Isolation and Characterization of the Cytosolic and Chloroplast Forms of Spinach Leaf Fructose Diphosphate Aldolase*

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Two different isoenzymes of fructose-P₂ aldolase can be resolved by chromatography of crude spinach leaf extracts on DEAE-cellulose columns. The acidic isoenzyme comprises about 85% of the total leaf aldolase activity. The two forms differ in primary structure as judged by their distinctive amino acid compositions, trypic peptide patterns, and immunological properties. Only the acidic isoenzyme was detected in extracts of isolated chloroplasts, suggesting that this molecule represents the chloroplast form of spinach leaf aldolase while the basic isoenzyme is of cytosolic origin.

The cytosolic (basic) isoenzyme and chicken aldolase A₂ are similar in the following respects. 1) They have similar specific catalytic activity (10–15 unitst/mg); 2) they are both highly sensitive to inactivation by very limited digestion with bovine pancreatic carboxypeptidase A; 3) they both have subunit molecular weights of 40,000; 4) they both have derivatized (blocked) NH₂-terminal structures; 5) they are both resistant to thermal denaturation at 50 °C; and 6) they both regain catalytic activity following reversible denaturation at pH 2.3 or in 5.8 M urea. Also, the cytosolic aldolase cross-reacted immunologically with the single aldolases present in spinach seeds and in wheat germ. Furthermore, this isoenzyme readily "hybridized" with chicken aldolase A₂, in vitro. These observations demonstrate the close homology between the cytosolic aldolases derived from plant and animal origins.

The chloroplast aldolase had a specific catalytic activity of about 8 unitst/mg and, like its cytosolic counterpart, was severely inactivated by limited digestion with carboxypeptidase A. However, this isoenzyme was distinct from the cytosolic aldolase in the following characteristics: 1) its "small" subunit size (Mₚ = 38,000); 2) its derivatized NH₂-terminal structure; 3) its high sensitivity to thermal denaturation at 50 °C; and 4) its inability to refold into an enzymatically active conformation following denaturation at pH 2.3 or in 5.8 M urea. The distinctive properties of the chloroplast aldolase may be expected for an enzyme which is synthesized as a higher molecular weight precursor on cytosolic polysomes and is then proteolytically processed to the "mature" form during its migration into the chloroplast organelle.

In eucaryotic cells, a number of specific biochemical reactions take place in more than one intracellular compartment (6). For example, in green plant leaves, many of the reactions of glycolysis occur in the chloroplast as well as in the cytosol. The glycolytic enzymes of the chloroplast participate in the conversion of atmospheric CO₂ to glucose through the "Calvin" cycle, while the cytosolic forms function in the breakdown of glucose through the classical glycolytic pathway (3). Both the chloroplast and cytosolic glycolytic enzymes are encoded for by the nuclear genome (3, 4). Thus, by analogy with a number of other proteins (4), the glycolytic enzymes destined to take up residence in the chloroplast should be synthesized as higher molecular weight precursors on cytosolic polysomes. Proteolytic processing of these precursors to the mature forms would be envisioned to occur during their uptake into the chloroplast organelle (4).

We have been using the glycolytic enzyme fructose-P₂ aldolase as a model system to investigate the mechanisms which regulate the levels of specific enzymes in eucaryotic cells. We have recently initiated an analysis of the properties and synthesis of the chloroplast and cytosolic forms of aldolase in green plant leaves. Previous work by others (5–9) showed that the two forms of leaf aldolase differ in charge. Also, Anderson and associates reported that the cytosolic and chloroplast forms of aldolase isolated from pea leaves differ somewhat in amino acid composition (10) even though the two aldolases were reported to be indistinguishable on the basis of subunit molecular weight (M₀ = 38,000) and NH₂- and COOH-terminal structure (11). We previously isolated two spinach leaf aldolases which had different subunit molecular weights (12). In these earlier studies we suggested that the "smaller" aldolase may have been produced by proteolytic modification of the "larger" enzyme (12). However, in the present work, we show that the two aldolases differ considerably in primary structure and, hence, are the products of different genes. The aldolase composed of 40-kDa subunits is of cytosolic origin and resembles the aldolases of animal species in a number of respects. The smaller aldolase is composed of 38-kDa subunits, is located in the chloroplast, and possesses a number of features which distinguish it from the cytosolic aldolases derived from both plant and animal sources.

