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Influence of Solvent and of Cation Size on the Conformations of Lasalocid A–Lanthanide(III) Ion Complexes: Circular Dichroism and Fluorescence Studies†

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ABSTRACT: The interaction of lanthanide(III) nitrates (La^{3+} to Lu^{3+}) with the carboxylic ionophore lasalocid A (LS) has been studied by circular dichroism (CD) and fluorescence spectroscopic techniques in acetonitrile and in methanol. Analysis of the CD data in acetonitrile has revealed the coexistence of both 1:1 (ionophore:cation) and 2:1 complexes in solution. For 1.22 Å > ionic radius > 1.13 Å, 1:1 complexes are preferred, and for 1.13 Å > ionic radius > 1.03 Å, 2:1 complexes are preferred. Induced CD bands for Ln^{3+} ions have been observed upon binding to LS in acetonitrile. The LS–Ln^{3+} complexes are less stable in methanol than in acetonitrile. CD spectral changes showed that the conformations of the complexes in methanol are different from those in acetonitrile. The complexes have rather open conformations in methanol compared to those in acetonitrile. The results underscore the importance of ionic radius, solvent environment, and ionization state of LS in determining the conformations of the ionophore–cation complexes.

Among the carrier ionophores, a carboxylic polyether antibiotic, lasalocid A (LS)[1] (Figure 1), has been the subject of extensive studies because of its diverse cation binding abilities with physiologically important monovalent and divalent cations and its transporting properties (Alpha & Brady, 1973; Degani et al., 1973; Degani & Friedman, 1974; Haynes & Pressman, 1974; Shen & Patel, 1976; Patel & Shen, 1976). By use of nuclear magnetic resonance (NMR) spectroscopy, it has been shown to transport Pr^{3+} ions across egg yolk lecithin (EYL) model membranes as a diffusive carrier with the transporting species forming a nonequimolar 2:1 complex (Fernandez et al., 1973). The conformation of the LS–Pr^{3+} complex in methanol has been studied with the aid of circular dichroism (CD), fluorescence, and NMR spectroscopic methods (Chen & Springer, 1978). These studies have shown that Pr^{3+} ion binds mainly to the salicylic acid moiety of the ionophore molecule forming a tris complex (LS_3Pr). However, Richardson and Dasgupta (1981) have, from their ultraviolet (UV) fluorescence, and circular polarization of luminiscence (CPL) studies on the interaction of Pr^{3+}, Nd^{3+}, Eu^{3+}, Tb^{3+}, and Gd^{3+} ions with LS anion in methanol, arrived at the conclusion that LS–Ln^{3+} complexes are of 1:1 stoichiometry and the coordination to the cation is provided by the carbonyl group, the tetrahydrofuran, the tetrahydropyran, and the two hydroxyl group oxygens besides the carboxylate anion. In view of the recent observations that the conformations of the LS–cation complexes are solvent polarity dependent (Vishwanath & Easwaran, 1983, 1985; Shastri & Easwaran, 1984), it is of interest to investigate the stoichiometries and conformations of LS–cation complexes in different solvents of varying polarity. The lanthanide series offers the unique advantage of controlling the size of the cation in steps of about 0.2 Å without any changes in the charge, which is another important factor that governs the conformations of the LS–cation complexes (Alpha & Brady, 1973; Shastri, 1985). In this paper, we present the results of our CD and fluorescence studies on the interaction of lanthanide nitrates with LS in acetonitrile and methanol.

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[1] Abbreviations: CD, circular dichroism; CPL, circular polarization of luminescence; EYL, egg yolk lecithin; LS, lasalocid A (free acid); NMR, nuclear magnetic resonance; UV, ultraviolet.

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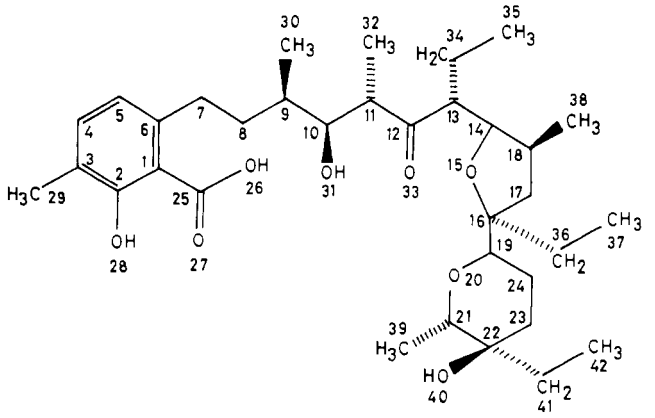

FIGURE 1: Chemical structure of lasalocid A.

Materials and Methods

Sodium salt of lasalocid A and *d*-10-camphorsulfonic acid were obtained from Sigma Chemical Co. Acetonitrile was obtained from Fluka Chemicals (99.9% pure), and the spectroscopic grade (Uvasol) methanol was obtained from E. Merck. Free acid of LS, used in our studies, was prepared from the sodium salt as reported in the literature (Alpha & Brady, 1973). Lanthanide nitrates were prepared from their corresponding oxides (Alfa Inorganics) by treating with concentrated nitric acid and recrystallizing several times from double-distilled water.

