RESOLVING POLARITY AND HYDROGEN BOND ENVIRONMENT AT PROTEIN LIPID-BINDING SITES USING HIGH FIELD EPR

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Introduction

Nitroxide spin labeling in combination with EPR spectroscopy has found many applications in studying structure and dynamics of proteins and biological membranes. Recently, there has been a substantial interest in utilizing High Field EPR to characterize local effects of polarity and hydrogen bonding in proteins and model phospholipid systems. Specifically, with the help of HF EPR it has been shown that two components of the nitroxide magnetic tensors, g_x, and A_z, are the most sensitive to the local electrostatic effects and also to formation of hydrogen bonds. In previous experiments carried out with 95 GHz EPR, this dependence was observed as a systematic shift of g_x and A_z with the solvent polarity [1-3]. We report on investigating local polarity and hydrogen bond formation for a phosphatidylcholine lipid molecule confined at binding site of human serum albumin.

Experimental

We have investigated effects of proctic and aproctic solvents of various polarity on the rigid-limit 220 GHz EPR spectra from 5-doxyl stearic acid (5-DSA). Our previous experiments at 130 GHz clearly show spitting in the g_x region, characteristic for existence of at least two spectral components. We have assigned the component with a higher g_x, g_{x1}, to spin label molecules with no hydrogen bonds formed and the component with a lower g_x, g_{x2} to the molecules with a single hydrogen bond. Using improved g-factor spectral resolution of 220 GHz EPR experiment we have confirm the existence of these two forms of the same nitroxide. We have obtained 240 GHz rigid-limit spectra from n-doxyl-DPPC lipid bound to human serum albumin.

Results and Discussion

240 GHz EPR spectra from HAS-bound n-doxyl PC (n=5,7,12 and 16 –position of the label from the polar head of the lipid) clearly show variation in polarity and hydrogen bond formation along the lipid chain. The spectra in g_x region can be interpreted as superposition of three components: g_{x1}: non-hydrogen bonded label, g_{x2}: one hydrogen bond formed and g_{x3}: a highly polar environment with possibly two hydrogen bond per nitroxide molecule formed. Spin-label in position 5 shows high fraction of g_{x3} and g_{x2} components indicating that the HSA lipid-binding channel as being polar in this region. Label at position 16 (the tail of the lipid) also reports on relatively polar environment. Positions 7 and 12 are the most non-polar with label either in non-hydrogen bonded form or forming one bond.

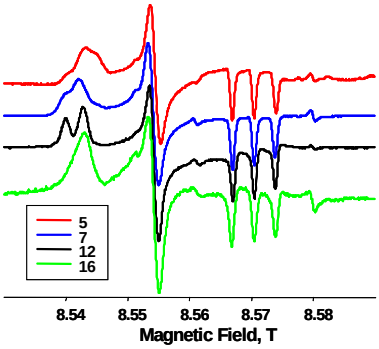

Fig.1 240 GHz EPR spectra from HAS-n-doxyl PC.

Conclusions

High g-factor resolution of very high field EPR provides unique opportunity to differentiate effects of hydrogen bond formation and dielectric effect of solvent on nitroxide EPR spectra. The sensitivity of the g_x region of the spectra to solvent effects is far superior to that of the A_z feature.

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References

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