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THE CIRCULAR POLARIZATION OF FLUORESCENCE OF THE IONOPHORE LASALOCID A (X-537A) AND SOME OF ITS METAL COMPLEXES

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The conformation of the ionophore lasalocid A (X-537A) and its complexes with metal ions was probed by the circular polarization of their luminescence (CPL). The CPL of each complex in methanol was found to be different than when in *n*-hexane. Furthermore, the different metal ion complexes investigated had a different CPL spectrum in each solvent. These findings indicate wide variability in the conformation of the complexes depending on the metal ion and the solvent. From the spectral behaviour of the CPL it was concluded that at least some of the complexes exist in more than one form in solution. A comparison between the CPL and CD spectra indicates a change in the conformation of the ionophore in the vicinity of the salicylate chromophore upon electronic excitation.

1. Introduction

The antibiotic X-537A, isolated from species of streptomyces [1] was shown to form lipophilic complexes with monovalent and divalent ions [2–4]. Its molecular structure suggests that the complexes with metal cations are formed by coordination with different oxygen atoms. This was shown to be the case in the crystals of its silver [5], barium [6], and sodium [7] salts. In all these cases the ionophore is encircling the cation to which it is coordinated and the external face is hydrophobic. This seems to be the explanation to the high solubility of the metal-complexes in nonpolar solvents and to their high permeability through biological membranes. It is still uncertain however whether its biological significance arises from its ability to increase the fluxes of calcium [2,8,9], sodium [10] or biological amines [11–13].

A few studies on the spectroscopic properties of metal ion complexes with X-537A have been reported [2,3,14–16]. To explain the differences between the CD spectra of complexes with different cations it was proposed [16] that the metal complexes of X-537A may exist in three different conformations, depending on the cation it is bound to, and on the polarity of the solvent. The polarity of the solvent determines also the stoichiometry of the complexes, thus in polar solvent the complexes are monomeric even with divalent cations: MX^+, while in nonpolar solvents like toluene or hexane divalent cations form dimeric, electrically uncharged complexes: MX_2 [16,17].

In the following we report the measurements of the circular polarization of luminescence, CPL, of complexes of X-537A with metal ions in different solvents. The light emitted by a chiral fluorescent molecule may be partly circularly polarized [18–20]. This phenomenon is related to the asymmetry of the molecule in the electronically excited state in the same way that CD is related to the molecular asymmetry in the ground state. The fluorescence anisotropy

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factor, g_{em}, is defined as $g_{em} = \Delta f/(f/2)$ [20], where f is the total fluorescence intensity and Δf is the intensity of the circularly polarized component, in analogy to the definition of the absorption anisotropy factor, g_{abs}, used in CD, i.e., $g_{abs} = \Delta\epsilon/\epsilon$.

The salicylic acid moiety is fluorescent. Though it is not inherently chiral, asymmetry may be induced in it by the asymmetric folding of the molecule as a whole.

Since the fluorescence involves usually only one electronic transition, CPL spectra may be easier to interpret and have more specific information than CD. This advantage manifests itself in the present study.

2. Experimental

2.1. Materials

X-537A was a gift from Dr. J. Berger of Hoffman-La Roch. The metal cations we used were: Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+} as the chlorides or bromides and were of analytical grade. Methanol and *n*-hexane were of spectroscopic grade. The complexes in methanol were prepared by mixing solution of the salts, *n*-tributylamine and X-537A to make a final molar proportion of 2 : 1.3 : 1, respectively. The complexes in *n*-hexane were prepared by shaking *n*-hexane solution of X-537A of about 1 mM with aqueous 20 mm solutions of the various salts. By this procedure the cation is extracted into the organic solution where it forms a complex with X-537A [16]. All measurements were carried out at 22°C.

2.2. Methods

Absorption spectra were obtained with a Zeiss Model PMQII spectrophotometer. Corrected fluorescence spectra were measured with a Hitachi–Perkin–Elmer spectrofluorimeter, Model MPF 3. Circular dichroism was measured with a Cary Model 60 spectropolarimeter, with a 6002 CD attachment. The circular polarization of fluorescence was measured with an instrument that was designed and constructed at the Weizmann Institute [21]. The light source was a 100 W high-pressure mercury lamp (Osram, HBO 100 w/2). The wavelength of excitation was 310 nm, selected by a Bausch and Lomb high intensity monochromator with a band pass of 30 nm. A Schott UG 11 glass filter introduced in the exciting beam was used to remove stray light with wavelengths above 360 nm. The 2 mm sample cuvette was followed by an elasto-optic light modulator (Morvue Model PEM-3), which modulated the circularly polarized component in the fluorescence. The wavelengths of fluorescence were selected with a Jarrel–Ash double monochromator (Model 82-410) with a band pass of 10 nm. A 2M KNO_2 solution in a 1 cm cell was put in the fluorescence beam to cut off stray light of wavelength shorter than 390 nm.

