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PROTON MAGNETIC RESONANCE DETECTION OF IONOPHOR MEDIATED TRANSPORT OF PRASEODYMIUM IONS ACROSS PHOSPHOLIPID MEMBRANES

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SUMMARY

This study describes the use of proton magnetic resonance spectroscopy (PMR) to detect the penetration of paramagnetic cations into the internal compartment of lipid vesicles. It has been previously demonstrated that when a non-penetrating paramagnetic cation such as Pr^{3+} is added to an aqueous lecithin dispersion, the proton magnetic resonance signal of the $-N^+(CH_3)_3$ shows two distinct peaks which arise from the groups in the inside and outside of the vesicles, respectively. When Pr^{3+} is added to lecithin liposomes containing the ionophore X-537A, the PMR signal of the trimethylammonium protons, shows only one distinct peak. This single peak is the result of the interaction of Pr^{3+} with the $-N^+(CH_3)_3$ groups of both the inner and outer layers of the liposome. This suggests that the antibiotic allows the diffusion of the lanthanide into the interior of the vesicle. Experiments performed in black lipid films show that indeed the ionophore makes the membrane permeable to Pr^{3+}, thus supporting the interpretation of the PMR data.

The ability of paramagnetic cations to modify the PMR spectra of a very large number of biological molecules, has been widely studied[1].

It has been recently shown that Eu^{3+} (ref. 2) and Pr^{3+} (ref. 3) allow the discrimination between the external and the internal surfaces of egg lecithin liposomes, as detected by PMR. The distinction depends on the property of these cations to induce shifts in the signals of certain groups in contact with them, and also on their inability to penetrate into the internal compartment of the lipid vesicles.

In particular[3], when $PrCl_3$ is added to the external aqueous phase of lecithin liposomes, the signal from the trimethylammonium protons exhibits two components. One component arises from the groups in the internal surface of the liposome which are not affected by the cation, and thus are not displaced in the spectrum. The other component arises from the groups in the surface wich are in contact with Pr^{3+} and hence shifted downfield.

If the liposome were made permeable to Pr^{3+}, the $-N^+(CH_3)_3$ groups on both, the external and the internal surface would be exposed to the cation, and thus only

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one peak (the shifted one) would appear. This possibility was explored with the use of the ionophorous antibiotics.

It has been reported that the carboxylic acid antibiotic X-537A transports alkali and alkaline earth cations across membranes[4–9]. In addition, it binds La^{3+} (ref. 10). It thus appeared possible that such an antibiotic would complex and transport paramagnetic cations into the interior of liposomes. If such transport occurred it could be ascertained by the changes in the PMR spectra of $-N^+(CH_3)_3$ as discussed above. Further, the ionophore-mediated transport of Pr^{3+} across a phospholipid membrane can be studied in black lipid membranes.

Egg yolk lecithin from Sigma was purified according to the method of Singleton *et al.*[11]. The purity was checked by thin-layer chromatography. Praseodymium chloride was purchased from K and K Laboratories Inc. and ^2H_2O (99.7%) from Sigma Chemical Co. *n*-Octane was A.R. grade reagent from Matheson, Coleman and Bell. The antibiotic X-537A was a gift from Dr S. Estrada of this laboratory. All other chemicals were reagent grade. Glass redistilled water was used throughout.

For the PMR experiments a chloroform–methanol (9:1, v/v) solution of egg lecithin was evaporated to dryness with N_2 in a glass tube. After the addition of the required amount of water (^2H_2O) the mixture was sonicated with an ultrasonic disintegrator (Branson Instruments) for 20 min in an ice-cooled vessel. The experiments with X-537A were performed by adding the antibiotic to the chloroform–methanol solution of lecithin before drying with N_2. The pH of the dispersions was 6.0 ± 0.3 unless otherwise noted. The PMR spectra were recorded with a Varian A 60 spectrometer at 33 °C, 1 h after preparing the dispersions. Chemical shifts were measured taking the $(CH_2)_n$ signal of lecithin as internal standard. The experiments were repeated at least seven times.

