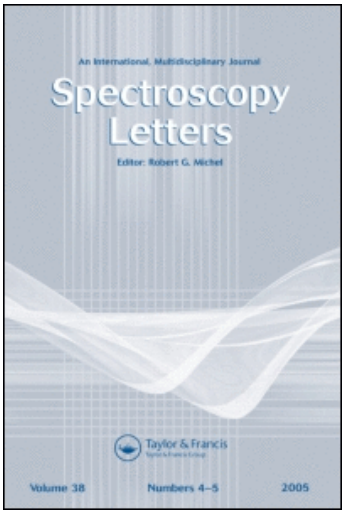


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# NMR and FT-Raman Studies on the Interaction of Lanthanide Ions with Sphingomyelin Bilayers

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# NMR AND FT-RAMAN STUDIES ON THE INTERACTION OF LANTHANIDE IONS WITH SPHINGOMYELIN BILAYERS

Key Words:NMR, FT-Raman, Sphingomyelin Bilayers, Lanthanide ions

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ABSTRACT:

The interactions of lanthanide ions with sphingomyelin bilayers have been studied by using 2D NOESY spectroscopy and FT-Raman spectroscopy methods. The results indicate that lanthanide ions, as well as divalent calcium, combine mainly to the phosphate group in the polar headgroup and do not change the conformation of O-C-C-$N^+$ backbone in the choline group of sphingomyelin bilayers. The polar headgroup is still

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extending parallel to the bilayer surface and O-C-C-$N^+$ group is still in its gauche conformer.

## INTRODUCTION

With the extensively application of rare earth in agriculture, medicament, especially the applica-tion of Gd-DTPA as nuclear magnetic resonance image reagent in clinical practice(1), the studies on the toxicology and the interactive mechanism of rare earth ions and their complexes in biological body, as well as the study on the use as informative probes in stead of divalent calcium ion in biolo-gical and biochemical research have attracted intensive concern(2).



Phospholipids bilayers have served as a model of biomembrane in research in last two decades. The effects of metal ions on the polar headgroup of bilayers are the focus(3-6) because the polar head-groups are located in the intermediary position between the hydrophobic and hydrophilic regions in the membrane bilayers in aqueous solution and therefore, the conformational changes would cause the structural changes of lipids bilayers and thus signicantly influence the physiological function of membrane protein. Controvercial conclusions were drawn from different experiments(3-5). We have used 2D NOESY and Raman spectroscopies in the investigation of the effects of trivalent lanthanide ions and their complexes on the conformation of polar headgroup of dipalmitoylphosphatidylcholine (DPPC) bilayers(7). To extend this work, we report here the results of 2D NOESY and FT-Raman spectroscopies studies on the influences of lanthanide ions on sphingomyelin (SPM, a major component of several biological tissues such as brain and nerve cells) bilayers which have identical polar headgroup as DPPC bilayers.

## EXPERIMENTAL:

Sphingomyelin was commmercially obtained from Sigma Chemical Co.. $D_2O$(99.8%), $CaCl_2$ (A.R.) were products of Beijing Chem. Plant. $Ln_2O_3$ were products of YaoLong Chemical Plant. Deuterochloride(DCl) was obtained from Aldrich Chemical Co. Inc. All other reagents were reagent grade.

$LnCl_3$ solutions were prepared by dissolving their oxides into DCl solution for NMR experi-ments or in HCl solution for Raman easurements and eliminating the excess acid. pH of these solutions were around 5.4. The concentrations were determined by EDTA titrations, using arsenazo as indicator. Sphingomyelin liposomes were prepared as described elsewhere(8). The samples for Raman measurements were prepared as described in Ref.(9).



NMR experiments were carried out on Varian Unity 400 Spectrometer at 50 °C. $T_1$ values were measured by using the inversion recovery sequence. 2D NOESY spectra were performed with the pulse sequence $(\pi/2\text{-}t_1\text{-}\pi/2\text{-}\tau m\text{-}\pi/2\text{-}t_2)n$. To suppress the J cross peaks, 10% random was introduced in mixed time(10).