The CD spectra were recorded on a Jasco J-20 spectropolarimeter calibrated with a standard solution of *d*-10-camphorsulfonic acid. The concentration of the ionophore used in the CD experiments was 0.5 mM. The titration experiments were performed, keeping the concentration of the ionophore constant, by gradual addition of the salt solution to give rise to various molar ratios. The titration graphs were constructed by plotting the changes in the molar ellipticities ($\Delta[\theta]^{\lambda}$ expressed in deg·cm^2·dmol^{-1}) against molar ratio. The binding constants for the complexes were calculated according to the methods described earlier (Reuben, 1973; Vishwanath & Easwaran, 1982; Sankaram & Easwaran, 1982). The binding constants K_1 and K_2 correspond to the equilibria

$$M + S \rightleftharpoons MS \qquad K_1 = [MS]/([M][S])$$

$$MS + S \rightleftharpoons MS_2 \qquad K_2 = [MS_2]/([MS][S])$$

where M, S, MS, MS$_2$, K_1, and K_2 stand for the cation, the ionophore, the 1:1 ionophore–cation complex, the 2:1 ionophore–cation complex, the binding constant for the 1:1 complex, and the binding constant for 2:1 complex, respectively.

The fluorescence spectra were recorded on a Hitachi 650 spectrofluorometer using a rectangular quartz cell of 1-cm path length. In the fluorescnece quenching experiments, where the concentration of LS was about 20 μM, broad-band excitation ($\Delta\lambda_{ex} \simeq 20$ nm) centered at the UV absorption maximum of LS (310 nm) was used, and the entire emission spectrum was recorded at each titration point. The percent quenching (%Q) of LS fluorescence was calculated according to

$$\% Q = 100(F_0 - F)/F_0$$

where F_0 is the uncorrected fluorescence intensity of the free LS in solution and F is the fluorescence intensity of LS in solution at a given molar ratio of LS:Ln^{3+}.

Results

CD Studies. Addition of lanthanide salt solution to LS in acetonitrile induces considerable changes in the CD spectrum. The changes observed in the CD spectrum of LS in acetonitrile

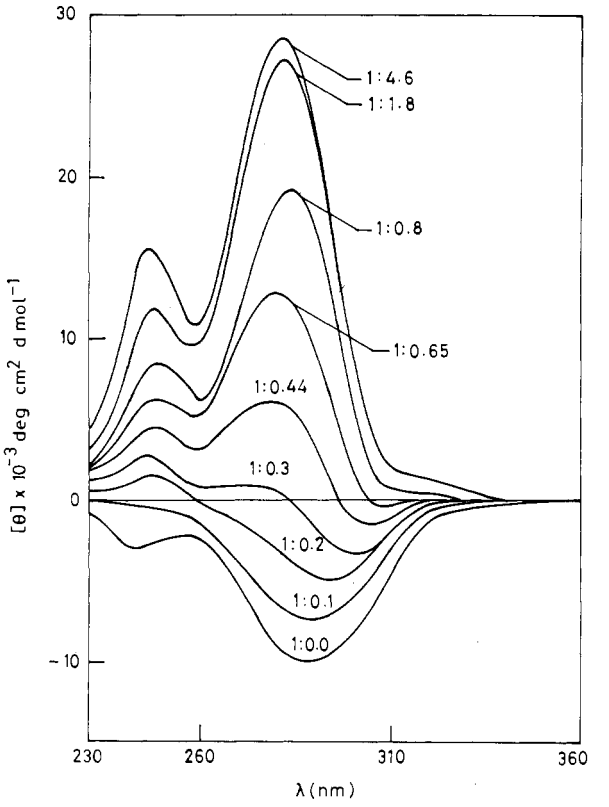

FIGURE 2: CD spectra for LS–La(NO$_3$)$_3$ complexation in acetonitrile at 25 °C: [LS] = 0.5 mM.

on gradual addition of La(NO$_3$)$_3$ salt solution are shown in Figure 2 as an illustrative example. As can be seen from this figure, the magnitude of the 290-nm band first decreases in intensity, changes its sign (i.e., becomes positive), then reappears as a new band at 280 nm with increasing intensity, and stabilizes at a molar ratio of 1:2. Similar changes are observed for the 245-nm band also, though they are rather small and more gradual. The CD spectra of LS–La^{3+}, LS–Tb^{3+}, and LS–Lu^{3+} complexes at those molar ratios where maximal changes have been observed are shown in Figure 3.

Salt-induced CD spectral changes have also been observed in methanol. The CD spectra of free LS and its Pr^{3+} complex in methanol at a molar ratio of 1:10 are shown in Figure 4. Upon addition of Pr(NO$_3$)$_3$ solution, the magnitude of the 290-nm band decreased whereas that of the 245-nm band increased. Similar spectra have been obtained upon addition of other lanthanide nitrate solutions although the magnitude of changes differed from one lanthanide to another.

The lanthanide ions exhibit absorption bands of low extinction coefficients in the visible region due to f–f transitions but do not show any CD bands since they are not in an asymmetric environment. However, at sufficiently high concentration of the metal ion ($\simeq$40 mM), they showed induced CD bands in the visible region when complexed with LS in acetonitrile. No CD bands were observed under the same experimental conditions in methanol. Figure 5 shows the CD spectrum of LS–Pr(NO$_3$)$_3$ complex in acetonitrile at a molar ratio of 1:1.