3. Results

The spectra of optical absorption, fluorescence, circular dichroism (CD) and circular polarization of luminescence (CPL) for the Sr^{2+}-X-537A complex are presented in fig. 1. The positions of the peaks in the absorption and fluorescence spectra of complexes of X-537A with Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+} and Ba^{2+} are similar to those of the strontium complex given in fig. 1. The intensities of the fluorescence and CD vary from one complex to another and are in agreement with those reported previously [3,16].

The CD spectra in fig. 1 are expressed in two ways: in terms of $\Delta\epsilon$ and of $\Delta\epsilon/\epsilon$. The conventional plot of $\Delta\epsilon$ versus wavelength exhibits two broad peaks centered at 245 and 290 nm as was described previously [3,14,16]. The presentation of the CD spectra in the form of the absorption anisotropy factor, g_{abs}, defined as $\Delta\epsilon/\epsilon$ has the advantage that g_{abs} is expected to be constant for each allowed electronic transition [22], which may be helpful in resolving CD spectra into the various component electronic transition. It can thus be seen from the g_{abs} plot in fig. 1 that three regions of different g_{abs} can be identified: in the ranges of 240–260, of 270–280 and of 320–350 nm. It may be noted that g_{em} is different from the g_{abs} at the long wavelength absorption of the complex.

The CPL spectra of X-537A complexes with various metal ions in methanol solution are presented in fig. 2. The spectrum of the free anion is also given for comparison. It is seen that there are large differences in the CPL of the different complexes. While the complexes with Na^+, K^+, Rb^+, Mg^{2+} exhibit very small g_{em} the Cs^+ complex exhibits a large positive g_{em}

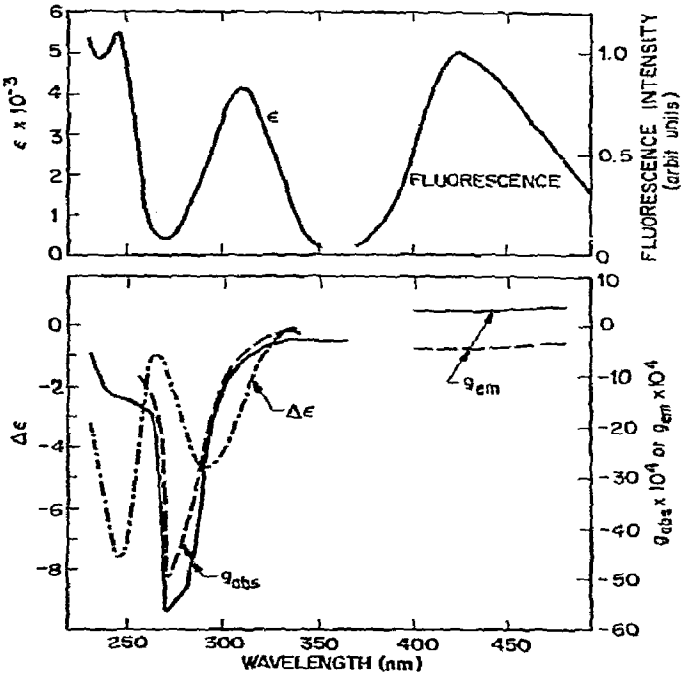

Fig. 1. Spectroscopic data for the complex of X-537A with Sr^{2+} ions. Upper part: absorption curve of the complex in methanol and its corrected fluorescence spectrum. Lower part: Δε in methanol (—·—·); absorption anisotropy factor, g_{abs}, and emission anisotropy factor g_{em}, in methanol (——) and in *n*-hexane (– – –). Excitation wavelength in the fluorescence and CPL measurements: 310 nm. Room temperature (≈22°).

while those containing Ca^{2+}, Sr^{2+} and Ba^{2+} exhibit large and negative g_{em} values.

