Volume 205, number 1 FEBS 3954 September 1986

Stucture of phospho*enol*pyruvate carboxylase from maize leaves

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Received 9 June 1986

Phospho*enol*pyruvate carboxylase (PEPC) from maize leaves has an M_r of 400000. The native enzyme molecule is a homotetramer. The amino acid composition of PEPC is determined. The enzyme contains 8 half-cystine residues per subunit. The role of half-cystine residues and the steric arrangement of the enzyme protein molecule are discussed.

*Phospho*enol*pyruvate carboxylase* *Photosynthesis* *Amino acid composition* (Zea mays)

1. INTRODUCTION

Phospho*enol*pyruvate carboxylase (EC 4.1.1.31) has an important role in nonphotosynthetic CO_2 fixation in a number of organisms. In algae, bacteria and C_3 plants the enzyme plays an anaplerotic role, while in C_4 and Crassulacean plants it catalyzes the initial carboxylation reaction in the photosynthetic fixation of atmospheric CO_2 [1]. The PEPC is present as two isoenzymes in green leaves of maize [2,3]. Major PEPC I isoenzyme has more allosteric and regulatory properties than PEPC II [2–5]. The kinetic and regulatory properties of the PEPC from C_4 plants have been studied in detail [1–9]. The role of essential amino acid residues (histidine, arginine, cysteine) has been also studied [6,9–11].

Although the PEPC from maize leaves is one of the best studied enzymes with this activity, the data about the structure of this enzyme have not yet been described. The structure of the PEPC protein molecule (major PEPC I isoenzyme) is studied here.

Abbreviations: DTNB, 5,5′-dithiobis(nitrobenzoic) acid; DTT, dithiothreitol; ME, 2-mercaptoethanol; PEPC, phospho*enol*pyruvate carboxylase; -SH, sulfhydryl

2. MATERIALS AND METHODS

The PEPC I isoenzyme was prepared from green leaves of maize *(Zea mays* L., cv. CE 205-S) by a modified procedure described in [3]. PEPC prepared by this procedure was further rechromatographed on a DEAE-cellulose column and on a Sepharose 4B column.

The PEPC activity was measured as described in our previous paper [3].

The M_r values of PEPC and its subunits were determined by gel filtration on a Sepharose 4B column (2 × 50 cm). Urease (483 000), catalase (232 000), γ-globulin (157 000) and bovine serum albumin (68 000) were used as standards.

Disc electrophoresis on polyacrylamide gel at pH 8.9 was carried out by the method of Slustr [12] in 7.5% polyacrylamide gel. The proteins were detected by Amido black 10B in 7% acetic acid and the enzyme activity by Fast violet B [13].

Enzyme subunit analysis was done after treatment with SDS by a modified Weber and Osborn method [14]. Bovine serum albumin (68 000), ovalbumin (43 000) and haemoglobin (15 500) were used as standards. Subunit analysis of the enzyme was also done on Sepharose 4B column (2 × 50 cm). Purified enzyme was redissolved in Tris-HCl

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00145793/86/$3.50

buffer (pH 9.0 or 6.0) containing 5 mM ME without SDS and applied on a Sepharose 4B column. Elution was carried out by the buffer, in which the enzyme was dissolved.

2.1. *Amino acid analysis*

Prior to amino acid analyses protein samples (PEPC I) were thoroughly dialyzed against distilled water and lyophilized. An amount of 1 mg PEPC was hydrolyzed for 20 or 70 h in 6 M HCl at 110°C. After evaporation of the acid, the amino acid content was determined in the hydrolysate on a Durrum 500 instrument. The content of half-cystine and methionine was determined after oxidation to cysteic acid and methionine sulfone, respectively, with performic acid according to Schram et al. [15]. The half-cystine content was also determined by the method of Zahler and Cleland [16] with DTNB as a specific reagent.

The number of free -SH groups in the PEPC molecule was estimated according to Riddles et al. [17] and Zahler and Cleland [16].

3. RESULTS

The maize PEPC preparation obtained is homogeneous on discontinuous polyacrylamide gel electrophoresis at alkaline pH and on discontinuous SDS electrophoresis in the presence of ME. Amino acid composition of PEPC is summarized in table 1. We do not quantify the tryptophan content determined after alkaline hydrolysis of PEPC, but its content is less than five residues per enzyme subunit. The enzyme has an M_r of 400 000 as determined by gel filtration on a Sepharose 4B column. By discontinuous SDS electrophoresis and by SDS-gel filtration in the presence of ME a subunit of M_r 100 000 was found. The native enzyme molecule is homotetramer. Upon alkali (pH 9.0) or acid (pH 6.0) exposure, the PEPC partially dissociated into dimer and monomer. The mono-, di- and tetramer can be found on Sepharose 4B gel filtration when the reduction is incomplete (fig.1). A trimer is never present. As the partial dissociation into dimer or monomer takes place in the presence of ME under nondenaturing conditions (fig.1), noncovalent bonds participate relatively insignificantly in the stabilization of the tetramer structure. No PEPC activity is detected with the mono- and dimer. Only tetramer exhibits the enzyme activity