EXPERIMENTAL PROCEDURES

Isolation of the Two Spinach Leaf Aldolases—Fresh spinach leaves (a total of 1000 g) were washed in water and were homogenized in 2
Characterization of Cytosolic and Chloroplast Aldolases

volumes of 10 mM Tris-HCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, 1 mM phenylmethanesulfonyl fluoride, 3 mM 1,10-phenanthroline, pH 7.5, in a Waring blender. The homogenate was filtered through cheesecloth, and the filtrate was titrated to pH 5.0 with 7 N acetic acid. After stirring at 4 °C for 30 min, the precipitated protein was collected by centrifugation at 10,000 g and was discarded. The supernatant was adjusted to pH 7.5 with concentrated NH4OH and was diluted with 2 volumes of distilled water. Then, approximately 200 ml of packed Whatman DEAE-cellulose was applied to the dialyzer bag containing 5 mM Tris-HCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, pH 7.0. Then, the sample was applied to a DEAE-cellulose column (2 × 10 cm; Whatman P-11) previously equilibrated in the same buffer. The column was washed with buffer until the A260 of the eluate was less than 0.65. Then, the aldolase was specifically eluted from the column with containing 1 mM fructose-P2.

The “acidic” isoenzyme could also be purified by affinity elution from phosphocellulose columns. However, the ability of this isoenzyme to bind to various batches of phosphocellulose varied considerably, resulting in highly variable yields of the enzyme from one preparation to another. As an alternative, we found that chromatography of the enzyme preparation on DEAE-cellulose, followed by pooling only the peak fractions, resulted in substantially higher yields of the enzyme with specific catalytic activities comparable to those of the enzyme isolated by affinity elution from phosphocellulose.

The aldolase preparations were dialyzed against 10 mM Tris-HCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, pH 7.5, and were stored at 4 °C until used. These preparations were judged to be greater than 97% pure on the basis of electrophoretic analysis in sodium dodecyl sulfate-polyacrylamide gels.

Isolation of Intact Chloroplasts—Spinach leaf chloroplasts were isolated in a similar manner as described from the DEAE-cellulose column, as described previously (13). Fifty grams of cut leaves were homogenized in 200 ml of 50 mM Tris-HCl, 5 mM magnesium acetate, 10 mM 2-mercaptoethanol, 500 mM sucrose, pH 7.8, in a Waring blender for 4 min. The homogenate was filtered through a layer of Miracloth (Calbiochem, catalog no. 475855), and the filtrate was centrifuged at 5,000 g for 2 min. The chloroplast pellet was gently suspended in 60 ml of the above buffer and the sample was centrifuged as before. Then, the pellet was suspended in 15 ml of 10 mM Tris-HCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, pH 7.5, and were stored at 4 °C until used. These preparations were judged to be greater than 97% pure on the basis of electrophoretic analysis in sodium dodecyl sulfate-polyacrylamide gels.

Enzyme Assays, Protein Determinations, and Electrophoretic Methods—Samples were assayed for aldolase activity at 25 °C by the continuous spectrophotometric assay previously described (14). Activity units are defined as micromoles of fructose-P2 cleaved/min and specific activities as units/mg of protein. Protein concentrations of crude and purified aldolase preparations were estimated by the Coomassie blue staining procedure described by Bradford (15), using chicken muscle aldolase A as a standard.

Electrophoretic analysis of crude and purified aldolase preparations was performed in 5% polyacrylamide slab gels containing 0.1% sodium dodecyl sulfate using the gel reagents and buffer systems suggested by Laemmli (16) or in nondenaturing 5% “basic” polyacrylamide slab gels as previously described (17, 18). Gels were stained for protein with Coomassie blue. Electrophoresis was performed on cellulose acetate strips followed by staining the strips specifically for aldolase activity was accomplished using the procedure described by Susor et al. (19).

Immunochemical Methods—Antisera to the spinach leaf aldolases were prepared in rabbits. Each rabbit received 0.3 mg of pure enzyme in complete Freund’s adjuvant by multiple subcutaneous injections. Two weeks later, the injection schedule was repeated, and blood samples were collected at 2-week intervals. “Booster” injections were given periodically to maintain anti-aldolase titers. Antisera were stored frozen at −20 °C in small aliquots.

