# THE PARTICIPATION OF PHOSPHATIDYLCHOLINE 1st AND 2nd FATTY ACYLS IN LIPID-PROTEIN INTERACTIONS IN Ca-ATPase. A SPIN LABEL STUDY

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**Introduction:**

Lipid-protein interactions were widely investigated by spin-labeling technique using spin-labeled phosphatidyl-choline, containing spin-labeled acyl moiety at the 2nd position of glycerol backbone (1-4). The suggested method resulted in the synthesis of phospholipids spin-labeled at the 1st position of glycerol backbone (5-7). This work proposed to compare the participation of the 1st and the 2nd spin-labeled fatty acyls in lipid-protein interactions in Ca-ATPase preparations by spin-labeling. With this aim in mind EPR spectra of two preparations of rabbit sarcoplasmic reticulum Ca-ATPase with different phospholipid content were studied. These preparations were labeled with spin-labeled phosphatidylcholines containing doxyl nitroxyl moieties either in the 1st or in the 2nd positions of glycerol backbone (8, 9).

**Results and Discussion:**

Rabbit sarcoplasmic reticulum (SR) Ca-ATPase preparations were employed with a low (≈30%) and high (≈70%) SR phospholipid content for comparing the EPR spectra of Ca-ATPase, spin-labeled with nitroxyls 1 or 2 (see Table legend). According to the SDS gel electrophoresis data, more than 95% of the total content of the protein in the preparations obtained belonged to the component with a molecular mass of 110 kDa (Ca-ATPase). Figure 1 shows typical EPR spectra of spin-labeled (spin label 1) Ca-ATPase preparations containing different levels of phospholipids. Such EPR spectra point to the anisotropic rotation of spin-labeled fatty acyl moieties, the distance between the external extrema of EPR spectrum, 2 A'//, for the more delipidized (≈30%) Ca-ATPase II being greater than that for the Ca-ATPase preparation I. Figure 2 shows the spectra of the spin-labeled phosphatidylcholine 2 in Ca-ATPase I and II. Table lists the parameters of these EPR spectra. The 2 A'// parameter of the spectra of spin label 1 increase as the content of the lipids in the preparation diminishes. In the case of delipidized Ca-ATPase II which is a lipoprotein complex (≈25 phospholipid molecules per Ca-ATPase protein molecule) it reaches the value greater than that for spin label 2 for which this parameter is equal for both preparations within the limits of error.

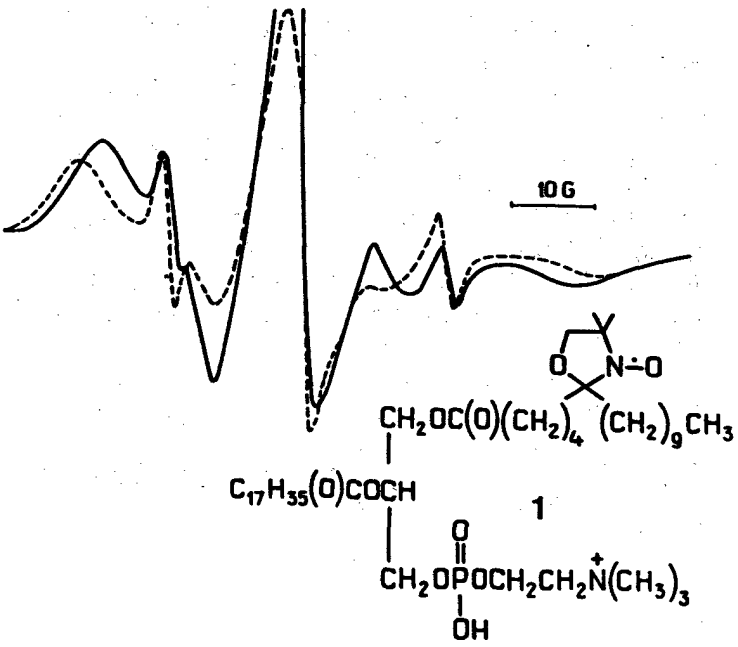


**Figure 1**: EPR spectra of rabbit SR Ca-ATPase preparations I (≈70% of SR phospholipids, solid line) and II (≈30% of SR phospholipids, dotted line) spin-labeled with phosphatidylcholine nitroxyl radical 1.

Ca-ATPase preparations I and II were labeled as follows: spin label 1 or 2 (1 mg per 40 mg of SR protein) was added into cholate solution employed at the stage of SR vesicles solubilisation (8, 9). Medium contained 50 mM of Tris HCl (pH 7.5) and 50 mM of KCl. Registration conditions are: scan 100 G, $H_M$= 1.25 G, P=5 mWt, 27°C.

