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Chloroplast class I and class II aldolases are bifunctional for fructose-1,6-biphosphate and sedoheptulose-1,7-biphosphate cleavage in the Calvin cycle

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Abstract Class I and class II aldolases are products of two evolutionary non-related gene families. The cytosol and chloroplast enzymes of higher plants are of the class I type, the latter being bifunctional for fructose-1,6- and sedoheptulose-1,7-P_2 in the Calvin cycle. Recently, class II aldolases were detected for the cytosol and chloroplasts of the lower alga *Cyanophora paradoxa*. The respective chloroplast enzyme has been shown here to be also bifunctional for fructose-1,6- and sedoheptulose-1,7-P_2. Kinetics, also including fructose-1-P, were determined for all these enzymes. Apparently, aldolases are multifunctional enzymes, irrespective of their class I or class II type.

Key words: Fructose-1,6-biphosphate aldolase; Sedoheptulose-1,7-biphosphate aldolase; Class I aldolase; Class II aldolase; Calvin cycle; Bifunctional enzyme

1. Introduction

Many enzymes of the sugar phosphate metabolism in plants are compartmentalized both in the plastids and in the cytosol [1,2]. The reactions of glycolysis and gluconeogenesis are accomplished by two sets of isoenzymes, one set in each compartment. Enzymes of the oxidative pentose phosphate pathway are all present in the chloroplasts of spinach leaves, but only the two key dehydrogenases of this pathway exist as isoenzymes in the cytosol, while activities for the regenerative part of the cycle are missing there [3]. The compartment-specific isoenzymes of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are well documented [4].

In this study, we wanted to clarify the compartmentation and properties of aldolase reactions in green leaf cells. In the Calvin cycle of higher plants, aldolase catalyzes both the reversible condensation of dihydroxyacetone-3-P (DHAP) and glyceraldehyde-3-P (GAP) to fructose-1,6-bisphosphate (FBP) and of DHAP and erythrose-4-phosphate (E4P) to sedoheptulose-1,7-bisphosphate (SuBP). FBP aldolase activity is also part of the glycolytic and gluconeogenic reaction sequence, while the function of SuBP aldolase activity in plants is limited to the Calvin cycle.

Aldolases are classified as class I and class II aldolases depending on their requirement of divalent ions in catalysis and many other criteria such as reaction mechanism, subunit structure, pH profile and substrate affinity [5,6]. They are distributed very differently among animals, plants, many algae and bacteria. Class I and class II FBP aldolases share little if any sequence homology [7–9] and must, therefore, be considered as independent lineages in evolution.

While the bifunctional role of class I aldolase for the reversible FBP and SuBP condensation in the Calvin cycle of higher plants has been well-clarified [10,11], this question has to be re-iterated for organisms which perform the Calvin cycle with a class II aldolase, e.g. the algae *Cyanophora paradoxa* (Glaucocystophyta). In this alga, two class II FBP aldolases were reported for the cytosol and the plastids, respectively [12], but their specificity with FBP and SuBP has not yet been clarified. In the present paper, we wanted to determine whether the class II aldolase activity in *C. paradoxa* has also a dual function with FBP and SuBP in the Calvin cycle and how the respective plastid enzymes of class I and class II type differ in their substrate specificity from their cytosolic counterparts.

2. Materials and methods

2.1. Materials

Spinach (*Spinacia oleracea* L.) was grown in the open. An axenic culture of *C. paradoxa* was kindly provided by Prof. Dr Hans Bothe (Universität Köln, Germany). Cells of *C. paradoxa* (Glaucocystophyta) were grown autotrophically in a liquid medium [13], complemented with 5% CO_2, at 6000 Lux of white incandescent light (cycles of 14 h light and 10 h dark).

2.2. Enzyme preparations

Soluble proteins of spinach leaves were isolated by homogenizing 40 g of deribbed leaf tissue in 40 ml of grinding medium consisting of 10 mM potassium phosphate, pH 7.5 and 10 mM 2-mercaptoethanol in a warring blender. The resulting slurry was squeezed through 4 layers of cheese cloth and centrifuged for 40 min at 40 000×*g*. After diluting with distilled water to a conductivity of less than 3 mS/cm, the supernatant was loaded onto a DEAE-Fractogel column (2.5×10 cm) equilibrated with grinding buffer. Proteins were eluted by a linear 200 ml gradient of 0–300 mM KCl in grinding buffer and collected into 2.5 ml fractions.

