THE LOCATION, ALIGNMENT, AND MOTION OF AN ANTIVIRAL DRUG - AMANTDINE IN DMPC BILYERS STUDIED BY SOLID-STATE NMR

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Introduction

Amantadine is a licensed drug for the treatment of influenza A viral inflection and the M2 proton channel is known to be the drug target. However, amantadine also partitions into lipid bilayers and makes the inhibition mechanism more complicated. Studying of the amantadine interaction with membranes in a native-like environment by solid state NMR can help us to understand the inhibition mechanism of the proton channel. This report describes the location, alignment and mobility of amantadine in a model membrane of DMPC bilayers utilizing ^{15}N labeled amantadine.

Results and Discussion

It is shown in figure 1, that both the lipid and amantadine molecules are aligned very well, that the amantadine is constrained within the lipid bilayer and restricted rotational freedom with the motional axis of amantadine parallel to the bilayer normal. When in the presence of the paramagnetic ion Mn^{2+} as shown in Fig 2, the signals from the headgroup of the DMPC almost disappear due to the paramagnetic broadening effect, however, the signals from the amantadine molecules are still observable (Fig 2 (C)), this suggests that amantadine partitions into the hydrophobic core of the lipid bilayer. It has been further noticed that the various ^{13}C signals from amantadine experience different broadening effect and the carbon signals close to the amino group decreases faster suggesting that the amino group points to the bilayer surface.

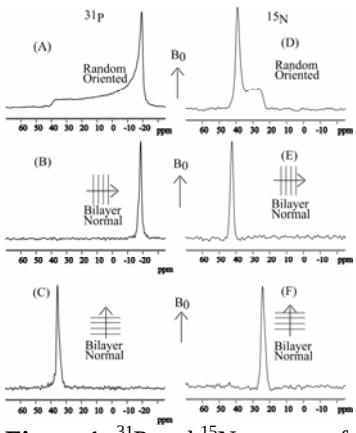

Figure 1: ^{31}P and ^{15}N spectra of 20% (molar ration) amantadine in DMPC of (A,D) powder, (B,E) bilayer normal perpendicular to B_0, (C,F) bilayer normal parallel to B_0. Hydrated powder samples were prepared by cosolubilizing DMPC and amantadine in TFE at different molar ratios, the solution was lyophilized to obtain dry powder and then incubated at 97% relative humidity for 3 days. Aligned samples were prepared by codissolving 10mg DMPC and different amounts of amantadine in 1.5ml TFE and spread onto 50 glass slides. The sample was vacuum dried and then incubated at 97% (K_2SO_4) relative humidity for 3 days.

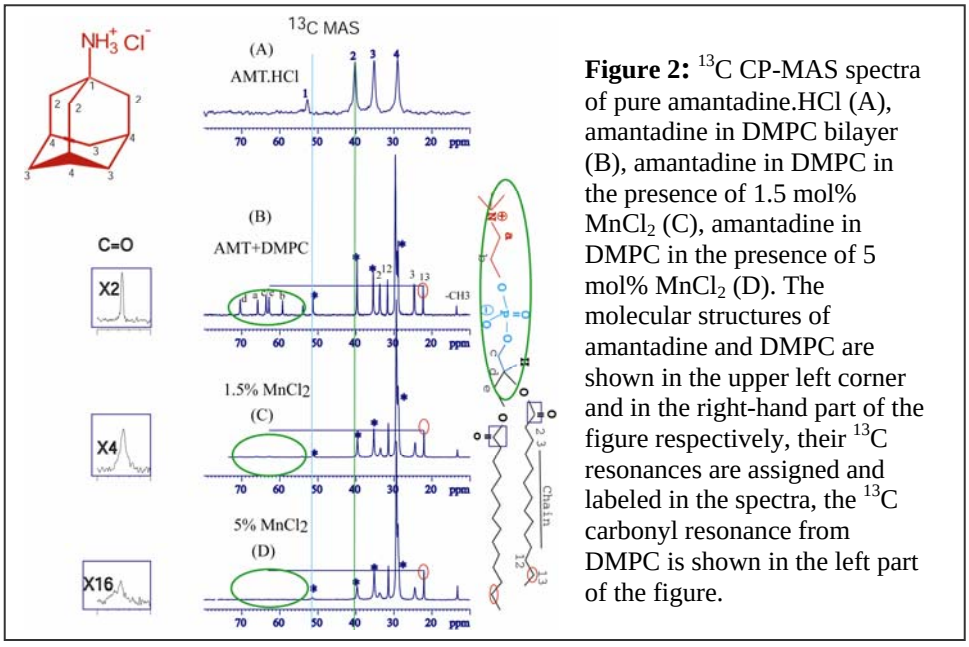

Figure 2: ^{13}C CP-MAS spectra of pure amantadine.HCl (A), amantadine in DMPC bilayer (B), amantadine in DMPC in the presence of 1.5 mol% $MnCl_2$ (C), amantadine in DMPC in the presence of 5 mol% $MnCl_2$ (D). The molecular structures of amantadine and DMPC are shown in the upper left corner and in the right-hand part of the figure respectively, their ^{13}C resonances are assigned and labeled in the spectra, the ^{13}C carbonyl resonance from DMPC is shown in the left part of the figure.

Conclusions

The solid-state NMR spectra show that the amantadine is constrained in the lipid bilayers and undergoes axial motion parallel to the bilayer normal. Most of the amantadine is partitioned into the hydrophobic core of the lipid bilayer with the amino group protruding towards the lipid bilayer surface.