Bimolecular (black) lipid membranes (BLM) were formed and their electrical properties studied by the method of Mueller and Rudin[12] as previously described in detail by Montal[13]. The membranes were formed from a solution of 4% egg phosphatidylcholine in *n*-octane. All data presented are representative of 3 to 5 experiments. All the experiments were performed at a temperature of 29 ± 1 °C.

PMR EXPERIMENTS

Fig. 1a shows the $-N^+(CH_3)_3$ proton signal (−119.5 Hz) from a 4% aqueous (^2H_2O) lecithin dispersion. As previously reported by Fernández and Cerbón[3], upon addition of 5 mM $PrCl_3$, the polar head group signal splits into two components (Fig. 1b): peak I (−119.0 Hz) which remains at the normal position for $-N^+(CH_3)_3$ protons and peak E (−128.5 Hz) which is shifted downfield. As Pr^{3+} does not penetrate into the liposomes, it is possible to adscribe peak E to the external groups in contact with the lanthanide, and peak I to the internal ones not interacting with it.

In the absence of Pr^{3+} the trimethylammonium signal is the same in the presence or the absence of X-537A (Fig. 1a). Furthermore, at low concentration of ionophore used the PMR signal of its protons cannot be detected. A distinct change in the PMR spectra of liposomes containig X-537A, becomes apparent upon addition of 5 mM $PrCl_3$ to the outside water phase of the system. Under this experimental condition, the trimethylammonium signal exhibits a single peak whose line position is −127.5 Hz *i.e.* it is shifted downfield (Fig. 1c). This spectrum differs from the one

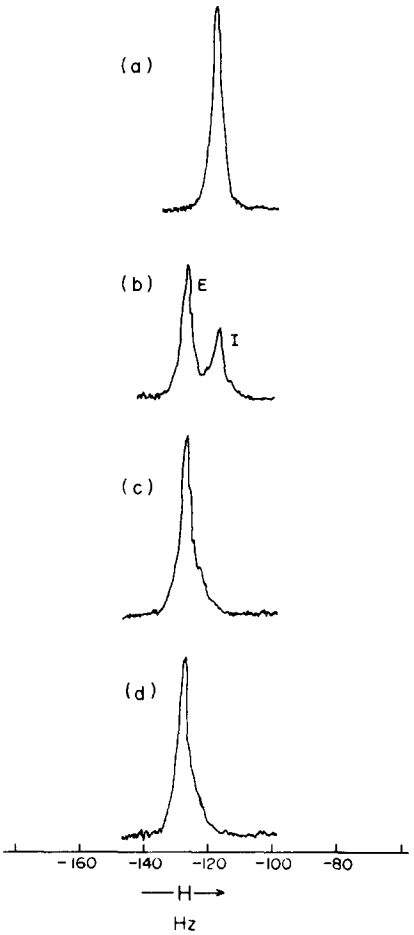

Fig. 1. The trimethylammonium proton magnetic resonance signal of a 4% (w/v) aqueous dispersion of egg yolk lecithin: (a) Sonicated in ^2H_2O. The presence of X-537A does not modify the signal position (−119.5 Hz). (b) Upon addition of 5 mM $PrCl_3$ following sonication in ^2H_2O. Two peaks appear: peak E (−128.5 Hz) corresponding to the external trimethylammonium groups, and peak I (119.0 Hz) representing the internal ones. (c) Upon addition of 5 mM $PrCl_3$ following sonication in ^2H_2O in the presence of X-537A (22.5 μg/mg lipid). The signal exhibits only one component at −127.5 Hz. (d) Sonicated in a 5 mM aqueous $PrCl_3$ solution. Signal position: −127.5 Hz. All the experiments were carried out at pH 6. Line positions were determined relative to the $(CH_2)_n$ signal of lecithin. The accuracy was about ±0.5 Hz.

obtained in the absence of ionophore (Fig. 1b) and suggests that both external and internal surfaces of the liposome are interacting with Pr^{3+}. When lecithin is sonicated in an aqueous solution of 5 mM $PrCl_3$, the paramagnetic ion will interact with both the internal and the external polar groups of the vesicles. Under this condition the recorded spectrum (Fig. 1d) shows a trimethylammonium signal (−127.5 Hz) which is identical to the one shown in Experiment 1c. These results may suggest therefore that X-537A acts as an ionophore for Pr^{3+} in lecithin liposomes.