FT-Raman spectra were recorded on Bruker RFS-100 FT-Raman spectrometer equipped with a InSb detector. Excitation was provided by a 1.064 μm CW Nd:YAG laser. The power of laser beam was 100 mW. Experimental temperature was maintained at 18 °C.

## RESULTS AND DISCUSSION:

### 1. The Coordination Site of Lanthanide Ions to Sphingomyelin Bilayers

Table 1 shows the $T_1$ values of sphingomyelin bilayers with the presence and absence of $Gd^{3+}$. $Gd^{3+}$ is a paramagnetic relaxation reagent.

TABLE 1. The spin-lattice times($T_1$) and assignments of $^1H$ in sphingomyelin bilayer in absence and presence of $Gd^{3+}$.

| Chemical Shift | Assignment | T1(sec)* | |
|---|---|---|---|
| ppm | | 0.1 M SPM | 0.01 M SPM + 0.02 mM $Gd^{3+}$ |
| 0.88 | $CH_3$ | 0.85 | 0.83 |
| 1.28 | $(CH_2)_n$ | 0.71 | 0.67 |
| 2.00 | $CH_2CON$ | 0.70 | 0.65 |
| 3.22 | $N(CH_3)_3$ | 0.43 | 0.32 |
| 3.66 | $CH_2N$ | 0.42 | 0.33 |
| 4.29 | $CH_2OP$ | 0.36 | 00.1 |
| 5.29 | CH=CH | 0.70 | 0.60 |

*The average of three times measurements, SD= ± 0.03 s



The scalar contribution of this ion to spin-lattice relaxation time($T_1$) is negligible. Hence, the spin-lattice relaxation rate is proportional to $r^{-6}$, where r is the internuclear distance between the metal ion and nuclear in question. The greatest decrease in spin-lattice relaxation time was found in Table 1 of proton atoms near the phosphate moiety in polar headgroup. Therefore, we concluded that Gadolinium(III) ion was combined to the phosphate group of the polar headgroup of sphingomyelin bilayers. The lanthanide induced shifts(LIS) of some other lanthanide ions to the various proyons of sphingomyelin molecule were also measured as shown in Table 2. The largest LIS data were observed at the protons close to phosphate group. These data were in acordence with $T_1$ values. Therefore, lanthanide ions combine mainly to the phosphate group in the polar headgroup of sphingomyelin bilayers.

TABLE 2 The lanthanide induced $^1H$ chemical shift(ppm) of sphingomyelin bilayers*

| | $Ce^{3+}$ | $Pr^{3+}$ | $Nd^{3+}$ | $Eu^{3+}$ |
|---|---|---|---|---|
| $N(CH_3)_3$ | 0.06 | 0.16 | 0.18 | - 0.11 |
| $CH_2N$ | 0.10 | 0.25 | 0.21 | - 0.24 |
| $CH_2OP$(choline) | 0.20 | 0.46 | 0.30 | - 0.57 |
| $CH_2OP$ | 0.16 | 0.21 | 0.22 | - 0.40 |
| CHOCO | 0.01 | 0.05 | 0.09 | - 0.08 |

* 10 mmol.dm$^{-3}$ $Ln^{3+}$/ 20 mmol.dm$^{-3}$ SPM



2. The Effect of Metal Ions on the Conformation of O-C-C-$N^+$ backbone

As described above, lanthanide ions were combining to the polar headgroup region of bilayers, concisely, to the phosphate group. The effect of lanthanide ions on the conformation of polar head-group is of great interest. We are involved in using 2D NOESY and FT-Raman spectroscopies studies on this issue.