Salt titrations were done for all the 13 lanthanide ions. The variation in the molar ellipticity at 280 and 320 nm as a function of Ln^{3+} ionic radius is shown in Figure 6. The titration data obtained at 280 nm have been analyzed for obtaining the stoichiometries and binding constants as described under Materials and Methods. The results are summarized in Table I. Such calculations could not be performed for Ho^{3+}, Er^{3+}, and Tm^{3+} ions since the molar ellipticity values

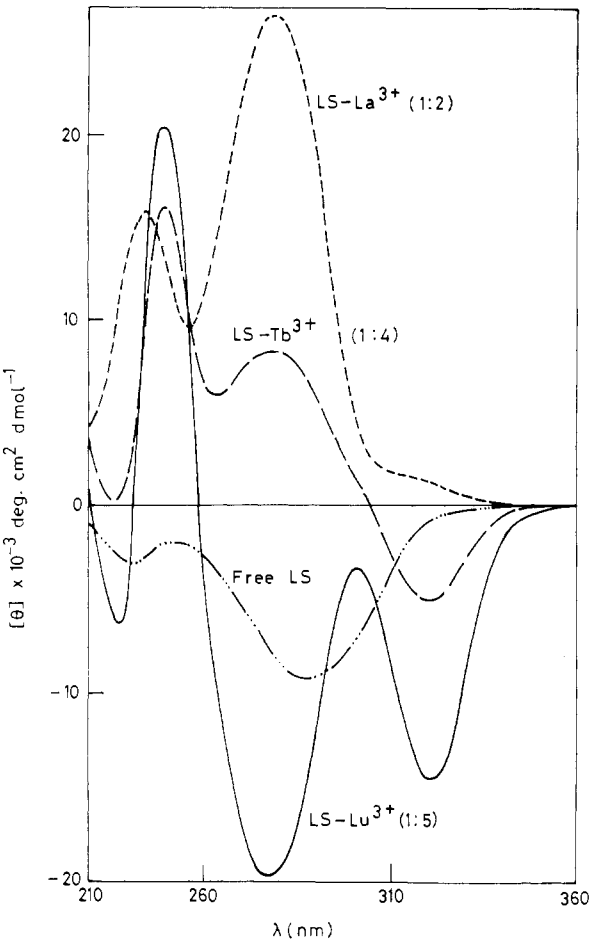

FIGURE 3: CD spectra of free LS, LS–La$(NO_3)_3$ (1:2), LS–Tb$(NO_3)_3$ (1:4), and LS–Lu$(NO_3)_3$ (1:5) complexes in acetonitrile at 25 °C: [LS] = 0.5 mM.

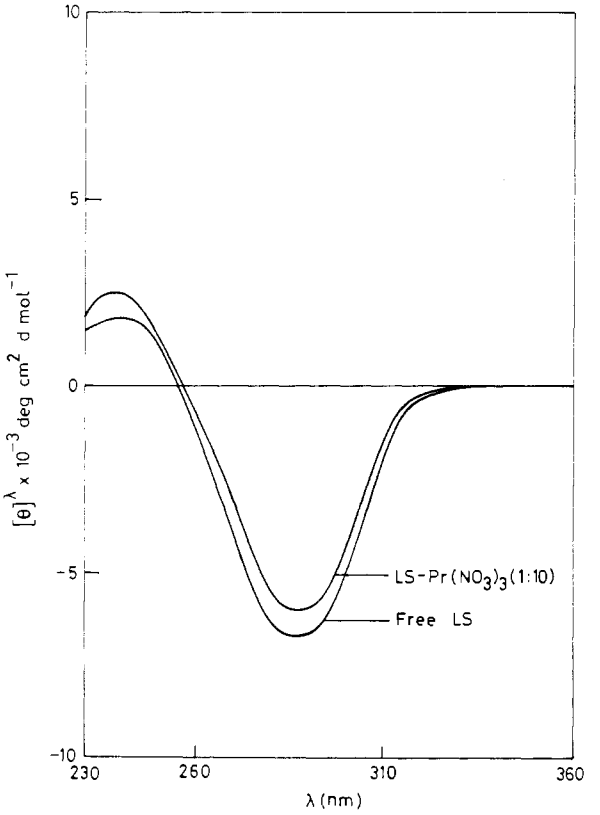

FIGURE 4: CD spectra of free LS and its $Pr(NO_3)_3$ complex at a molar ratio of 1:10 in methanol at 25 °C: [LS] = 0.5 mM.

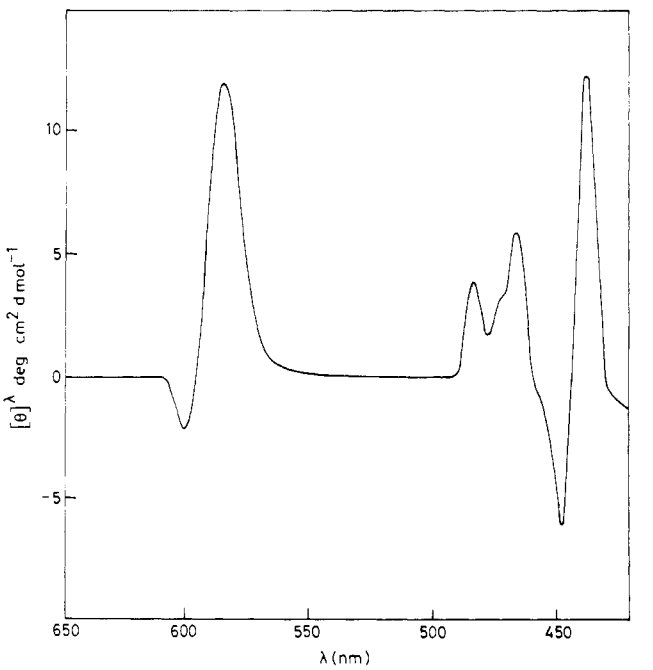

FIGURE 5: Induced CD spectrum for $Pr(NO_3)_3$ upon complexation with LS at a molar ratio of 1:1 in acetonitrile at 25 °C: [$Pr(NO_3)_3$] = 40 mM.