The CPL results in *n*-hexane solution also show large differences for the various metal ions but they are significantly different from those in the methanol solution. Particularly noticeable are the Sr^{2+} and Ba^{2+} complexes which have large negative g_{em} values in methanol and positive values in *n*-hexane.

It may be noted that for some metal complexes the CPL is not constant within the measured spectral range (figs. 2 and 3). This is particularly noticeable for the Ca^{2+} complex in *n*-hexane and for X^- and its Cs^+ and Ca^{2+} complexes in methanol solution.

The application of the CPL for the determination of the stoichiometry of X-537A: metal-ion complexes is illustrated in fig. 4 for the case of Ba^{2+} in ethanol. As can be seen, the equivalent point occurs at a Ba^{2+} to ionophore ratio of 0.95 ± 0.05, corresponding to a composition of the complex of BaX^+. This result is in variance with the CD result of Alfa and Brady [14] where a 1 : 2 metal-to-ionophore ratio was found. The nature of this discrepancy is not clear. It may be noted that in this case it is difficult to follow the titration fluormetrically since the fluorescence yield for the Ba^{2+} complex is very similar to that of the free X-537A anion [15,23]. Similar titration of Ba^{2+}-X-537A in methanol also yielded a 1 : 1 stoichiometry, in agreement with previous data [3].

4. Discussion

The circular polarization of luminescence is usually sensitive to the environment of the chromophores. By use of this technique, differences in the structure of the ionophore X-537A when free or when complexed to a variety of cations in a polar and a nonpolar solvent could be demonstrated. Furthermore, the results indicate that for at least some of the ion complexes of X-537A more than one structure occurs

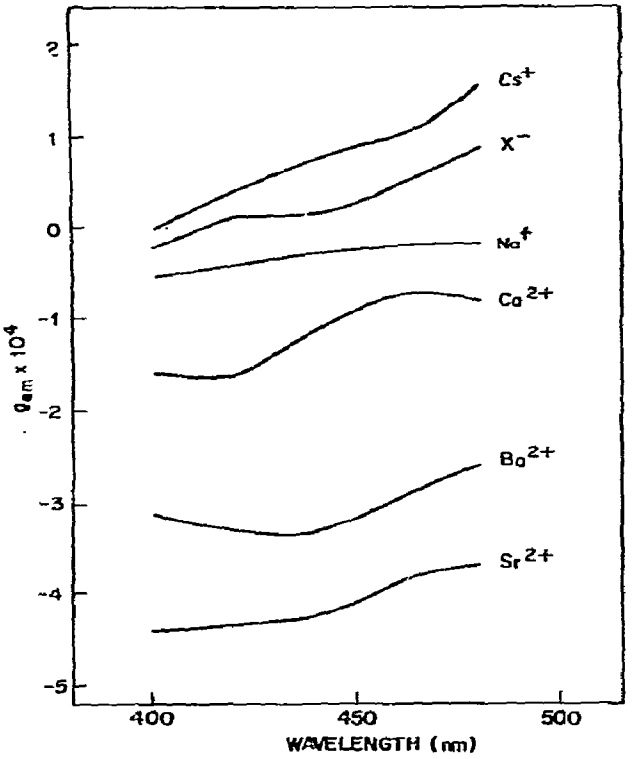

Fig. 2. CPL spectra of X-537A (denoted X^-) and of its salts with the various cations in methanol. The emission anisotropy factor g_{em} of the complexes with K^+, Rb^+ and Mg^{2+} is zero. Concentration of X-537A is $\approx 10^{-3}$ M throughout. The molar ratio of metal ion: X-537A: tributylamine is 2 : 1 : 1.3. Excitation wavelength 310 nm. Room temperature (≈22°).