Fig.1 shows the 2D NOESY spectra of sphingomyelin bilayers with or without the presence of lanthanide ion $Eu^{3+}$. In Fig.1A, sphingomyelin bilayers with the absence of $Eu^{3+}$ ion, we found that proton of $N(CH_3)_3$ in polar headgroup has cross-peak not only with the methylene groups of choline group but also with the methylenes of hydrocarbon chains. As we known, the cross relaxation occurred through two pathways: spin diffusion and close spatial interaction. Experimental performed by Xu et al(11) with deuterated lipids had shown that cross relaxation is not mediated by spin diffusion, but by close spatial interaction. This means that the spatial distance between the protons of $N(CH_3)_3$ and methylene groups of hydrocarbon chains is within 5 A($\tau m$ = 500 ms) which allows the

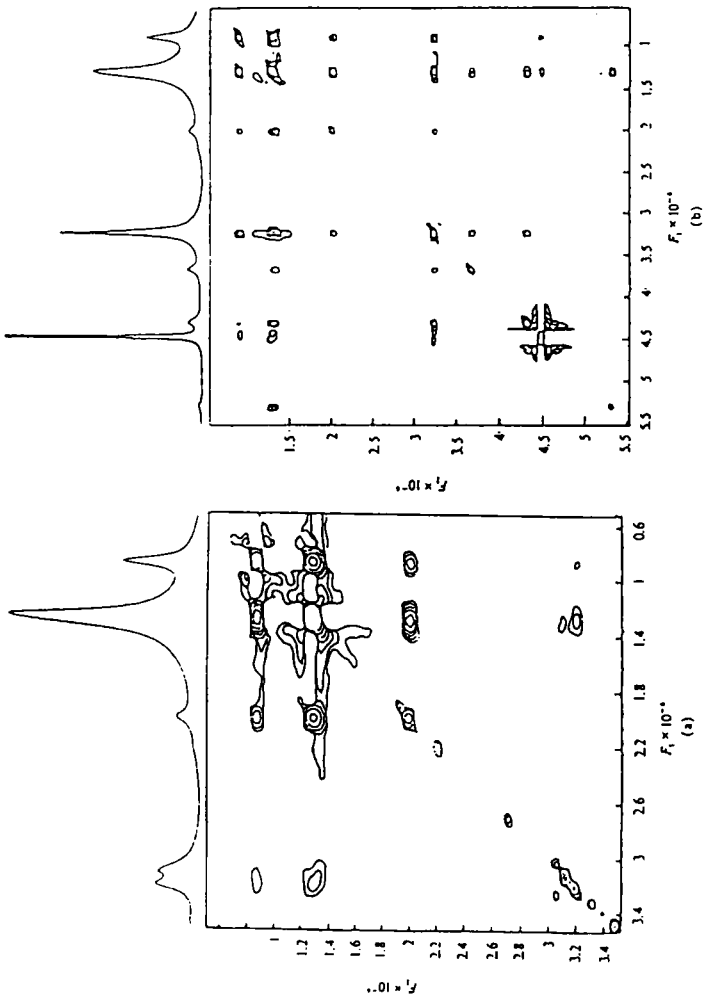


Fig. 1. 2D proton NOESY spectra of sonicated sphingomyelin liposomes (A) and sphingomylein liposomes in the presence of $Eu^{3+}$ ion (B). The molar ratio of $Eu^{3+}$/SPM is 1:5. The mixing time, $\tau m$ is 500 ms and data set consisted of 512 blocks with 512 data points per block. Each block represents 32 acquisition.

development of NOE cross-peak between the protons in two groups. This is in agreement with that the polar headgroup of sphingomyelin bilayers in liquid-crystalline state is extending parallel to the membrane surface. In the presence of $Eu^{3+}$ ions, a paramagnetic shift ion, which can only combine to the outer surface of sphingomyelin bilayers, and thereby only shifting the proton signal of outer surface to high field, two peaks of $N(CH_3)_3$ were observed in Fig. 1 and both of them have cross-peaks with the methylene groups of hydrocarbon chains. This strongly demonstrates that the presence of lanthanide ions does not alter the spatial distance between $N(CH_3)_3$ and methylene groups of hydrocarbon chains. Therefore, the polar headgroup in the presence of lanthanide ions is still extending parallel to the membrane surface.