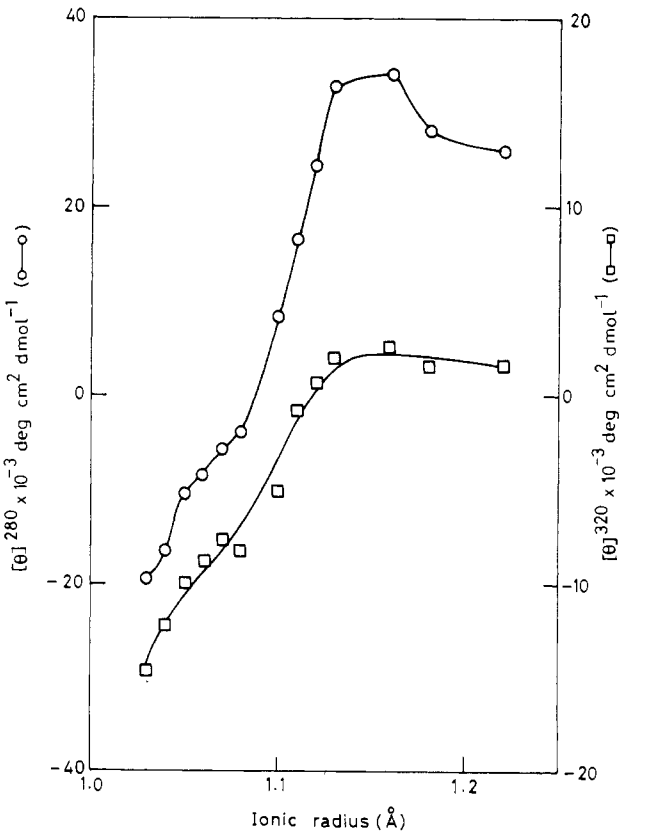

FIGURE 6: Variation in molar ellipticity at 280 (○) and 320 nm (□) as a function of ionic radius of the lanthanide ions.

at 280 nm for these complexes are close to that of free LS. Therefore, the binding constants for these complexes have been calculated by utilizing the titration data at 320 nm. This analysis has shown that LS forms both 1:1 and 2:1 complexes with all the 13 lanthanides in acetonitrile, and Figure 7 shows the dependence of log K (binding constant) on the size of the cation. The titration data did not fit with any other combinations of 1:1, 2:1, and 1:2 complexes. The CD titration graphs for LS–Pr^{3+} and LS–Lu^{3+} systems that form the two extremes

Table I: Binding Constants for Various LS–$Ln(NO_3)_3$ Complexes in Acetonitrile at 25 °C (from CD Analyses at 280 nm)

cation	ionic radius (Å)[a]	obsd molar ellipticity, $[\theta]^{280}_{obsd}$ ($\times 10^{-3}$ deg·cm²·dmol⁻¹)	limiting molar ellipticities $[\theta]_{1:1}$ ($\times 10^{-3}$ deg·cm²·dmol⁻¹)	limiting molar ellipticities $[\theta]_{2:1}$ ($\times 10^{-3}$ deg·cm²·dmol⁻¹)	binding constants K_1 ($\times 10^{-3}$ M⁻¹)	binding constants K_2 ($\times 10^{-3}$ M⁻¹)	RMSE, $[\theta]$ ($\times 10^{-3}$ deg·cm²·dmol⁻¹)
free LS		−10.0					
La^{3+}	1.22	27.2	37.0	17.0	323.00	1.64	0.48
Pr^{3+}	1.18	28.4	38.0	8.0	243.00	1.26	0.42
Nd^{3+}	1.16	33.4	52.0	20.0	199.00	2.44	0.86
Sm^{3+}	1.13	32.0	44.0	5.0	123.00	5.76	1.36
Eu^{3+}	1.12	24.0	36.0	7.0	322.00	9.09	0.79
Gd^{3+}	1.11	16.5	28.0	5.0	9.09	3.22	1.42
Tb^{3+}	1.10	−4.2	22.0	5.0	1.96	1.23	1.55
Dy^{3+}	1.08	−5.8	9.0	−8.0	4.76	1.25	0.45
Ho^{3+} [b]	1.07	−7.3	−7.0	−5.0	109.00	7.97	0.11
Er^{3+} [b]	1.06	−8.5	−8.0	−3.0	10.20	4.76	0.38
Tm^{3+} [b]	1.05	−9.8	−9.0	−14.0	2.44	4.77	0.32
Yb^{3+}	1.04	−16.3	−5.0	−10.0	1.24	10.00	0.43
Lu^{3+}	1.03	−19.4	−8.0	−17.0	1.24	9.09	0.53

[a] Ionic radii taken from Shannon (1976). [b] Analyses have been done at 320 nm, and the $[\theta]_{obsd}$ values correspond to this wavelength.

