial sources and were the highest purity grades available.

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Complexes of the stoichiometry A_2M between A23187 and divalent cations were formed by equilibrating solutions of the ionophore as the free acid in chloroform against aqueous solutions of divalent metal salts. The pH of the aqueous phases was brought to a sufficiently basic value by addition of the non-complexed bases Tris or tetraethylammonium hydroxide to insure nearly complete saturation of the ionophore with cation. After complex formation, the chloroform layer was evaporated in a nitrogen stream and the ionophore–metal complex redissolved in fresh solvent to remove traces of water which may have partitioned into the initial solution. The free acid form of the ionophore could be recovered from cation complexes in chloroform solution by repeated extraction against aqueous 1 M HCl.

NMR spectra were recorded at probe temperature in the Bruker HX-270 spectrometer located in the Chemistry Department, University of Wisconsin. Samples contained 15–20 mM A23187 in chloroform-d. CD spectra were recorded at 25°C in the Cary 61 spectropolarimeter located in the Chemistry Department, Purdue University, on samples containing 100 μM A23187 in chloroform. Data obtained as degrees of rotation was converted to units $\Delta\Sigma$ according to the relationship:

$$\Delta\Sigma = \frac{\text{degrees rotation}}{33\,bc}$$

where b is the cuvette path length and c the molar concentration. Values were calculated using the nominal ionophore concentration whether it existed as the free acid or in dimeric cation complexes.

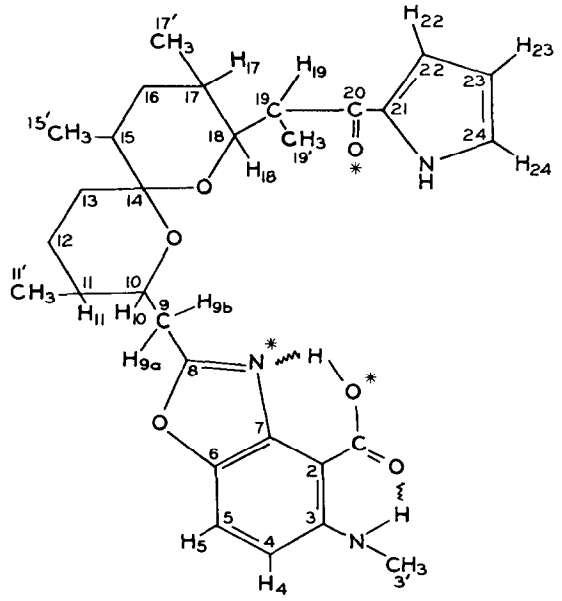

Fig.1. The structural formula of A23187. The numbering system follows that in [3]. Those atoms marked by an asterisk are liganding atoms in dimeric complexes with divalent cations. The hydrogen bonding scheme indicated is the probable one for the free ionophore in chloroform solution (see text for discussion).

3. Results

3.1. *NMR spectroscopy*

Substantial conformational variations between free and complexed A23187 probably arise by rotation about carbon–carbon bonds in the 'hinge regions' of the molecule [2] (see fig.1). Using a Karplus-Bystrov-type analysis of vicinal coupling constants [8,9] to determine dihedral angles between protons 9a,b and 10 in the benzoxazole hinge region and between protons 18 and 19 in the pyrrole hinge region, we obtained the data shown in table 1 for the complexes A_2Mg and A_2Zn, and compared it with the A_2Ca data [2]. Note the strategic location of these ions in the cation affinity pattern of this ionophore (vide infra). The results indicate that, allowing for the uncertainties recognized for this technique [8,9], the conformations of individual molecules of A23187 in the stoichiometrically equivalent complexes are substantially the same for this group of divalent cations. An NMR study of the A23187 Mg^{2+} complex [10] also suggests a similarity between solution conformations of A_2Mg and A_2Ca. It had further been observed [2] that the pyrrole NH proton is shifted dramatically from 9.70 ppm (relative to tetramethylsilane) in the free acid form of A23187 to 13.97 ppm in the A_2Ca complex, reflecting formation of intracomplex hydrogen bonds; the other complexes examined displayed shifts of nearly equal magnitude (to 13.64 and 13.30 ppm for the A_2Mg and A_2Zn complexes, respectively) indicating an equivalent nature as well for this aspect of the complex structures.