It must be noticed that the peaks from Fig. 1b and Fig. 1c, corresponding to liposomes whose internal and external surfaces are affected by Pr^{3+}, show a slight

asymmetry. This fact could be due to a different extent of interaction of Pr^{3+} with the internal and the external surfaces of the vesicles. The liposomes for geometrical reasons have a lower packing density and larger free volume in their outer lipid layer[14]. The use of a higher frequency spectrometer could help to resolve the signal. Lee *et al.*[15] and Levine *et al.*[16] have found that, in the absence of any paramagnetic ions, the 100 MHz PMR spectra of sonicated synthetic lecithins show two partially resolved components arising from the trimethylammonium protons of the polar groups in the inside and outside of the vesicles. Jendrasiak[17], with the use of 220 MHz PMR has obtained spectra of pure aqueous dispersions of egg lecithin showing two components of the trimethylammonium signal differing in a very small frequency.

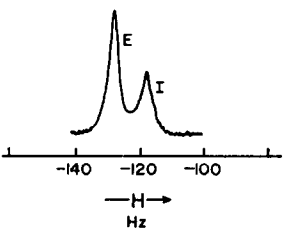

Fig. 2. The trimethylammonium proton signal of a 4% (w/v) aqueous dispersion of egg yolk lecithin, sonicated in the presence of X-537A (22.5 μg/mg lipid) at pH 3. Subsequently, 5 mM $PrCl_3$ was added. The signal is split into two components: peak E (−128.0 Hz) and peak I (−119.5 Hz). Line positions were measured relative to the $(CH_2)_n$ signal of lecithin.

The experiments described above were carried out at pH 6. If the pH is lowered to a value of 3 and $PrCl_3$ is added to liposomes containing X-357A, the spectrum again exhibits two peaks (Fig. 2) as in Expt 1b in which no penetration of Pr^{3+} occurred. This result indicates that protons, at a concentration as low as 10^{-3} M inhibit the ionophore-induced permeability to Pr^{3+}. It may be presumed that if the lanthanide is transported by the ionophore, the effect of protons could be due to competition. Calcium, at the same concentration as Pr^{3+} (15 mEquiv), does not prevent penetration of the lanthanide as detected by PMR. Further experiments are being performed in this laboratory to elucidate the nature of the complexes between X-537A and different cations by means of their PMR spectra.

LIPID BILAYER EXPERIMENTS

The addition of X-537A to a bimolecular lipid membrane in the presence of Pr^{3+} results in a conductance increment; if in the presence of X-537A a Pr^{3+} concentration gradient is established across the film, a cationic membrane potential is generated. The membrane potential depends linearly on the logarithm of the Pr^{3+} concentration ratio across the membrane as illustrated in Fig. 3A. The line through the experimental points is drawn with a slope of 21 mV/decade which is very close to the expected theoretical Nernst slope for a trivalent cation of 20 mV/ decade at a temperature of 29 °C. The transference number for Pr^{3+} as derived from this experiment is nearly 1.0 indicating that X-537A has conferred to the bimolecular lipid membrane the property of Pr^{3+} selectivity.