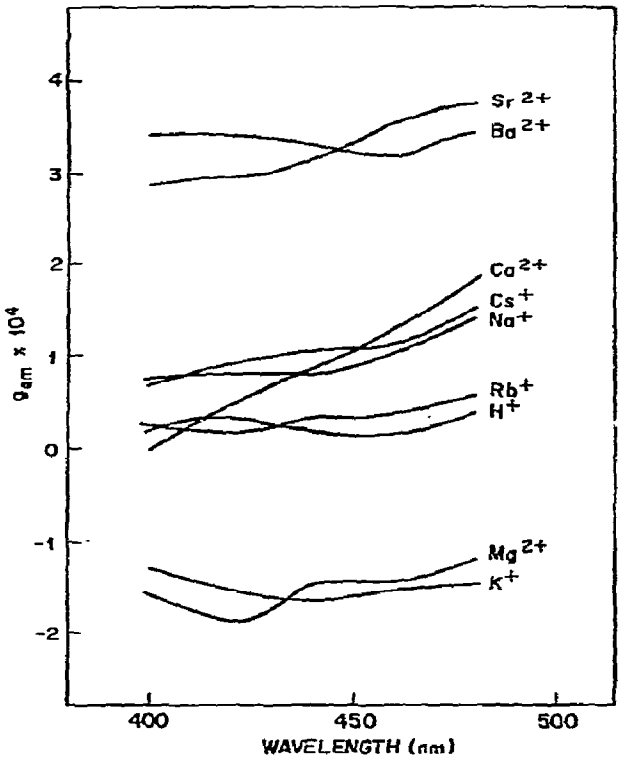

Fig. 3. CPL spectra of X-537A (free acid) and of its salts with the various cations in *n*-hexane. Concentrations are $\approx 10^{-3}$ M. Excitation wavelength 310 nm. Room temperature (≈22°).

in solution.

The emission of X-537A and of its complexes should be attributed to its salicylate chromophore. The fluorescence spectrum of the strontium complex in methanol (see fig. 1), as well as that of the complexes with the other ions studied in both methanol and *n*-hexane, have a rather pronounced Stokes shift. The anion of the ionophore behaves similarly [16], the fluorescence spectrum was reported to be blue shifted by as much as 40 nm when the ionophore was studied in acid solution [16]. This behaviour is similar to that of other salicylates and is most probably due to the ionization of the phenolic hydroxyl group taking place upon electronic excitation, the emitting species thus having lower energy than the species to which excitation took place. We do not believe however that the ionized phenolic hydroxyl is complexed to the metal cations. The reasons for this are the following:

The X-ray structures of the sodium complex of the ionophore [7] and of its barium complex [6], show that the phenolic hydroxyl is not adjacent to the complexed ion. Any distortion of the molecular conformation to bring this group into the vicinity of the ion will require a major reshuffling of the bonds around this ion and these will not be able to proceed during the lifetime of the excited state. Futhermore, the emission spectra of the free anion and of the various complexes are very similar to one another which makes it unlikely that the ions complex directly to the phenolate oxygen.

The circular dichroism spectrum of the strontium complex of X-537A is presented in fig. 1 in two ways: as $\Delta\epsilon$ and as the anisotropy factor g_{abs}. We believe that the latter representation is more useful since g_{abs} is expected to be constant across each allowed electronic transition; variations in g_{abs} across the spectrum

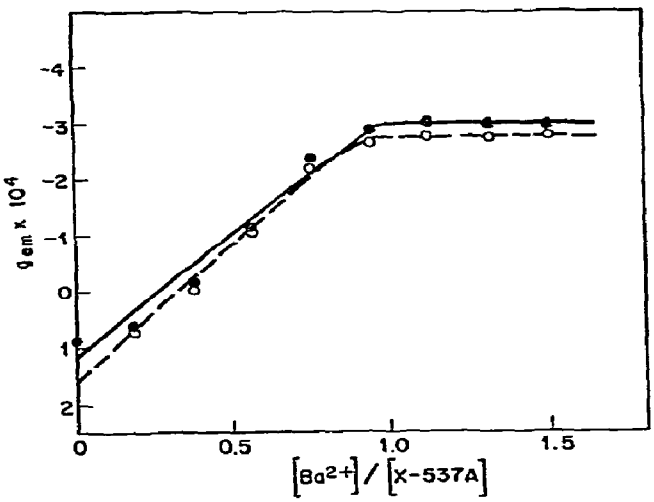

Fig. 4. Titration curve of X-537A with $BaBr_2$ in ethanol. Wavelength of excitation 310 nm. Wavelength at which g_{em} was measured is 410 nm (——) and 430 nm (– – –). Concentration of X-537A was $\approx 10^{-3}$ m, and that of tributylamine 1.3×10^{-3} M. Room temperature (≈22°).

may be more readily applied to the assignment of transitions in the spectrum. Thus, there is clearly a strongly dichroic band in the absorption spectrum of the Sr^{2+}: X-537A complex in the range of 270–280 nm, which is distinct from those occurring at shorter or longer wavelengths. This is most probably due to the carbonyl chromophore present in the ionophore and is masked in the absorption spectrum by the more intense band of the salicylic acid chromophore which peaks at longer wavelengths. It may be noted that Degani and Friedman [16] have already suggested that the carbonyl chromophore contributes to the Δε band centered at 290 nm. However, the presentation of the optical activity in terms of g_{abs} demonstrates the carbonyl contribution more clearly. From the constancy of g_{abs} above 320 nm we can conclude that a single chromophore, i.e. the salicylate, contributes to the CD at this spectral range and that the contribution of the carbonyl above 320 nm is negligible.

