THE HIGH ISOELECTRIC POINT OF THE PRECURSOR OF THE SMALL SUBUNIT OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE AND A POSSIBLE ROLE FOR THE TRANSIT PEPTIDE

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1. Introduction

Ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) (RuBPCase) is a soluble chloroplast enzyme and is composed of 8 large and 8 small subunits [1]. The large subunit is coded in chloroplast DNA [2] and the small one in nuclear DNA [3]. The small subunit may be synthesized as a precursor [4–7], which is post-translationally transported into the chloroplast; the extra sequence, transit peptide [8], is removed by a specific protease, and the resultant small subunit assembles with the large subunit to form RuBPCase [9]. More recently, the amino acid sequence of the transit peptide of the precursor from *Chlamydomonas reinhardtii* was determined [10]. However, the function of the peptide is not well understood.

Here, the isoelectric point of the precursor of the small subunit is reported and compared with that of the small subunit, using two-dimensional gel electrophoresis. Four variants of the small subunit as well as of the precursor were detected. The isoelectric points of the precursor variants were considerably higher than those of the small subunits. The isoelectric points of the variants of the major small subunit were 6.25 and 6.85, while those of the precursor were 8.5 and 9.1. The higher isoelectric point of the precursor suggests a possible role for the transit peptide which enables the precursor to bear a positive charge and to interact electrostatically with the negatively-charged envelope of the chloroplast.

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2. Materials and methods

2.1. *Materials*

RuBPCase, anti-RuBPCase IgG, small subunit, and anti-small subunit IgG were prepared as in [11]. [^{35}S]-Methionine (600 Ci/mmol) was obtained from New England Nuclear.

2.2. *Preparation of [^{35}S]methionine-labeled RuBPCase from pea leaves*

Pea seedlings (*Pisum sativum* var. Alaska) grown for 7 days in darkness were illuminated for 48 h with white light of ~10 000 lux. Of 10 μCi [^{35}S]methionine (600 Ci/mmol) 10 μl containing 2% Tween 80 was spread over the leaf surface of intact seedlings illuminated for an additional 6 h. The labeled proteins were extracted and immunoprecipitated by anti-RuBPCase IgG as in [11]. The immunoprecipitates gave 2 bands of M_r ~55 000 and 14 000 in SDS gel electrophoresis and were identified as RuBPCase.

2.3. *Preparation of [^{35}S]methionine-labeled precursor by a cell-free system*

Cytoplasmic RNA from pea seedlings illuminated for 48 h was extracted, translated in a wheat germ cell-free system, and immunoprecipitated as in [11]. The immunoprecipitates gave a main band of M_r 20 000 corresponding to the precursor of the small subunit [7].

2.4. *Isoelectric focusing*

The in vivo labeled and immunoprecipitated RuBPCase and the in vitro synthesized and immunoprecipitated precursor were dissolved in 8.5 M urea/2% ampholyte (LKB, pH 3.5–10), and subjected to

isoelectric focusing ($V \times h$ = 5500). After focusing, the gels were equilibrated with an SDS-containing buffer (2.3% SDS/5% 2-mercaptoethanol/10% glycerol/62.5 mM Tris–HCl (pH 6.8)) for 2 h, and applied to 15% slab gels (acrylamide:bis = 30:0.18) as in [12]. The gel was fixed, stained, and fluorographed [13]. For the determination of the isoelectric points of the in vitro synthesized precursor variants, the immunoprecipitates were dissolved in 8.5 M urea/2% ampholyte (Pharmacia, pH 8–10.5) and subjected to isoelectric focusing ($V \times h$ = 4700). The gel was fixed in 15% trichloroacetic acid, and extensively washed with ethanol/acetic acid/H_2O (25:8:65). The gel was cut into 5 mm pieces, and dried for 1 h at 80°C. To each piece, 0.3 ml H_2O_2 was added and incubated at 60°C for 16 h. After adding a scintillator, ACS II (Amersham), the radioactivity of the solution was assayed.

3. Results and discussion

The presence of 3 different, large subunit polypeptides is confirmed for all RuBPCases from a wide variety of plants, but the number of different, small subunit polypeptides is varaible. For example, there is one kind of polypeptide present in the small subunit of RuBPCase in *Triticum monococcum* and there are 4 in *Nicotiana excelsior* [14]. On the basis of peptide maps, the 3 large subunit polypeptides are known to be the results of modification of a single gene product [15]. The variants of the small subunit are believed to be products of separate genes [15]. The presence of 2 variants of the small subunit in pea has been reported [16]. To see the differences between the compositions of the small subunits and their precursors, the labeled RuBPCase and the in vitro synthesized precursors were resolved by 2-dimensional polyacrylamide gel electrophoresis (isoelectric focusing in the first dimension, SDS gel electrophoresis in the second). Two fluorograms are shown in fig.1a,b.

RuBPCase exhibited 1 broad spot corresponding to a M_r 55 000 and 4 spots corresponding to a M_r 14 000 as shown in fig.1a. The former is the large subunit and the latter corresponds to the variants of the small subunit. The isoelectric points of the variants were 5.8, 6.25, 6.85 and 7.5, respectively. Two variants, with pI 6.25 and 6.85, were more extensively labeled than the others. The most extensively labeled spot had pI 6.85. Two variants of the small subunit detected [16] seem to correspond to the variants of pI 6.25 and 6.85.