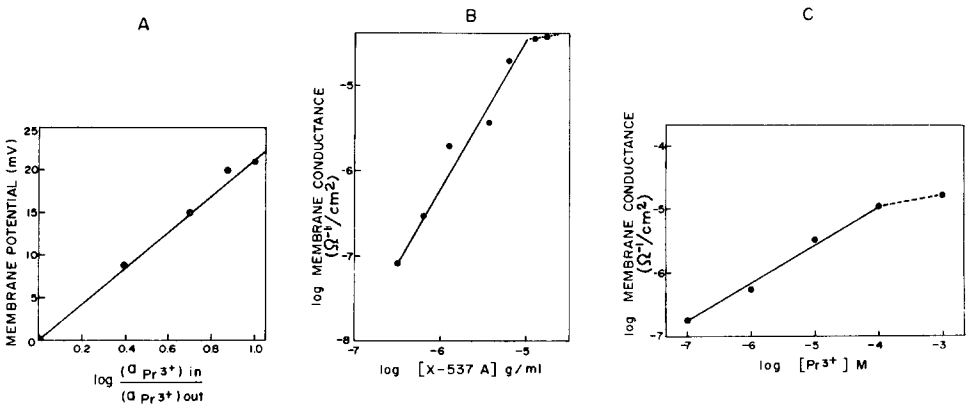

Fig. 3. Effects of X-537A on the membrane conductance properties of the bimolecular lipid membrane in the presence of Pr^{3+}. (A) Potential difference across the bimolecular lipid membrane treated with X-537A as a function of the ratio of activities of $PrCl_3$ in the two aqueous phases. Membranes were formed in 10^{-5} M $PrCl_3$ at pH 6.6 and X-537A was then added to the inner compartment to a concentration of 6.25 μg/ml. The bimolecular lipid membrane attained a conductance of approximately $3.8 \cdot 10^{-5}$ $\Omega^{-1} \cdot cm^{-2}$. Concentration differences were established across the membrane by addition of small amounts of concentrated $PrCl_3$ solution to the inner compartment, and the resulting membrane potential measured. The slope of the line is 21 mV/decade. (B) Relation between membrane conductance and concentration of X-537A in the aqueous phase at constant $PrCl_3$ concentration (1 mM, pH 6.4) and constant voltage (50 mV). From the slope of the linear portion of the line we have $C \alpha C^2_{X-537A}$. (C) Relation between membrane conductance and concentration of $PrCl_3$ in the aqueous (pH 6.9), at constant X-537A concentration (6.25 μg/ml), and constant voltage (50 mV). From the slope of the line one obtains $G \alpha C_{PrCl_3}$.

The dependence of the membrane conductance on ionophore and ion concentration are illustrated in Figs 3B and 3C, respectively. There is a quadratic dependence of the membrane conductance on X-537A concentration (Fig. 3B) and a linear dependence on the concentration of Pr^{3+} (Fig. 3C). This suggest that the charged permeant species is a complex formed between two ionophore molecules and one Pr^{3+}.

These results indicate that in the presence of X-537A lipid bilayers are selectively permeable to Pr^{3+}, thus supporting the interpretation of the PMR data. Studies in this laboratory[18] have demonstrated that X-537A confers to the bimolecular lipid membrane the property of H^+ and Ca^{2+} selectivity. The relative affinity of X-537A to Pr^{3+} with respect to H^+ and Ca^{2+} has been derived from biionic potential measurements at 10^{-3} M concentration. The selectivity obtained under these conditions is the following: $H^+ \geqslant Pr^{3+} > > Ca^{2+}$. The fact that H^+ associates with X-537A to approximately the same extent as Pr^{3+} at pH 3 supports the interpretation given to the H^+ inhibition of X-537A mediated transport of Pr^{3+} in liposomes as being the result of competition between H^+ and Pr^{3+} for the ionophore. The lack of competition between Ca^{2+} and Pr^{3+} observed in the vesicles can also be well accounted for by the selectivity sequence found in bilayers.

The PMR measurements reported here have been used as an indicator of changes in the steady state before and after the transport of a paramagnetic ion. This same spectroscopic method may be used in the future to obtain kinetic data

of transport by means of equilibrium perturbation and analysis of the subsequent relaxation[19]. The method could be of use in both model and biological membranes.

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