For the separation of proteins from *C. paradoxa*, about 4 g of wet packed material was harvested by centrifugation. They were homogenized with a bead beater in the presence of 10 g beads (0.5 mm in diameter) in 10 ml of grinding medium for six times 3 s with intermittent cooling. After centrifuging for 45 min at 40 000×*g*, the supernatant was processed for enzyme separation on a DEAE-Fractogel in the same way as with spinach.

2.3. Enzyme assays

Aldolase activities were assayed according to a modified procedure of Rutter [5]. The 1 ml assay consisted of 100 mM Tris-HCl, pH 7.5, 1 U each of triosephosphate isomerase and glycerol-3-phosphate dehydrogenase, 4.5 mM $MgCl_2$, enzyme aliquot and 1 mM FBP, 1 mM F1P or 20 mM SBP, respectively. One unit of activity is defined as the cleavage of 1 μmol substrate per min equivalent to the oxidation of 2 μmol NADH in case of FBP and of 1 μmol in case of the other two substrates. For measuring the class II aldolase activity, 5 or 10 mM

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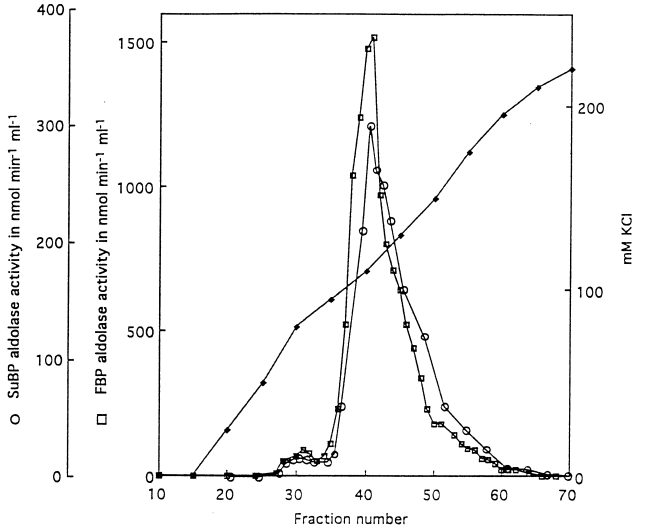

Fig. 1. Separation of FBP and SuBP aldolases from spinach by ion exchange chromatography on a DEAE-Fractogel.

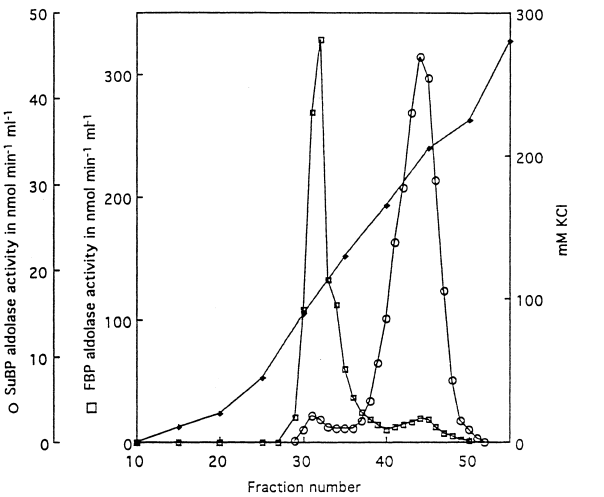

Fig. 2. Separation of FBP and SuBP aldolases from *C. paradoxa* by ion exchange chromatography on a DEAE-Fractogel.

EDTA was added to the enzyme preparation 5 min before adding the assay mixture. The final concentration of EDTA was 1 mM. This concentration did not influence the activity of the coupling enzymes. This activity was then compared with the activity without EDTA treatment.

3. Results

When chloroplast and cytosolic isoenzymes of aldolase either from spinach leaves or cells of *C. paradoxa* were separated by anion exchange chromatography on a DEAE-Fractogel (Figs. 1 and 2), two peaks of FBP aldolase activity are observed, the first peak representing the cytosolic isoenzyme and the second peak the plastid isoenzyme, respectively [12,14,15]. The FBP aldolase activity of the cytosol enzyme from spinach leaves was only about 4% of the total activity but occasionally accounted for up to 15%. The FBP aldolase activity of the cytosol isoenzyme in cells of *C. paradoxa* was slightly higher than the plastid isoenzyme.