The Raman spectra of sphingomyelin bilayers in 600-950 $cm^{-1}$ regions with or without the presence of lanthanide ions and divalent calcium ion were shown in Fig.2. The characteristic bands observed in this region are 717,763,770,875 and 889 $cm^{-1}$. The Raman band at 717 $cm^{-1}$ has been assigned to $C_4N^+$ totally symmetric stretching mode(12). The band at 763 $cm^{-1}$ is the result of OPO diester symmetric stretch(13), while 875 and 889 $cm^{-1}$ have been assigned to carbon-carbon stretch in flid-like and crystallinity state of interfacial region(13). The predominant changes of spectral feature in Fig.2 are the shift of 763 $cm^{-1}$ to lower wavenumbers and 770 $cm^{-1}$ to higher. Two bands occurred of OPO diester symmetric stretch may be resulted from different length of hydrocarbon chains in sphingomyelin molecules. When at higher concentration of lanthanide ions, the lower band at 763 $cm^{-1}$ disappeared. This result demonsrtrates that the binding site of metal ions to bilayers is the phosphate moiety of polar

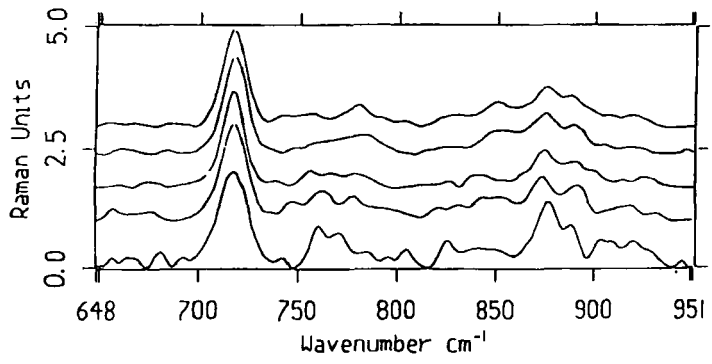


Fig.2 Raman spectra of sphingomyelin bilayers with or without the presence of metal ions in 650-950 $cm^{-1}$ region. The lipid was dispersed in : a. Distilled water, b. 10 mM $LaCl_3$ solution, c. 40 mM $LaCl_3$ solution, d. 0.1 M $LaCl_3$ solution, e. 0.2 M $CaCl_2$ solution.



headgroup. This is in accord with the results of $^1$H-NMR spin-lattice time and lanthanide induced chemical shift measurements. The band at 717 $cm^{-1}$ that originated from the totally symmetric C-N stretching mode of $C_4N^+$ group, characteristic of O-C-C-$N^+$ backbone in its gauche conformation(12) remains invariant in the presence of $La^{3+}$ and $Ca^{2+}$ ions. No new band observed at 770 $cm^{-1}$ that originated from the totally symmetric C-N stretching mode of $C_4N^+$ group when the backbone of O-C-C-$N^+$ in ist trans conformation(12). The constancy of both the intensity and the frequency of 717 $cm^{-1}$ band and no 770 $cm^{-1}$ band occurred indicate that no conformational chage occurred in polar headgroup of sphingomyelin bilayers combined with rare earth ions and calcium ion. The conformation of choline group is still in its gauche form which allows the polar headgroup extend parallel to the surface of sphingomyelin bilayers. Another apparent change observed in this region is the increase of the intensity ratios of

$I_{889}/I_{875}$ in the presence of metal ions. The peak intensity ratio of $I_{889}/I_{875}$ was used to monitor the physical state changes in interfacial region(13). The increase of the intensity ratio of $I_{889}/I_{875}$ suggested that the interfacial region of sphingomyelin bilayers be in an ordered state. Certainly, This conclusion may be dependent on the further investigation.

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