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Carbonic anhydrase activities in pea thylakoids

A Photosystem II core complex-associated carbonic anhydrase

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Abstract

Pea thylakoids with high carbonic anhydrase (CA) activity (average rates of 5000 µmol H+ (mg Chl)−1 h−1 at pH 7.0) were prepared. Western blot analysis using antibodies raised against the soluble stromal β-CA from spinach clearly showed that this activity is not a result of contamination of the thylakoids with the stromal CA but is derived from a thylakoid membrane-associated CA. Increase of the CA activity after partial membrane disintegration by detergent treatment, freezing or sonication implies the location of the CA in the thylakoid interior. Salt treatment of thylakoids demonstrated that while one part of the initial enzyme activity is easily soluble, the rest of it appears to be tightly associated with the membrane. CA activity being measured as HCO−3 dehydration (dehydrase activity) in Photosystem II particles (BBY) was variable and usually low. The highest and most reproducible activities (approximately 2000 µmol H+ (mg Chl)−1 h−1) were observed in the presence of detergents (Triton X-100 or n-octyl-β-D-glucopyranoside) in low concentrations. The dehydrase CA activity of BBY particles was more sensitive to the lipophilic CA inhibitor, ethoxyzolamide, than to the hydrophilic CA inhibitor, acetazolamide. CA activity was detected in PS II core complexes with average rate of 13,000 µmol H+ (mg Chl)−1 h−1 which was comparable to CA activity in BBY particles normalized on a PS II reaction center basis.

Abbreviations: AA – acetazolamide; CA – carbonic anhydrase; EZ – ethoxyzolamide; IC – ‘inorganic carbon’, sum of CO2 and bicarbonate; PS II – Photosystem II; tCA – thylakoid-associated carbonic anhydrase

Introduction

The requirement of inorganic carbon (IC) for the proper electron transport activity in thylakoids was noticed more than 40 years ago (Warburg and Krippahl 1958). It is generally accepted that one site of IC binding is located on the acceptor side of Photosystem II (PS II) regulating electron transfer (Van Rensen 1993). However, the involvement of IC on the donor side of PS II has also been reported although the specific binding site(s) are less thoroughly characterized (Stemler and Govindjee 1973; Stemler and Jursinic 1983; Stemler et al. 1984; Klimov et al. 1995; Wincencjuisz et al. 1996; Allakhverdiev et al. 1997; Baranov et al. 2000). In spite of the fact that the term IC is used in this context almost as a synonym of bicarbonate, there was an early indication that CO2 may be an active species regulating PS II electron transport (Sarojini and Govindjee 1981). A principally new approach originated from work of Stemler (1980) who considered the role of both of the IC species. His concept implies a coupling of electron transport with the conversion between CO2 and bicarbonate; the latter reaction is known to be catalyzed by the enzyme carbonic anhydrase.

A soluble enzyme was considered to be the dominating CA in the higher plant chloroplast (Graham et al. 1984). A membrane-associated CA has been
Table 1. CA activity (measured as the rate of CO₂ hydration) of thylakoids before and after treatments disintegrating the membrane. Each row represents a separate thylakoid preparation. For incubation with triton X-100, 0.023% Triton was used giving a detergent/chlorophyll ratio of 1:1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CA activity, W–A Units (mg Chl)^{−1}</th>
<th>CA activity after treatment</th>
<th>CA activity before treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triton X-100 (30 min at 0°C)</td>
<td>6.4</td>
<td>16.7</td>
<td>2.60</td>
</tr>
<tr>
<td>Sonication at 22 kHz during 30 s</td>
<td>18.4</td>
<td>36.3</td>
<td>1.97</td>
</tr>
<tr>
<td>Freezing to −20°C for 30 min</td>
<td>2.4</td>
<td>11.2</td>
<td>4.7</td>
</tr>
</tbody>
</table>

It was suggested in some works that a thylakoid CA is associated with photosystems (Pronina and Borodin 1993), mainly with PS II (Vaklinova et al. 1982; Stembler 1986; Ignatova et al. 1993). A thylakoid-bound CA located close to (or integrated in) the primary photosynthetic machinery attracts special interest. Addition of external CA has been found to activate the Hill reaction in chloroplast fragments (Vaklinova et al. 1984). Different, both inhibitory and stimulating, effects of light (Stembler 1986; Lazova and Vaklinova 1989; Moskvin et al. 1996) on the thylakoid-associated CA activity have been reported. The thylakoid CA activity was further shown to be dependent on the surrounding redox-potential that had not been observed before for any other known CA (Moubarak-Milad and Stembler 1994).

It was suggested in some works that a thylakoid CA is associated with photosystems (Pronina and Borodin 1993), mainly with PS II (Vaklinova et al. 1982; Stembler 1986). A CA localized in thylakoids of Chlamydomonas reinhardtii was discovered and shown to be directed to the thylakoid lumen (Karlsson et al. 1998) and later Park et al. (1999) showed that this CA, denoted Cah3 was associated with PS II-enriched membrane preparations. Recently, it was shown that Cah3 is required for both stability and function of the water-oxidizing complex in C. reinhardtii (Villarejo et al. 2002). In addition, Lu and Stembler (2002) demonstrated that an antibody raised against Cah3 reacts with a protein in PS II-enriched thylakoid fragments from maize mesophyll chloroplasts.

In a preliminary communication, we presented data showing the presence of CA activity in core complexes of PS II (Moskvin et al. 1998a, b). In the present study we show the presence of at least two CA activities in thylakoid membranes and demonstrate that CA activity measured in PS II membrane fragments (BBY particles) from garden pea is enriched upon isolation of PS II core complexes.

Materials and methods

Pisum sativum plants were grown in a greenhouse in soil, at 22/18 °C (day/night) and 400 µmol of photons m⁻² s⁻¹ illumination provided by tungsten halogen lamps. Leaves from two upper tiers of 12–14-day-old plants were harvested for isolation of thylakoids according to Moskvin et al. (1995) with additions of one extra resuspension-centrifugation step to ensure a complete removal of soluble contaminants. With exception of the results presented in Table 1, all of the experiments were performed with thylakoids frozen in liquid nitrogen (with addition of 20% glycerol to the suspension medium) and stored at −70 °C. Total leaf extracts were prepared as a positive control for western blot analysis. Fresh leaves were frozen with liquid nitrogen and homogenized in a mortar with pestle. The leaf powder was incubated in the medium for measuring of the CA activity as described below (with addition of Triton X-100 up to 0.02%) on ice during 1 h with shaking periodically. After that, the suspension was centrifuged at 14,000 × g during 3 min, and the supernatant was used as a source for soluble CA. CA activity of the supernatant was measured, and it was diluted to obtain the CA activity (per volume unit) equal to that of the thylakoid suspension.

PS II membrane fragments (BBY particles) were prepared from pea leaves according to Bertold et al. (1981). The fragments were suspended in a medium containing 50 mM MES (pH 6.5), 400 mM sucrose, 35 mM NaCl, 20% glycerol, frozen in liquid nitrogen and stored at −70 °C. PS II core complexes