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BRIEF COMMUNICATION

Effects of 1-alkyl-1-ethylpiperidinium bromide detergents on Mg^{2+}-ATPase activity in *Phaseolus vulgaris* and *Zea mays*

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Abstract

1-alkyl-1-ethylpiperidinium bromides inhibited light induced Mg^{2+}-adenosintriphosphatase activity of isolated thylakoids in both *Phaseolus vulgaris* L. and *Zea mays* L. The short chain detergents (C_5 to C_7) were less effective in maize than in bean.

For the point of view of photosynthetic carbon metabolism, higher plants may be divided into four groups, *i.e.* C_3, C_4, CAM and C_3-C_4 plants. They differ in anatomical, cytological, biochemical and physiological characteristics, among others in phosphorylation. Our previous paper (Šulková and Devínský 1992) reported that cationic detergents 1-alkyl-1-ethylpiperidinium bromides (AEPBr) inhibit light activated adenosine triphosphatase in the presence of Mg^{2+} (Mg^{2+}-ATPase) bound to thylakoid membranes in leaves of *Phaseolus vulgaris*. The purpose of this work was to compare the inhibition of hydrolytic activity of Mg^{2+}-ATPase from plant species with different carbon fixation pathway, *i.e.* C_3 (*Phaseolus vulgaris*) and C_4 (*Zea mays*).

Plants of *Phaseolus vulgaris* L. cv. Helia and *Zea mays* L. were grown in Knop's liquid medium in a growth chamber (temperature 25 ± 1 °C, irradiance 10 W m^{-2} with 12 h photoperiod, relative humidity 60 %). Thylakoid membranes were prepared from chloroplasts isolated from 12- to 15-d old leaves using a slightly modified method of Ivaschenko and Ponomarenko (1987). Osmotically broken chloroplasts were sonicated (*UC 005 AJ 1* sonicator, *Tesla*) for 8 min. The sonicate was then centrifuged for 15 min at 18 000 *g*.

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Thylakoid sediment was resuspended in 25 mM TRIS-HCl buffer (pH 7.8) containing 20 mM KCl. Aliquots were taken for chlorophyll (Chl) estimation by the spectrophotometric method of Arnon (1949). ATPase activity was measured in the presence of methanol (Sakurai *et al.* 1981). The effect of detergents on the Mg^{2+}-ATPase hydrolysing activity was assayed in a medium that contained 40 mM TRIS-HCl (pH 8.0), 1.5 mM $MgCl_2$, 30 % (v/v) of methanol, the particular detergent (1.0 mol m^{-3}) and the enzyme corresponding to 0.2 - 0.3 mg(Chl) cm^{-3}. For ATP hydrolysis the modified method of Komatsu-Takaki (1987) was used. After a 10 min preincubation of the reaction mixture at 37 °C, the latent ATPase was activated by irradiation with intense "white light" for 60 s. 10 s after the end of irradiation 0.1 cm^3 40 mM ATP was added and the reaction proceeded in the dark for 60 s at 37 °C. The reaction was stopped by adding 1 cm^3 of 15 % (m/v) trichloracetic acid and the released phosphate (P_i) was determined according Tauszky and Shorr (1953). All AEPBr were synthetized according to Lacko *et al.* (1977). At least six independent experiments were conducted, each with three replicates.

All the tested 1-alkyl-1-ethylpiperidinium bromides with alkyl chain length from C_5 to C_{18} at the concentration of 1.0 mol m^{-3} inhibited the hydrolytical activity of membrane bound thylakoid ATPase from both *P. vulgaris* and *Zea mays* (Fig. 1). Derivatives with alkyl chain length C_5 to C_7 were more effective inhibitors of bean than maize ATPase (28 and 63 % of control for C_5AEPBr). For C_8 - C_{18} AEPBr detergents no significant differences between activities of both ATPase were found. Average specific activities of control ATPases (without detergents) were 41.17 ± 15.47 mmol(P_i) kg^{-1}(Chl) s^{-1} for *P. vulgaris* and 91.22 ± 26.03 mmol(P_i) kg^{-1}(Chl) s^{-1} for *Z. mays*, respectively.

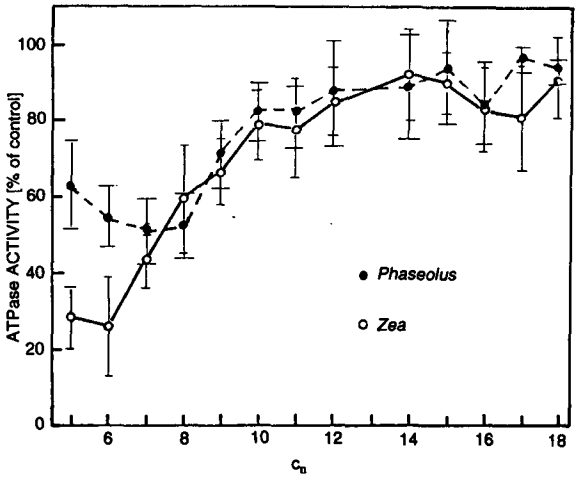

Fig. 1. Effect of 1.0 mol m^{-3} AEPBr detergents of different chain length (c_n = 5 to 18) from bean and maize thylakoids. ATPase activity is expressed as percent of that without detergents. The error bars represent the standard deviation from the mean of at least 6 isolations.

As reported by Kasamo (1982), detergents which inhibit the ATPase activity are not suitable as solubilizing agents. We have previously examined the efficiency of

AEPBr to solubilize ATPase from thylakoid membranes: C_5-C_7 AEPBr could not solubilize ATPase. The solubilization ability of the other employed detergents to ATPase increased only slightly with lengthening of the alkyl chain. Nevertheless, the C_{18}AEPBr had a most expressed effect (unpublished results).

Thylakoid membrane bound ATPase consists of two separable parts, an oligomeric hydrophilic protein called coupling factor one (CF_1), and a hydrophobic protein denoted CF_0. CF_1 can be extracted from intact CF_1CF_0, only it has a latent ATPase activity and is composed of five subunits: alfa, beta, gamma, delta, epsilon. The soluble maize and spinach CF_1 differ in the way of expressing Mg^{2+}-ATPase activity (in the presence of detergents, alcohols, SO_3^{2-} ions, Patrie and Miles 1987). The gamma subunit of spinach CF_1 is considerably more hypersensitive to trypsin proteolysis in the dark than that of maize (Richter and McCarty 1987). These results are in agreement with the hypothesis that disulfide bridge of the maize gamma subunit may be less accessible to thiol oxidants/reductants and thus the ATPase is less affected.

AEPBr detergents combine hydrophobic (alkyl chain) and hydrophilic (piperidinium) groups in their molecules. This combination is necessary for the inhibition of ATPase activity (Kasamo 1988). In contrast, many cationic detergents efficiently solubilize components of biological membranes. The efficiency of detergents and the degree of solubilization have often been studied (Barzatt 1983, Murphy and Woodrow 1984, Hermann *et al.* 1988) but the solubilization mechanism is not yet known. Detergents in a subcritical concentration may be bound in monomeric form to membrane proteins and lipids. The conformational changes or proteolysis of the polypeptide subunits alfa, gamma and epsilon in CF_1 may result from these interactions together with a modulation of the enzyme and decreased hydrolytic activity of ATPase (Arana *et al.* 1986). The present investigation indicated that AEPBr in 1.0 mol m^{-3} concentration inhibited Mg^{2+}-ATPase activity due to bounds with thylakoid membrane and that different degree of inhibition of ATPase activity in bean and maize may be caused by different characteristics of CF_1 proteins.

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