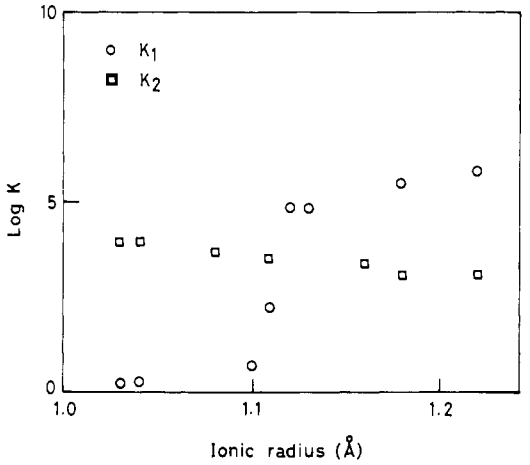

FIGURE 7: Dependence of log K_1 and log K_2 (binding constants) on the ionic radius of lanthanide ions.

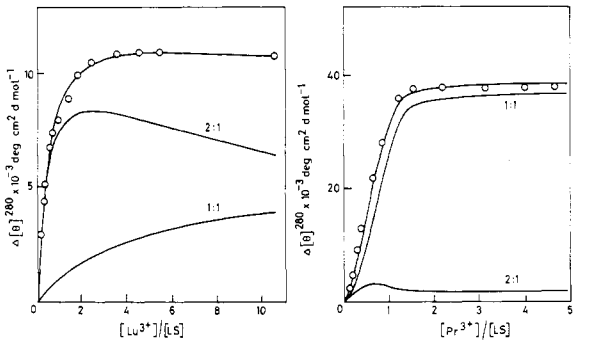

FIGURE 8: CD titration graphs for LS–$Pr(NO_3)_3$ and LS–$Lu(NO_3)_3$ complexation in acetonitrile: (O) experimental points; (—) computed curves.

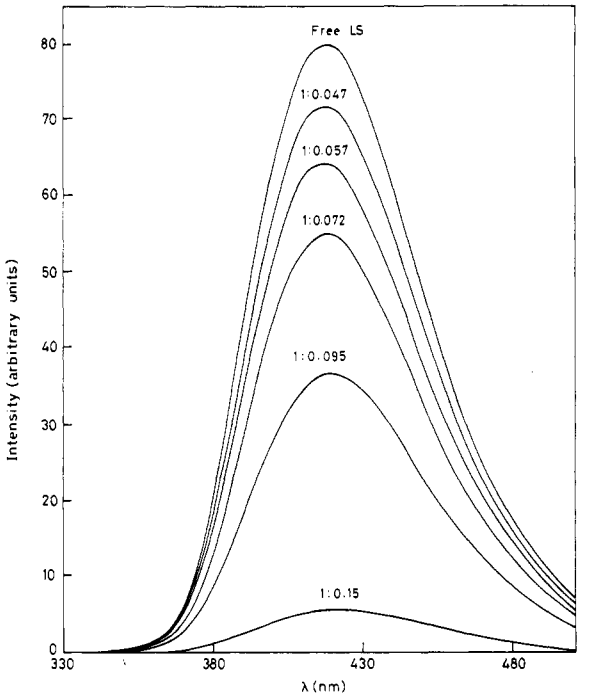

FIGURE 9: Fluorescence spectra for LS–$Pr(NO_3)_3$ complexation in acetonitrile at 25 °C: [LS] = 20 μM.

of the lanthanide series are shown in Figure 8 along with the resolved components.

Fluorescence Studies. The interaction of lanthanide nitrates with LS in acetonitrile and methanol has been studied by following the quenching of LS fluorescence intensity at 420 nm upon addition of lanthanide salt solutions. The fluorescence spectra of LS in acetonitrile- and Pr^{3+}-induced quenching are shown in Figure 9. All the lanthanides quench the LS fluorescence completely at approximately the same concentration. In the case of $Tb(NO_3)_3$, the decrease in LS fluorescence is accompanied by a concomitant appearance of a Tb^{3+} emission band centered at 487 nm with increasing intensity, and the corresponding spectra are shown in Figure 10. However, excitation at 310 nm for Tb^{3+} alone in acetonitrile does not produce any emission at 487 nm. The fluorescence titration graphs of LS complexation with La^{3+}, Pr^{3+}, Tb^{3+}, Dy^{3+}, Yb^{3+}, and Lu^{3+} ions in acetonitrile and methanol are shown in Figure 11. As it can be seen from this figure, the quenching effect produced in methanol by the addition of lanthanide ions is less pronounced compared to that observed in acetonitrile, and the quenching efficiency varied from one lanthanide to another. In addition, a hump with gradually increasing clarity is seen in the titration graphs for the quenching experiments carried out in methanol as one goes down the series.

Discussion

The changes observed in the molar ellipticity at 280 nm in the CD spectrum of LS in acetonitrile upon gradual addition of Ln^{3+} ions indicate a considerable change in the asymmetric environment surrounding the carbonyl group and suggest that the carbonyl group is involved in binding to the cation. The changes observed at 245 nm vary from one lanthanide to another and indicate the participation of the salicylic acid moiety as well in binding to the cations. This is further