However, since requisite protons are lacking, the

Table 1
Coupling constants (J_{ij}) in the hinge regions of A23187[a]

Protons i–j	Coupling constants ($J_{H_i-H_j}$ (Hz)			
	Free acid[b]	Ca^{2+} complex	Mg^{2+} complex	Zn^{2+} complex
9a–9b	15.2[c]	13.3[c]	13.1[c]	~12.0[c]
9a–10	7.3	11.2	11.4	11.7
9b–10	7.5	3.0	2.8	2.5
10–11	2.4	1.8	2.7	2.5
17–18	2.0	2.0	2.0	2.5
18–19	10.1	10.1	10.3	10.0
19–19′	6.8	6.8	6.3	–

[a] Coupling constants determined from 270 MHz proton NMR spectra in deuterochloroform solution. Protons numbered according to scheme given in fig.1. Protons 9a,b, 10 and 11 are in the benzoxazole hinge region; protons 17, 18, 19 and 19′ are in the pyrrole hinge region. Uncertainty in measured J-values, ± 0.3 Hz

[b] Data for free acid and Ca^{2+} complex from [2]

[c] Geminal coupling constant. All others are vicinal coupling constants

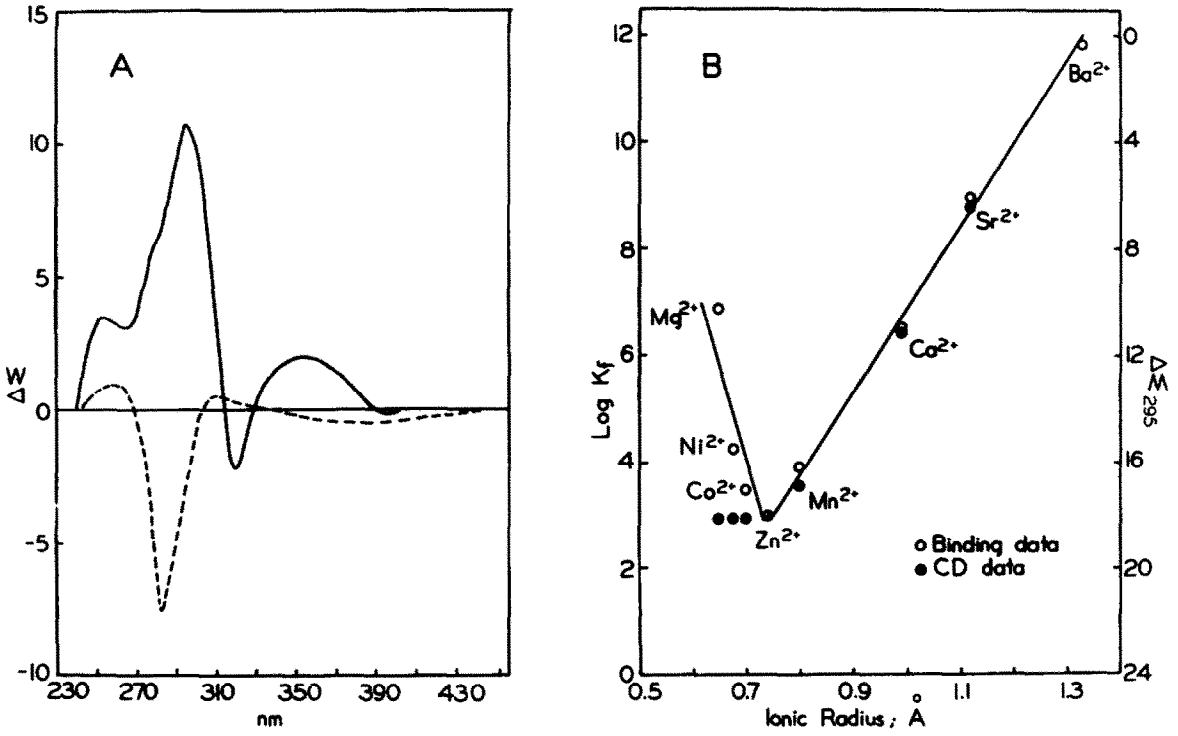

Fig.2. Comparison of circular dichroism data and complex stabilities as a function of divalent cation ionic radius. CD spectra were recorded as in section 2. (A) Dashed line, the CD spectrum of 100 μM A23187 in chloroform; solid line, the CD spectrum of 50 μM A_2Ca complex in chloroform. (B) Open circles (○), the negative logarithms of overall binding constants for formation of complexes A_2M with selected divalent cations [11]; solid circles (●), the ellipticity of the band at 295 nm in CD spectra of these complexes.