The value of the order parameter S in this series for spin label 1 also increases from 0.640 to 0.709, whereas for spin label 2 it is not responsive to the content of SR phospholipids. The parameter S value for spin label 1 in Ca-ATPase II is higher (S=0.709) than in Ca-ATPase I (S=0.640), which testifies to a greater interaction with the protein. The change of the value of isotropic superfine interaction constant, $a'_{iso}$ as a measure for the polarity of microenvironment is 0.4 G greater for spin label 1 than for spin label 2 in preparation I. A similar tendency in the change of EPR spectra parameters is also confirmed by the following data: for SR preparation (100% phospholipid content) the values were 2 A'// = 50.6±0.25 G ; S = 0.630±0.007; and $a'_{iso}$ = 14.55±0.14 G.

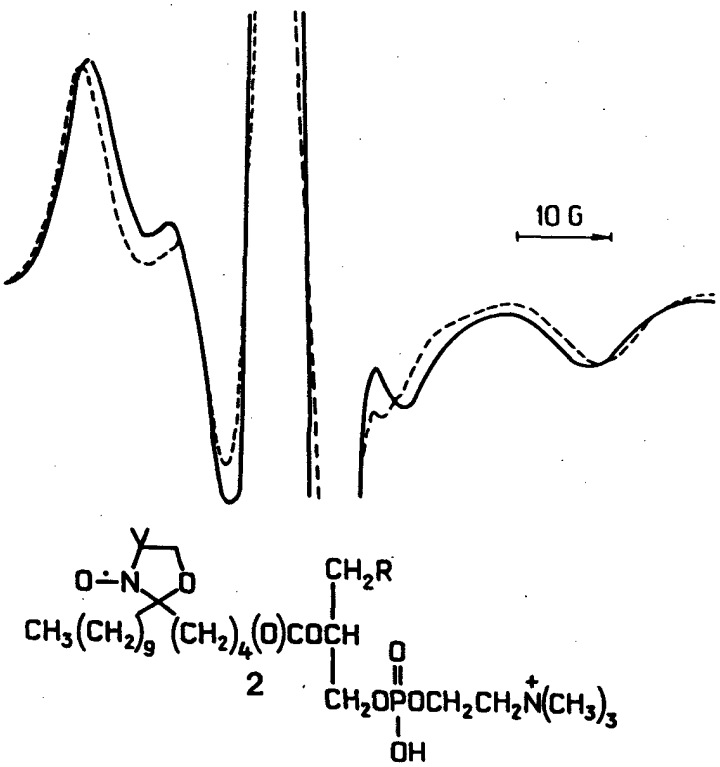


Figure 2: EPR spectra of rabbit SR Ca-ATPase preparations I and II spin-labeled with phosphatidyl-choline nitroxyl radical 2 (see Figure 1 legend).

Thus, the differences of EPR spectra parameters between Ca-ATPase I and II preparations are significant only for spin label 1. In our opinion (8, 9) all this, pointing to the differences in the microenvironment of spin labels, may testify to the different kind of the participation of the 1st and the 2nd phospholipid acyl residues in lipid-protein interactions.

Table. EPR spectra parameters of SR Ca-ATPase preparations I (≈70% of SR phospholipids) and II (≈30% of SR phospholipids), spin labeled with phosphatidylcholine nitroxyl radicals 1 or 2.

Rabbit SR Ca-ATPase preparations were isolated as described earlier (10). Initial SR vesicles contained 0.96±0.05 mcmol of phosphorus/mg protein at 37°C. Ca-ATPase preparation I was characterized by specific ATPase activity 13.0±1.0 mcmol Pi/min mg protein and contained 0.75±0.02 mcmol of phosphorus/mg protein. 0.5 mg of sodium cholate/mg protein was used for Ca-ATPase I isolation. At the isolation of Ca-ATPase preparation II 1.0 mg of sodium cholate/mg protein was employed. This Ca-ATPase preparation contained 0.28±0.02 mcmol of phosphorus/mg protein and had specific ATPase activity 5.4±0.2 mcmol Pi/min mg protein (10). 2 A'// and 2 $A'_{\perp}$ values were found from 4-8 experiments.

| SL | 2 A'//, G | $a'_{iso}$, G | S (1) |
|---|---|---|---|
| | | Ca-ATPase I (≈70%) | |
| 1 | 52.0±0.5 | 14.63±0.12 | 0.640±0.015 |
| 2 | 56.0±0.9 | 15.00±0.14 | 0.712±0.015 |
| | | Ca-ATPase II (≈30%) | |
| 1 | 58.6±0.12 | 15.66±0.12 | 0.709±0.006 |
| 2 | 54.7±1.8 | 14.76±0.13 | 0.703±0.014 |
| | P<0.01* | P<0.01* | P<0.01* |

*P is the significance of differences between preparations I and II.

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Acknowledgement: This work was supported in part by A. von Humboldt Grant ( for R.Z.). The authors are very grateful to Prof. W. Hasselbach for the help and useful discussions.