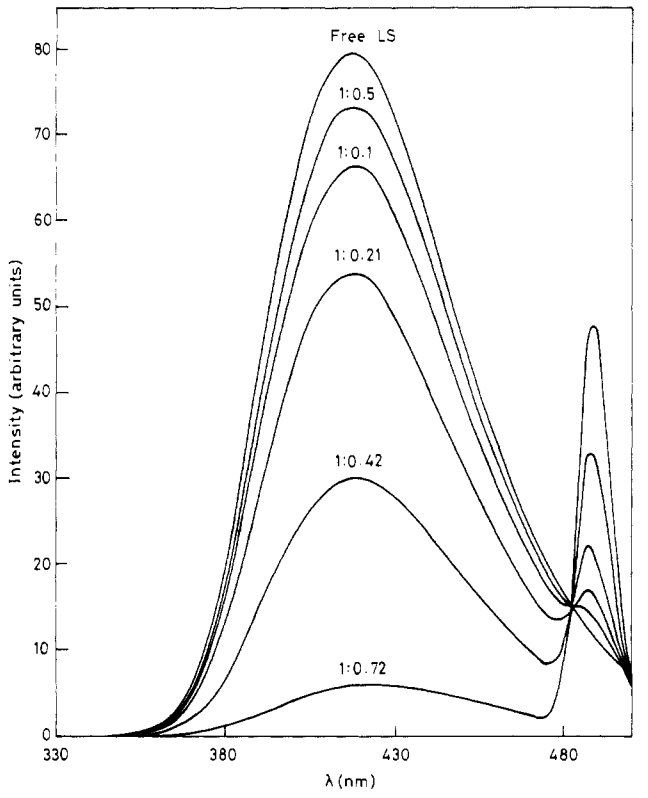

FIGURE 10: Fluorescence spectra for LS–$Tb(NO_3)_3$ complexation in acetonitrile at 25 °C: [LS] = 20 μM.

supported by the changes observed in the molar ellipticity at 320 nm and the complete quenching of LS fluorescence signal upon addition of lanthanide salt solutions in acetonitrile. The induced CD bands observed for Ln^{3+} ions in the presence of LS in acetonitrile show that they are in an asymmetric environment and strongly suggest that they are bound to LS. Further evidence to the participation of the salicylic acid group in binding to the cation comes from the observation of Tb^{3+} fluorescence upon excitation of LS at 310 nm. As can be seen from Figure 6, the molar ellipticity at 320 nm remains almost the same for the first few lanthanide ions in the series and then gradually changes to a negative value, indicating that the binding of a salicylic acid group to the cation necessitates changes in its conformation and orientation with reduction in the size of the cation. The CD data analyses show that in the beginning of the lanthanide series 1:1 complexes are predominantly formed whereas the formation of 2:1 complexes is favored as one goes from La^{3+} to Lu^{3+} (see Figure 7). Figure 7 reveals an interesting dependence of log K on ionic radius for both 1:1 and 2:1 complexes. Whereas the 2:1 complexes do not show much variation in their binding constants, the log K_1 changes in a stepwise fashion as a function of ionic radius. For 1.22 Å > ionic radius > 1.13 Å, 1:1 complexes are preferred whereas for 1.13 Å > ionic radius > 1.03 Å 2:1 complexes are preferred. In other words, when the size of the cation is large, LS forms a complex of 1:1 stoichiometry with a large binding constant. The coordination to the cation in these complexes probably comes from the tetrahydrofuran and the tetrahydropyran ring oxygens, the two hydroxyl group oxygens, and the carboxyl group of the salicylic acid moiety besides the carbonyl group oxygen. The other coordination sites are probably filled by the counterions (i.e., nitrate ions) and/or solvent molecules. Formation of the 1:1 complex does not require any considerable changes in the conformation and/or orientation of the salicylic acid group. However, as the size of the cation decreases the salicylic acid moiety has to fold in to provide maximum coordination to the cation in order to form a 1:1 complex which, in turn, would lead to changes in its conformation and orientation that are reflected in the molar ellipticity values at 320 nm. As a consequence, the stability and formation of 1:1 complex decrease due to steric and torsional strain, and the formation of 2:1 complex is favored, in which the cation is held between two LS molecules without involving the salicylic acid groups and possibly the counterions/solvent molecules as well. The two salicylic acid groups are likely to be in an anti configuration (Patel & Shen, 1976; Vishwanath & Easwaran, 1985). The proposed models for the 1:1 and 2:1 complexes in acetonitrile are shown in Figures 12 and 13, respectively. The conformations of these two complexes are similar to those of the LS–Ca^{2+} complexes. However, the LS–Ca^{2+} 2:1 complex has a binding constant of 2.2×10^4 M^{-1} and is more stable than any of the LS–Ln^{3+} 2:1 complexes. The LS–Ca^{2+} 1:1 complex has a binding constant of 4.8×10^2 M^{-1} and is weaker than the LS–Ln^{3+} 1:1 complexes (Vishwanath & Easwaran, 1983).

The CD spectral changes observed in methanol are very different compared to those observed in acetonitrile. In all the cases, we observed CD spectral shapes similar to that of free LS with differing molar ellipticity values. No induced CD bands could be detected for Ln^{3+} ions in methanol when bound to LS. The analyses of CD titration data to obtain stoichiometries and binding constants were not conclusive and often hampered by rather small differences in molar ellipticities between the complexes and free LS. Also, the binding constants obtained were not with satisfactory rms error for the molar ellipticity calculations. However, it appears that both 1:1 and 2:1 complexes are possible in methanol as well (data not shown), and the CD spectral changes observed suggest participation of the salicylic acid moiety in complex formation. The results obtained by earlier workers (Chen & Springer, 1978; Richardson & Dasgupta, 1981) on the interaction of LS anion with lanthanide ions in methanol demonstrate the participation of salicylic acid in binding to the cation. However, their results do not provide enough evidence for the participation of the carbonyl group and other oxygens coordinating to the cation (Richardson & Dasgupta, 1981). The reasons are twofold. First, the decrease in the molar ellipticity at 290 nm of the CD band upon addition of Pr^{3+} ion could be due to a decrease in the salicylic acid contribution to the combination band [^1L_b transition: Degani and Friedman (1974)]. Second, the observed CPL in the case of Eu^{3+} and Tb^{3+} ions upon LS complexation could have been as well induced by salicylic acid per se.