A comparison between g_{abs} at the long wavelength absorption band and g_{em} of the Sr^{2+}: X-537A complex indicates that there is a difference in the chirality of the salicylic acid chromophore when in the ground or in the electronically excited states. This is not surprising in view of the conclusion drawn above that the absorbing and emitting species differ in the ionization of the phenolic hydroxyl group. It is possible, however, that this difference between g_{abs} and g_{em} reflects also some change in interaction of the chromophore with the rest of the molecule upon electronic excitation.

A comparison between fig. 2 and fig. 3 clearly shows that there is a difference in conformation X-537A or its cation complexes when dissolved in methanol and in *n*-hexane. As has been demonstrated before [3,16,17] for various divalent cation X-537A complexes, they tend to be in monomeric form (i.e., containing one ionophore group per molecule) in methanol while they are in dimeric form (i.e., containing two ionophore groups per molecule) in *n*-hexane. This difference in molecular structure most probably affects the way in which the ionophore wraps itself around the complexed ions, which may be responsible for the differences in optical activity observed for the salicylate chromophore in the different solvents. There are indications from NMR relaxation that the sodium complex forms dimer in nonpolar solvents [17]. It is not known, however, what is the polymeric state of other monovalent cation complexes in *n*-hexane. If some of them are monomeric, the differences in g_{em} observed in methanol and *n*-hexane should be attributed solely to differences in conformation in the two solvents.

It is also conceivable that a direct exciton interaction between pairs of salicylate chromophores in the dimer affects the circular polarization of their fluorescence in some cases. The latter possibility is unlikely for the Ba^{2+} complex, the structure of which was determined by Johnson et al. [6], or for the head to tail Na^+ complex [7], because of the large separations between the salicylate groups, but may be important for dimers which have structures similar to the head-to-head complex of Na^+ observed by Schmidt et al. [7].

There are dramatic differences between the emission anisotropy factors of the ionophore when complexed to the different cations studied (figs. 2 and 3). These results most probably reflect differences in the conformation of the ionophore when bound to different ions in any one of the solvents studied. This conclusion is in line with the fact that the Ba^{2+} dimer and Na^+ dimer have different structures in the crystalline state.

Schmidt et al. [7], have concluded from their X-rays and NMR studies that large conformational changes do not occur in the backbone in different solvents or crystals structures. The dihedral angles do not vary more than 8°. The CPL results are expected to reflect the conformational changes in the environment of the salicylate group and it is not possible to state at this stage whether these small variations in backbone conformation contribute to the observed CPL differences.

It is instructive to examine the shape of the CPL spectrum for the ionophore and its cation complexes. For a single chromophore having an allowed electronic transition g_{em} is expected to be constant across the emission band [20]. Inspection of figs. 2 and 3 shows that this expectation is not fulfilled in quite a few cases. Thus, g_{em} of the ionophore and its Ca^{2+} and Cs^{+} complexes in methanol, as well as the g_{em} of the Ca^{2+}, Sr^{2+}, Cs^{+} and Na^{+} in *n*-hexane vary markedly with wavelength. Some of the other complexes also show variation in g_{em} with wavelength though to a lesser degree. These results suggest that the conformation of many, if not all, of the complexes is heterogeneous in solution, molecules of different conformation having somewhat different emission spectra and different g_{em} values. The superposition of the contributions of the different conformations to the g_{em} spectrum thus results in a variable g_{em} across the spectrum. It is pertinent to note that for the Na^{+} dimeric complex two very different structures were in fact identified by X-ray crystallography and it is conceivable that more structures may exist in solution. The possibility of the coexistence of different structures of X-537A should of course be born in mind in their study by various techniques, e.g., by optical of NMR spectroscopy.

The results presented show that the circular polarization of the fluorescence of X-537A and of its complexes with cations is sensitive to the conformation and state of aggregation of the ionophore. It is thus expected to be a useful tool in probing the structure of this ionophore under a variety of conditions such as which is present in biological membranes.

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