BIOLOGIA PLANTARUM 50 (3): 444-446, 2006

BRIEF COMMUNICATION

Anion effects on the structural organization of spinach thylakoid membranes

A. JAJOO* and A. KAWAMORI**

*School of Life Sciences, Devi Ahilya Vishwavidyalaya, Indore-452017, M.P., India**
*Faculty of Science, Kwansei Gakuin University, Gakuen 2-1, Sanda 669-1337, Japan***

Abstract

Changes in the conformation of spinach thylakoid membranes were monitored in 5-doxyl stearic acid (SAL)-treated thylakoid membranes in the presence of various anions (Cl^-, Br^-, I^-, NO_2^-, SO_4^{2-}, PO_4^{3-}). The presence of anions made the thylakoid membrane more fluid. The extent of change in membrane fluidity differed with different anion and was reversible.

Additional key words: electron spin resonance, membrane fluidity, spin labels, *Spinacia oleracea.*

The photosynthesis depends on a set of complex protein molecules and enzymes like proteases (Nair and Ramaswamy 2004) that are located in and around a highly organized membrane. The ionic environment of the thylakoid membrane influences and regulates many functions such as electron transport, energy transfer, photophosphorylation, photosynthetic carbon reduction, *etc*. Thylakoid stacking and unstacking depends on the ionic conditions of suspension (Izawa and Good 1966, Murakami and Packer 1971, Gross and Prasher 1974). A correlation between the thylakoid stacking and cation-induced decrease in photosystem 1 (PS 1) electron transport has been suggested by Barber and Chow (1979). Cations bind with the negatively charged phosphate groups of the phospholipids as well as with the carbonyl groups of the proteins resulting in altered membrane fluidity and phase separation of the bilayer (Radda 1975, Papahadjopoulos *et al*. 1977). Most of these studies emphasized the role of cations in maintaining the integrity of thylakoid membranes. Since all these studies were performed in thylakoids suspended in a low salt medium, the role of anions went unrecognized. In our previous work (Jajoo *et al*. 1994) we showed that the Mg^{2+}-induced lipid phase transition in thylakoid membranes is reversed by the addition of anions such as chloride and bicarbonate. These observations inspired us to investigate the effects of other anions on the structural integrity of the thylakoid membranes.

In this paper, we investigated the effects of chloride, bromide, iodide, sulphate, nitrite, and phosphate on the fluidity properties of the thylakoid membranes and their reversibility. 5-doxyl stearic acid (SAL) was used as a spin label which serves as a physical probe to report structural and environmental aspects at a localized molecular site in the thylakoid membrane.

The thylakoid membranes of market spinach (S*pinacia oleracea* L.) were prepared as described in Jajoo *et al*. (1994) and stored until use at 77 K in a suspending buffer (0.2 M sucrose, 20 mM MOPS, 20 mM NaCl, and 50 % (v/v) glycerol at pH 6.8) at chlorophyll concentrations of approximately 4 mg cm^{-3}, determined according to Porra (1991). All steps of preparations were performed under dim green light. ESR measurements were performed using *Varian E-109 system* X-band spectrometer. Rotational correlation time (Tc) and order parameter (S) were calculated following the method of McRae *et al*. (1982).

The spin probe intercalates into the membrane bilayer. The ESR signal from the probe is sensitive to perturbations near the surface of the membrane. The

---

Received 29 September 2004, *accepted* 9 August 2005.
Abbreviations: ESR - electron spin resonance; Hepes - 4-(2-hydroxyethyl-1-piperazine ethane sulphonic acid; MOPS - 4-morpholino-propane sulphonic acid; SAL - 5-doxyl stearic acid.
Acknowledgements: A.J. thanks the University grants Commission (UGC) for the project (3-221/2001/SRII). A postdoctoral fellowship to A.J. by Kwansei Gakuin University, Japan, is also thankfully acknowledged.
* Corresponding author; fax: (+91) 731 2360026, e-mail: ajajoo.sls@davv.ac.in

lipophilic domains of the thylakoid membranes were labeled with 5-SAL and the motion parameter of the label in the micro-environment was computed to understand lipid mobility in these membranes. The ESR spectra of 5-SAL in buffer show an isotropic hyperfine structure consisting of 3 equidistant lines indicating that the paramagnetic molecule is tumbling freely in the solution. Incorporation of the spin label in the thylakoid membranes shows an anisotropic spectrum indicating immobilization of the paramagnetic probe in the surroundings. In order to test that the probe was incorporated in the membranes 0.1 M $NiCl_2$ was added to