The results of the fluoresence studies also suggest that the LS–Ln^{3+} complexes are more stable in acetonitrile than in methanol (Figure 11). Although, no reliable information could be obtained from the CD studies in methanol as to the stoichiometries of the complexes, the increasing hump in the fluorescence titration graphs suggests that LS forms both 1:1 and 2:1 complexes in methanol as well. In addition, the salicylic acid group does not participate in binding to the cation in the 2:1 complexes as can be seen from the very low quenching observed till a molar ratio of 0.5. The failure to observe such a hump in the earlier studies is probably due to the use of LS anion. Under such conditions, lanthanide ions preferably bind to the salicylic acid group, and 2:1 complexes are not formed. From the fluorescence studies in methanol, it can be inferred that the carbonyl group and the other oxygens participate in coordination to the cations in the 1:1 and 2:1 complexes. The reason for not observing significant changes in the CD studies could be due to rather open

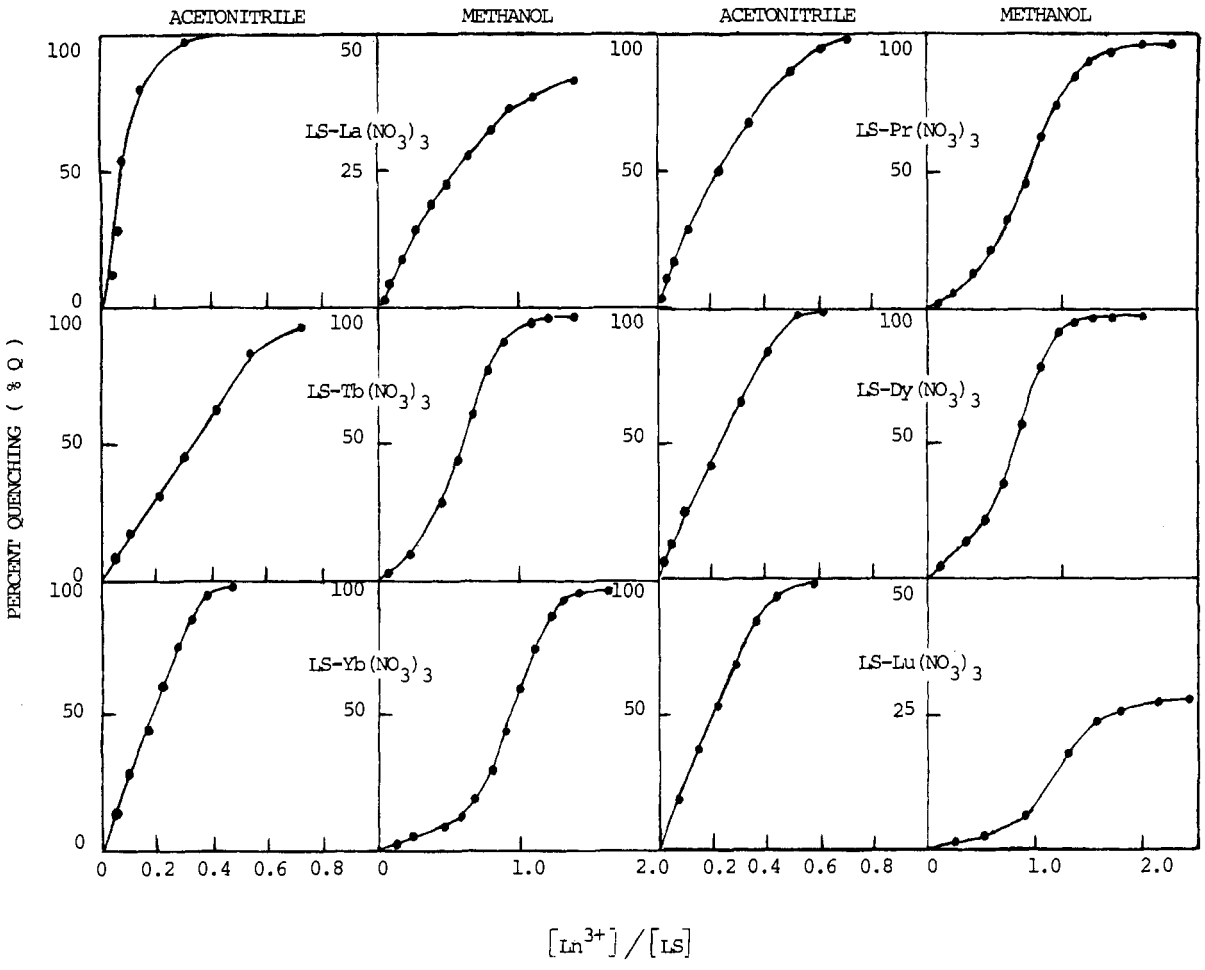

FIGURE 11: Fluorescence titration graphs for LS–La$(NO_3)_3$, LS–Pr$(NO_3)_3$, LS–Tb$(NO_3)_3$, LS–Dy$(NO_3)_3$, LS–Yb$(NO_3)_3$, and LS–Lu$(NO_3)_3$ complexation in aceonitrile and in methanol.

