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

Renat Zhdanov¹,², Pavel Komarov² and Vitaly Shvets²

Max-Planck-Institut für medizinische Forschung, Heidelberg D 6900, FRG (1) and Institute for
Biotechnology, Moscow 117246, USSR (2)

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 phosphatidylylcholines containing
doxyl nitroxy moiety 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 nitroxyIs 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
phosphatidylylcholine 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
phosphatidylylcholine nitroxy radical I.
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, H₂M=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, $a^\text{iso}$ 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 $2 \text{ A''/} = 50.6 \pm 0.25 \text{ G}$; $S = 0.630 \pm 0.007$; and $a^\text{iso} = 14.55 \pm 0.14 \text{ G}$.

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 ($\approx 70\%$ of SR phospholipids) and II ($\approx 30\%$ of SR phospholipids), spin labeled with phosphatidylcholine nitroxy radicals 1 or 2.

Rabbit SR Ca-ATPase preparations were isolated as described earlier (10). Initial SR vesicles contained 0.96+0.05 mmol of phosphorus/mg protein at 37°C. Ca-ATPase preparation I was characterized by specific ATPase activity 13.0±1.0 mmol Pi/min mg protein and contained 0.75±0.02 mmol 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 mmol of phosphorus/mg protein and had specific ATPase activity 5.4±0.2 mmol Pi/min mg protein (10). $2 \text{ A''/}$ and $2 \text{ A’/}$ values were found from 4–8 experiments.

<table>
<thead>
<tr>
<th>SL</th>
<th>$2 \text{ A''/}$, G</th>
<th>$a^\text{iso}$, G</th>
<th>$S$ (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52.0±0.5</td>
<td>14.63±0.12</td>
<td>0.640±0.015</td>
</tr>
<tr>
<td>2</td>
<td>56.0±0.9</td>
<td>15.00±0.14</td>
<td>0.712±0.015</td>
</tr>
</tbody>
</table>

Ca-ATPase I ($\approx 70\%$)

<table>
<thead>
<tr>
<th>SL</th>
<th>$2 \text{ A''/}$, G</th>
<th>$a^\text{iso}$, G</th>
<th>$S$ (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58.6±0.12</td>
<td>15.66±0.12</td>
<td>0.709±0.006</td>
</tr>
<tr>
<td>2</td>
<td>54.7±1.8</td>
<td>14.76±0.13</td>
<td>0.703±0.014</td>
</tr>
</tbody>
</table>

P<0.01* P<0.01* P<0.01*

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


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.