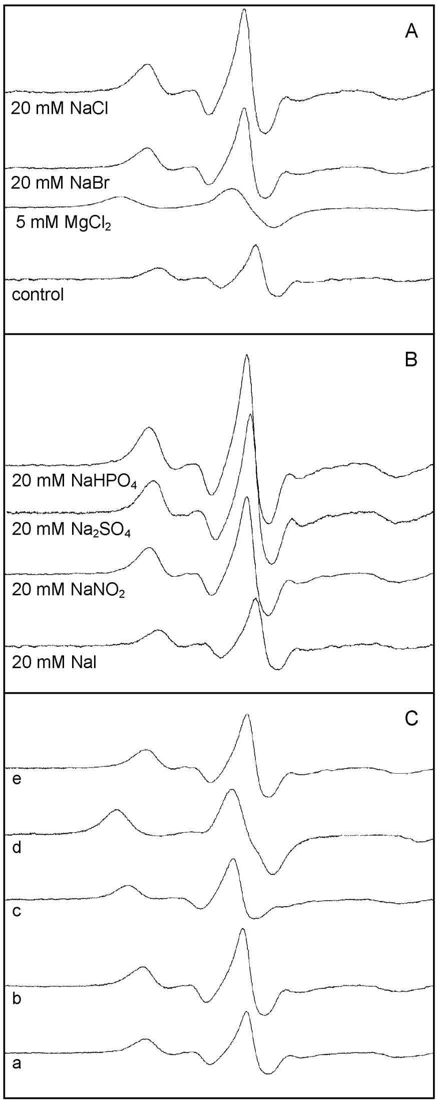

Fig. 1. ESR spectra showing effects (*A* and *B*) of various anions on 5-SAL treated thylakoid membranes and (*C*) their change after washing off the anions: *a* - control, *b* - thylakoids in which chloride has been washed out, *c* - thylakoids in which nitrite has been washed out, *d* - thylakoids in which sulphate has been washed out, *e* - thylakoids in which phosphate has been washed out. Experimental conditions: microwave field: 3386 G, microwave power 2 mW, modulation amplitude 5G.

Table 1. Rotational correlation time (Tc) and order parameter (S) as calculated in SAL-labeled thylakoid membranes after treatment with various anions. Means ± SE, *n* = 9. In all samples means differ significantly from control at $P < 0.01$.

Treatment	Tc	S
Control	4.77 ± 0.04	0.74 ± 0.02
5 mM $MgCl_2$	2.52 ± 0.02	0.86 ± 0.03
20 mM NaCl	5.92 ± 0.03	0.59 ± 0.02
20 mM NaBr	5.67 ± 0.02	0.63 ± 0.02
20 mM NaI	5.60 ± 0.01	0.69 ± 0.02
20 mM $NaNO_2$	5.62 ± 0.02	0.62 ± 0.02
20 mM Na_2SO_4	6.23 ± 0.04	0.53 ± 0.01
20 mM $NaHPO_4$	6.47 ± 0.04	0.50 ± 0.01

SAL-treated thylakoid membranes. The spectra recorded in the presence of $NiCl_2$ confirm the incorporation of SAL into the thylakoid membranes.