Results obtained for the variants of the precursors are shown in fig.1b. There are 4 detectable spots corresponding to M_r 20 000, a value which equals the

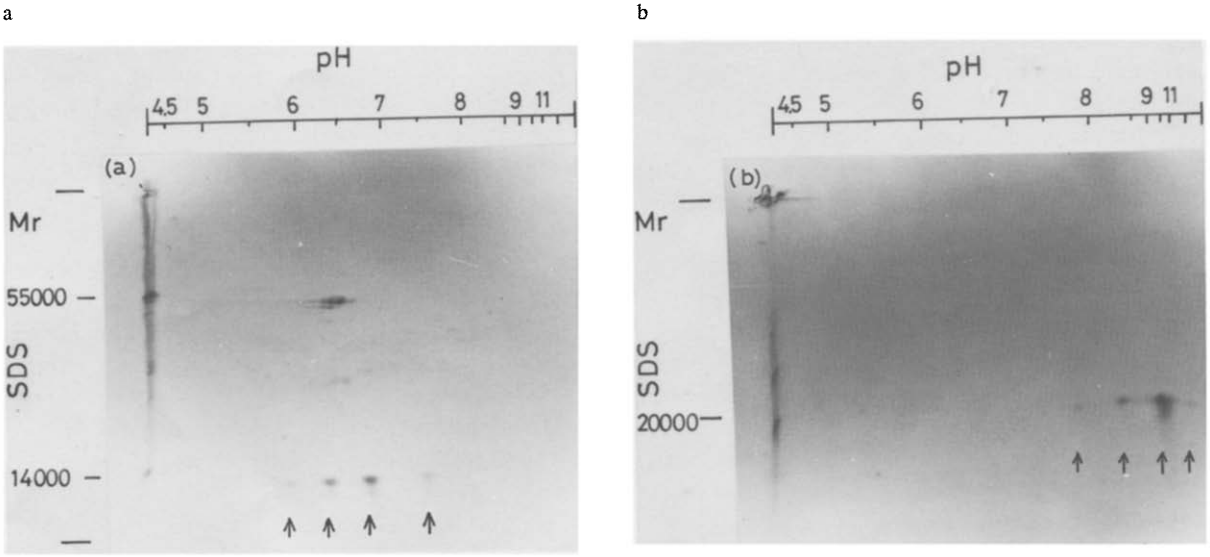

Fig.1. Two-dimensional gel electrophoresis of RuBPCase and of its precursor synthesized in vitro. (a) [^{35}S]Methionine-labeled RuBPCase was prepared and immunoprecipitated as in section 2. The immunoprecipitates were analyzed by isoelectric focusing and SDS–polyacrylamide gel electrophoresis, and fluorographed as described. (b) [^{35}S]Methionine-labeled precursor was prepared and immunoprecipitated as in [11]. The immunoprecipitates were treated as above.

M_r values of the precursor, in the region of isoelectric points higher than those of the small subunit. The intensities of these spots seem to be similar to those of the small subunits. The middle 2 spots are labeled more extensively than the 2 spots on either side. The more alkaline spot of the middle 2 was labeled more extensively than the acidic one. Such a correspondence between the properties of the small subunits and their precursors suggests that the in vitro synthesized precursor is translated from an mRNA similar to or identical with the functional mRNA present in vivo. This heterogeneity of the mRNA of the small subunit can probably be explained by the presence of 4 kinds of genes coding for the small subunit in the pea plant.

The parallel movement of all variants of the precursor to the alkaline side implies that the transit peptides contain many basic amino acid residues and that the amino acid sequence of the 4 variants is similar. This is true for the precursor of *Chlamydomonas reinhardtii* [10]. Amino acid sequence data on the precursor in *Chlamydomonas* suggest the same [10]. Probably this property is common to the transit peptide of the precursors of the small subunits.

To determine the isoelectric point accurately, isoelectric focusing using an alkaline pH-range ampholyte was used. The results shown in fig.2 indicate 2 major peaks. The isoelectric point of the intensively labeled spot was 9.1 and that of the other 8.5. These correspond to the 2 middle spots in fig.1b. The variants of the small subunit of pI 6.25 and 6.85 seem to correspond to the precursor variants of pI 8.5 and 9.1, respectively. Thus, the precursor is more basic than the small subunit.

Since the envelope of chloroplasts has been shown to have a strong negative charge [17], the high isoelectric point of the precursor suggests that the precursor is positively charged in the cytoplasm and interacts electrostatically with the envelope of the chloroplast. The attachment of the small subunit to the chloroplast envelope has been demonstrated [18,19]. Thus, the results presented strongly suggest that the biological role of the transit peptide is, at least in part, to enable the precursor to interact with the negatively charged envelope, due to the positive charge of the transit peptide in the cytoplasm.

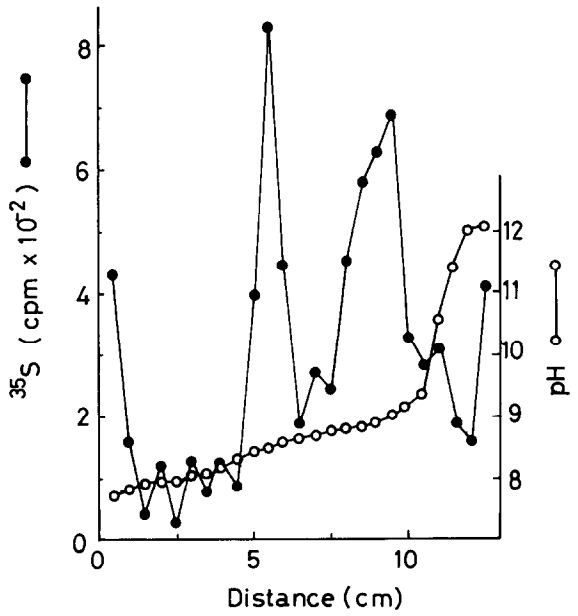

Fig.2. Isoelectric point of two major variants of the precursor of the small subunit. The precursor was prepared and analyzed as in section 2.

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