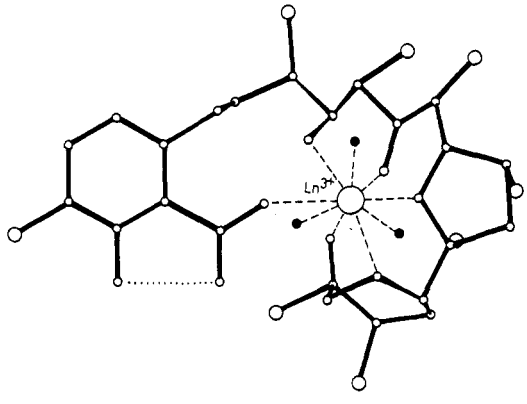

FIGURE 12: Conformational model for the 1:1 LS–Ln$(NO_3)_3$ complex in acetonitrile (● represents either solvent or anion).

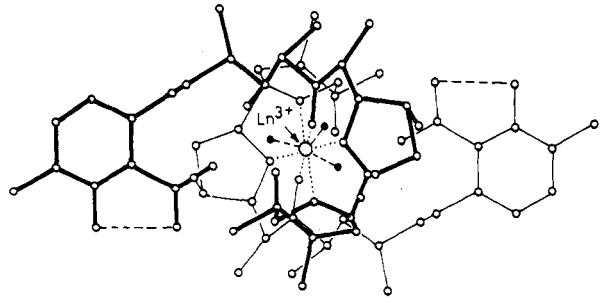

FIGURE 13: Conformational model for the 2:1 LS–Ln$(NO_3)_3$ complex in acetonitrile (● represents either solvent or anion).

structures for LS in both free and complexed forms in methanol owing to its better solvation properties compared to those in acetonitrile (Degani & Friedman, 1974). This is justified by the fact that induced CD bands for lanthanide ions are observed only in acetonitrile and not in methanol.

In conclusion, the present studies on the interaction of lanthanide nitrates with LS in solution show that LS forms both 1:1 and 2:1 complexes in acetonitrile and in methanol. The complexes are less stable in methanol and also have different conformations compared to those in acetonitrile. A strong dependence of stoichiometries and binding constants has been observed for the complexes in acetonitrile. The conformations of the complexes are critically dependent on the degree of ionization of LS, ionic radius of the cation, and the solvent environment. It is interesting to note that for ions like Ca^{2+}, which have radii less than 1.03 Å, LS is a very good ionophore and 2:1 complexes are preferred in a membrane mimetic solvent like acetonitrile. The possible conformations LS may adopt at the membrane–water interface require not only a knowledge of dependence of the stoichiometries the binding constants, and the conformations of LS–cation complexes on solvent polarity and ionic radius but also the mode of interaction of LS with membranes. Our results on this aspect will form part of another publication (Sankaram et al., 1987).

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Interaction of Carrier Ionophores with Phospholipid Vesicles†

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ABSTRACT: The interactions of carrier ionophores, nonactin, A23187, and lasalocid A with liposomes formed from the synthetic lipids dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine are investigated by differential scanning calorimetry and ^1H and ^{31}P nuclear magnetic resonance techniques. The results indicate that the mode of interaction of these ionophores is dependent on the fluidity of the bilayer and on the chemical nature of these ionophores. The ^{31}P NMR studies are suggestive of the formation of small particles that are probably intervesicular lipid–ionophore aggregates in multilamellar vesicles when they are incorporated with these ionophores at high concentrations. The results are interpreted on the basis of the chemical structure and conformations of the ionophores in membrane mimetic media. The ^1H NMR line-width measurements indicate that the aromatic rings containing the carboxyl groups of lasalocid A and A23187 are located near the membrane interface while the rest of the molecule is buried in the membrane interior.

A prerequisite for understanding of the ionophore-mediated cation transport across membranes is a detailed knowledge of the preferred conformations of the ionophores, their cation complexes in solution, and their interaction with model and biological membranes. During the last few years, several studies have been aimed at understanding the structure and conformations of ionophores and their cation complexes, and these results clearly demonstrated that the cation selectivity of the ionophores depends not only on the nature of ligands they contain but also on the conformation of the ionophore–cation complex as an entity and this, in turn, is dependent on size and charge of the cation, the counterion (i.e., anion), and the solvent environment (Alpha & Brady, 1973; Chen & Springer, 1978; Degani et al., 1973; Degani & Friedman, 1974, 1975; Devarajan et al., 1980; Easwaran, 1985; Hamilton et al., 1981; Haynes & Pressman, 1974; Johnson et al., 1970; Ovchinnikov et al., 1974; Sankaram & Easwaran, 1982, 1985; Shastri & Easwaran, 1984; Shastri et al., 1987; Vishwanath & Easwaran, 1982, 1983, 1985; Young & Gomperts, 1977). However, studies dealing with the interaction of these ionophores with model and biological membranes are limited though some studies on the interaction of valinomycin (VM)[1] with model membranes have been reported (Hsu & Chan, 1973; Grell et al., 1974; Walz, 1977, 1979; Feigenson & Meers, 1980; Sankaram & Easwaran, 1984). In this paper, we report our results on the interaction of nonactin (NA), lasalocid A (LS), and A23187 (see Figure 1 for chemical structures of the ionophores) with liposomes (both unilamellar and multilamellar vesicles) made from two synthetic lipids, namely, dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) studied with differential scanning calorimetry (DSC) and ^1H and ^{31}P nuclear magentic

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[1] Abbreviations: CD, circular dichroism; CSA, chemical shift anisotropy; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; LS, lasalocid A; MLVs, multilamellar vesicles; NA, nonactin; NMR, nuclear magnetic resonance; ULVs, unilamellar vesicles; VM, valinomycin.

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