The aldolase activity with SuBP of both organisms showed peaks at the same position as with FBP (Figs. 1 and 2). The ratios of FBP to SuBP activity were close to unity for the two plastid isoenzymes of spinach and of *C. paradoxa* (Table 1). The SuBP aldolase activity of the cytosolic isoenzyme of spinach was still substantial while this activity in cells of *C. paradoxa* was very low. The two class I aldolases of spinach showed also activity with F1P, another substrate of aldolases in animal tissues (e.g. [16]). This activity could only be measured for the class I aldolases of higher plants, but not for the class II enzymes of *C. paradoxa*, even at concentrations of up to 100 mM F1P.

The K_m(FBP) values (Table 1) were in the order of 1–20 μM in the case of the two class I aldolases of spinach and in the order of 1 mM in the case of the two class II aldolases from cells of *C. paradoxa*. The K_m(SuBP) were generally somewhat smaller for all these aldolases except for the cytosolic isoenzyme of spinach. The K_m(F1P) was in the order of 2–4 mM for the two spinach isoenzymes.

Both the FBP and SuBP aldolase activities of *C. paradoxa* were inhibited by EDTA (data not shown) and no additional SuBP aldolases could be found, excluding any class I aldolase activity in cells of *C. paradoxa*. On the other side, both aldolases from spinach leaves were not affected by EDTA in their FBP and SuBP aldolase activities.

In order to strengthen the view of one protein cleaving FBP, SuBP and F1P, we assayed the activities in the simultaneous presence of two substrates (Table 2). In some instances, substrate concentrations were lowered as compared to standard assay conditions (see Section 2) in order to achieve nearly equal activities for various substrates. The activity in the presence of two substrates was always within the limits of that of a single substrate (Table 2). This clearly implies that the various substrates competed for one binding at both class I and class II aldolases.

Table 1
Activities and K_m values for FBP, SuBP and F1P of the two class I aldolases from spinach and the two class II aldolases from *C. paradoxa*

	K_m (μM)			Activity (mU/ml)		
	FBP	SuBP	F1P	FBP	SuBP	F1P
Spinach (cytosol)	1	4	1800	380	140	80
Spinach (plastid)	20	6	4000*	1370	520	160
C. paradoxa (cytosol)	660**	230**	nma***	180	11	n.m.a.
C. paradoxa (plastid)	1000**	20**	nma***	103	130	n.m.a.

*Sigmoid kinetics. Value represents $S_{0.5}$.
**Substrate inhibition at 10 mM and above.
***n.m.a., no measurable activity up to 100 mM F1P.

Table 2
Activities of the cytosol and plastid aldolases from spinach and *C. paradoxa* in the presence of one or two substrates

	Activity (mU/ml)	
	Cytosolic enzyme	Plastidic enzyme
Spinach		
FBP aldolase	150	3500
SuBP aldolase	425	1125
F1P aldolase	100	437
FBP+SuBP aldolase	425	3475
FBP+F1P	100	3437
SuBP+F1P	425	425
C. paradoxa		
FBP aldolase	1150	35
SuBP aldolase	50	115
F1P aldolase	n.m.a.	n.m.a.
FBP+SuBP aldolase	700	55

4. Discussion

The class II aldolases of *C. paradoxa* are bifunctional for FBP and SuBP as are the class I aldolases from higher plants ([10,11], this paper). This implies that in both instances a basic reaction mechanism enables each class I and class II aldolases to react with either substrate. The detection of F1P aldolase activity for the class I aldolases only might be the consequence of that the K_m values for F1P of class II aldolases are possible to high to allow such an activity. In any case, no function for the F1P aldolase activity has yet been proposed in plant metabolism.

As to the physiology of the Calvin cycle, also the class II aldolase in the plastids of *C. paradoxa* has a 2-fold function in this cycle. No function of SuBP aldolase activity is yet known for the cytosolic plant enzymes. While the ratio in FBP and SuBP aldolase activities and the affinities are fairly similar in the two class I aldolases of higher plants, at least the activity ratio of FBP to SuBP is much more in favor of SuBP in the plastidic class II enzyme of *C. paradoxa* than in the respective cytosolic enzyme. The aldolase of the cyanobacterium *Anacystis nidulans* appears also to have a SuBP aldolase activity, however, with an unrealistically high K_m(SuBP) value of 10 mM [17]. The bifunctionality of the class II aldolases for FBP and SuBP in *Cyanophora paradoxa* is, therefore, the last documentation for multifunctional enzymes in the Calvin cycle.

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