Fig. 1*A* and *B* show the ESR spectra of the thylakoid membranes labeled with SAL after treatment with various anions. As reported earlier (Jajoo *et al.* 1994), addition of 5 mM Mg^{2+} makes the thylakoid membranes rigid as evident from the highly anisotropic spectra. Addition of 20 mM of different anions resulted in free motion of the probe (Fig. 1*A,B*). This was further confirmed by calculating rotational correlation time (Tc) computed from the ESR spectra. Tc is a measure of relative fluidity of the membranes. Addition of anions caused an increase in the value of Tc (Table 1) suggesting relatively free motion of the probe in the thylakoid membranes and indicating an increased fluidity of the thylakoid membranes. A comparatively low Tc value obtained in Mg^{2+} treated thylakoid membranes denotes a rigid lipid matrix. Different anions affected the Tc differently, but all the anions made the membranes more fluid to varying extent. An order parameter (S) calculated from the ESR spectra is a measure of the mean angular deviation of the rotating hydrocarbon chain from its time-averaged orientation in the bilayer. Values close to 1 correspond to highly anisotropic motion with only a small angular deviation while a value of zero would correspond to the other extreme of completely isotropic motion. S is used as a parameter describing the fluid nature of the membranes. The S values also showed differences in control and in anion-treated thylakoid membranes (Table 1). Chloride and other anions caused decrease in S indicating that the amplitude of motion which averages out anisotropy increased. The elevation in the local lipid fluidity implied that a melting of the lipophilic compartments could be associated with the action of anions. An important and new observation is that by using sulphate (divalent) and phosphate (trivalent), membranes were relatively more fluid than by using other monovalent anions (chloride, bromide and nitrite). The action of anions in causing state changes in the thylakoids is valency dependent (Jajoo *et al.* 1998).

Whether the changes in the thylakoid membrane organization caused by the anions on were reversible or irreversible, was further investigated. Anion-treated membranes were given two washings in a suspension medium composed of 0.2 M sucrose and 50 mM Hepes-NaOH buffer, pH 7.2. Anions are thought to be washed off after 2 washings. Similar washings were given to the control sample in order to eliminate any changes in the ESR spectra because of washings. Thylakoid membranes in which anions were removed by washing in buffer, restored their original conformation almost completely (Fig. 1*C*). The effects of all used anions in making the thylakoid membranes more fluid were reversed by removing the anion from the medium, suggesting thereby that the anions did not cause any irreversible change in the thylakoid membranes organization. The values of Tc and S in the anion washed thylakoid membranes were close to that of control thylakoids (data not shown).

Summing up, the found increase in thylakoid membrane fluidity was different by using of monovalent (Cl^-, Br^-, NO_2^-, I^-), divalent (SO_4^{2-}), and trivalent (PO_4^{3-}) anions. These differences and the reversibility of the anion effects suggest that anions might increase the fluidity of the thylakoid membranes by redistributing the surface charges.

References

Barber, J., Chow, W.S.: A mechanism of controlling the stacking and unstacking of chloroplast thylakoid membranes. - FEBS Lett. **105**: 5-10, 1979.

Gross, E.L., Prasher, S.H.: Correlation between monovalent cation-induced decreases in chlorophyll a fluorescence and chloroplast structural changes. - Arch. Biochem. Biophys. **164**: 460-468, 1974.

Izawa, S., Good, N.E.: Effect of salts and electron transport on the conformation of isolated chloroplasts. II. Electron microscopy. - Plant Physiol. **41**: 544-552, 1966.

Jajoo, A., Bharti, S., Govindjee: Inorganic anions induce state changes in spinach thylakoid membranes. - FEBS Lett. **434**: 193-196, 1998 .

Jajoo, A., Dube, A., Bharti, S.: Mg^{2+}-induced lipid phase transition in thylakoid membranes is reversed by anions. - Biochem. biophys. Res. Commun. **202**:1724-1730, 1994.

McRae, D.G., Baker, J.E., Thompson, J.E.: Evidence for the involvement of superoxide radical in the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene by pea microsomal membranes. - Plant Cell Physiol. **23**: 375-383, 1982.

Murakami, S., Packer, L.: The role of cations in the organization of chloroplast membranes. - Arch. Biochem. Biophys. **146**: 337-347, 1971.

Nair, J.S., Ramaswamy, S.K.: Chloroplast proteases. - Biol. Plant. **48**: 321-326, 2004.

Papahadjopoulos, D., Vail, W.J., Newton, C., Nir, S., Jacobson, K., Poste, G., Lazo, R.: Studies on membrane fusion. III. The role of calcium-induced phase changes. - Biochim. biophys. Acta **465**: 579-598, 1977.

Porra, R.J.: A simple method for extracting chlorophylls from the recalcitrant alga, *Nannochloris atomus* without formation of spectroscopically different magnesium-rhodochorins derivatives. - Biochim. biophys. Acta **1019**: 137-141, 1991.

Radda, G.K.: Dynamic aspects of membrane structure. - In: Parsons, D.S. (ed.): Biological Membranes. Pp. 81-95. Clarendon Press, Oxford 1975.