Table 1

Amino acid composition of phospho*enol*pyruvate carboxylase

Amino acid	Residue per mol of the PEPC subunit (100 000)	
	Actual	Nearest integer
Aspartic acid	80.4	80
Threonine	40.7	41
Serine	46.2	46
Glutamic acid	78.8	79
Proline	25.6	26
Glycine	71.6	72
Alanine	81.5	82
Valine	50.6	51
Methionine	13.7	14
Isoleucine	33.2	33
Leucine	57.6	58
Tyrosine	21.9	22
Phenylalanine	19.7	20
Histidine	11.7	12
Lysine	45.3	45
Arginine	49.7	50
Tryptophan	–	–
Half-cystine[a]	8.3	8

[a] An average of determinations by different methods (see section 2)

(fig.1). The enzyme contains about eight half-cystine residues per subunit (table 1). Four of the eight half-cystine molecules per subunit are cysteine residues. They were determined by the methods of Riddles et al. [17] and Zahler and Cleland [16] as free or partially free thiol residues. Two thiols are

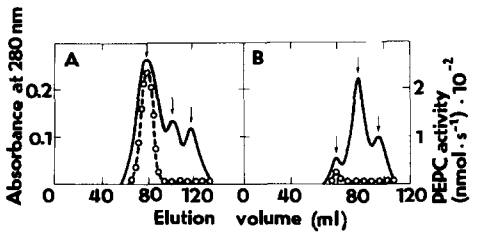

Fig.1. Gel filtration of PEPC on Sepharose 4B at pH 9.0 (A) and 6.0 (B). PEPC was preincubated in 50 mM Tris-HCl buffer containing 5 mM ME of a given pH (5 h) and applied on a Sepharose 4B column (2 × 50 cm). The elution was carried out by the above mentioned buffers. Proteins (——) and the PEPC activity (○---○). Locations of tetramer, dimer and monomer are indicated by arrows.

free accessible thiols. The next thiol (the third) is hidden in the PEPC protein molecule (SDS must be present for determination of this group) and the fourth thiol is determined when only dithiothreitol (DTT) without SDS is present. The incubation of PEPC with DTT resulted not only in the increase of numbers of the free accessible thiol groups but the PEPC activity was also increased (to 179% of control). Four half-cystine residues form the -S-S- bridges.

4. DISCUSSION

As far as the steric arrangement of the maize PEPC molecule is concerned, it is clear that the enzyme molecule is a homotetramer. This was also found by O'Leary [1]. Only the tetrameric molecule of the enzyme has the full catalytic activity. The dimer or monomer did not exhibit an enzymic activity (fig.1). The enzyme contains thirty-two-half-cystine residues per molecule, i.e. eight half-cystine residues per subunit. Four of the half-cystine residues in a subunit are cysteine residues and the SH groups of these residues are free or partially free. Some of these cysteine residues play an important role in a catalytic function of PEPC [6,10, 11]. Four half-cystine residues of a subunit would form an intrachain or interchain bond. Theoretically two possibilities for the arrangement of a tetrameric structure can be considered: 'square' and tetrahedron (fig.2). As four half-cystine residues form the -S-S- bridges, the tetramer cannot form the tetrahedron arrangement. In this case, only three half-cystine residues would form disulfide bridges. Thus, we suppose that the subunits are bound in the square arrangement. Two possibilities for a square arrangement of the tetrameric structure can be considered: the one with double -S-S- bridges between subunits (fig.2C) or the other with one interchain -S-S- bridge between subunits and one intrachain -S-S- bridge (fig.2B). As the most drastic conditions must be present for total dissociation of the PEPC tetrameric molecule into subunits (SDS, ME, heating) or for determination of all half-cystine residues (oxidation to cysteic acid) the square arrangement with double -S-S- bridges between subunits is, in all probability, present. However, a definite proof of this structure must be elucidated by further investigations.

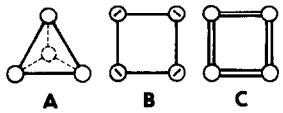

Fig.2. Possible steric arrangements of the native maize PEPC molecule. (A) Tetrahedron, (B) square with one interchain -S-S- bridge between subunits and one intrachain -S-S- bridge and (C) square with double -S-S- bridges between subunits.

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