NMR analysis cannot yield information relevant to the orientations about C_8–C_9 and C_{19}–C_{20} carbon–carbon bonds which are also hinge region atoms proximal to the benzoxazole and ketopyrrole groups, respectively.

3.2. *Circular dichroism spectroscopy*

Ionophore A23187 has several asymmetric centers and chromophoric groups, which make it highly suitable for CD measurements. Figure 2a shows CD spectra of A23187 as the free acid and as the calcium complex. The ionophore as the free acid displays a major minimum at 283 nm, which is transformed upon complex formation to a major maximum at 295 nm. As both aromatic chromophores are expected to contribute to the absorption in this region, the CD spectral alterations accompanying cation complexation support the requirement for a reorientation of one, and probably both, aromatic moieties with respect to nearby asymmetric centers.

As shown in fig.2b, the logarithm of the overall affinity constants increases linearly with decreasing ionic size upon passing from the larger divalent ions to Zn^{2+} [11]. Over this region of ionic radius, there is a parallel increase in the magnitude of the CD band at 295 nm. However, while cations smaller than Zn^{2+} form complexes of decreasing stability, the magnitude of the 295 nm CD band is not further changed.

4. Discussion

Since the orientations about the C_9–C_{10} and C_{18}–C_{19} bonds were equivalent within the sensitivity of NMR analysis, these results suggest that the CD spectral variations between the A_2Sr, A_2Ca, A_2Mn and A_2Zn complexes may arise from altered orientations about the C_8–C_9 and C_{19}–C_{20} bonds. By this interpretation, the cation binding cavity formed by two A23187 molecules is ideally sized to contain the cation Zn^{2+}. To the extent that this cavity maintains its essential dimensions, liganding sites, and geometric integrity, it can be described as isosteric (i.e., essentially unchanged) throughout the series of divalent cation complexes investigated here. The cavity is expanded in complexes with cations larger than Zn^{2+} by minor rotations about the C_8–C_9 and C_{19}–C_{20} bonds. However, for cations smaller than Zn^{2+}, rotations about these bonds cannot reduce the cavity size, since unfavorable contacts due to proximities of liganding and/or other A23187 atoms prevent the binding cavity from 'contracting' any further. Thus, for cations smaller than Zn^{2+}, the overall complex stability decreases progressively but no further rotations occur about C–C bonds. The analogy to small marbles rattling around in a box of fixed dimensions may be appropriate here in providing a description of A23187 complexes with cations of smaller ionic radii than Zn^{2+}.

By these arguments alone, it remains difficult to explain the reduction in complex stabilities (eight orders of magnitude) for cations larger than Zn^{2+}. Rotations about the C_8–C_9 and the C_{19}–C_{20} single bonds should not, by themselves, present a significant energy barrier. Furthermore, even minor rotations would alter the alignments of ligand groups and complexed cation, as well as of those groups involved in the intra- or intermolecular hydrogen bonds (and hence affect hydrogen bond strengths), but evidence for such effects did not emerge. Therefore, the possibility must be considered that the apparently extensive hydrogen bond network in the dimeric complexes effectively increases the energetic barriers to mobility about hinge region C–C bonds. Increasing the binding cavity size to accommodate cations larger than Zn^{2+} would then result in strain on these bonds rather than in simple rotation, with consequent complex destabilization.

Although further investigation is required before the high cation size discrimination displayed by A23187 is completely explained, the present findings demonstrate that the essential features of the Ca^{2+} complex structure are maintained over a wide range of divalent cations and complex stabilities. The unyielding conformational integrity of A23187 divalent metal complexes provides an interesting contrast to other ionophores where available data indicate significantly different structures for complexes with strongly or weakly bound cations.

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