Ouchterlony double diffusion tests were performed at room temperature in 0.8% agar gels prepared in 10 mM Tris-HCl, pH 7.4, which also contained 4% NaCl. Precipitin lines were allowed to form overnight, and the gels were extensively washed in the above solution. Gels were fixed and stained for protein with Coomassie blue.

Amino Acid Analysis—Amino acid compositions were determined from timed hydrolysates prepared in 6 N HC1 at 110 °C under reduced pressure (evacuated to remove oxygen). Analyses were performed in a Durrum (Dionex) D-500 instrument. Residues were calculated assuming molecular weights of 40,000 and 38,000 for the cytosolic and chloroplast aldolase subunits, respectively. Half-cystine was determined as cysteic acid following performic acid oxidation (20). Amino Acid Sequence Determinations—NH2-terminal sequences were determined with a Beckman 890C Sequencer using a 1 M Quandrol program (Beckman Program 072172C). Phenylthiolydantoina were formed by 1 N HCl treatment at 80 °C for 10 min followed by identification with a Hewlett-Packard 1084B high performance liquid chromatograph equipped with a Beckman C-18 microsphere column. Development was with an acetonitrile/methanol/acetate buffer system (21).

Tryptic Peptide Patterns—Approximately 1 mg of each aldolase was carboxymethylated with iodoacetic acid as described previously (22). The carboxymethylated proteins were dissolved in 20 ml of 0.1 M NH4HCO3, pH 8.5, and 10 µg/mg of aldolase of trypsin was added. The reaction mixtures were incubated at 37 °C and, after 4-h, a second aliquot of trypsin (equal to the first) was added. After incubation overnight (~15 h) the samples were lyophilized and redissolved in 500 µl of 0.1% trifluoroacetic acid. After centrifugation in the Beckman microfuge, aliquots of 50 µl were applied to a reverse phase C-8 column which was developed with a gradient of 0.1% trifluoroacetic acid (aqueous) and 2-propanol (organic) (linear, 90% 2-propanol) using a Beckman/Altex high performance liquid chromatograph. The peptide elution patterns were monitored by absorption at 280 nm.

RESULTS

Detection and Intracellular Localization of the Two Spinach Leaf Aldolase Isoenzymes—Two different aldolase activities...
Basic aldolases appear to be present in the chloroplast and total leaf homogenates. Thus, in the intact leaf, the acidic and the strips were stained for aldolase activity (see under “Experimental preparations were electrophoresed on cellulose polyacetate strips and the strips were stained for aldolase activity (see under “Experimental procedures” for details). Lane 1, total leaf extract; lane 2, chloroplast isoenzyme; lane 3, (1 + 2), mixture of the two isoenzymes. Protein standards used were: 1) muscle enolase (M_r = 50,000); 2) muscle creatine kinase (M_r = 43,000); 3) muscle aldolase (M_r = 40,900); 4) muscle glyceraldehyde-3-P dehydrogenase (M_r = 37,000).

The two aldolase isoenzymes were isolated as described under “Experimental procedures.” Aldolase activity in chloroplast and in total leaf extracts was concentrated and partially purified by adsorption to DEAE-cellulose followed by elution with 0.5 M NaCl. Aliquots of these preparations were electrophoresed on cellulose polyacetate strips and the strips were stained for aldolase activity (see under “Experimental procedures” for details). Lane 1, total leaf extract; lane 2, chloroplast extract.

The three enzymes in 10 mM Tris-HCl, 1 mM EDTA, 2-mercaptoethanol (pH 7.6) were diluted to 0.4 mg/ml and were adjusted to 1% lithium chloride. Bovine pancreatic carboxypeptidase A (Sigma, type II) was added at a concentration of 1 μg/ml, and the solutions were incubated at 25°C. At the times indicated, aliquots were assayed for aldolase activity. After incubation for 3 h, other aliquots were subjected to electrophoresis in a sodium dodecyl sulfate-polyacrylamide gel, and the gel was stained for protein (see inset). In each comparison the control sample appears on the left and the carboxypeptidase A-treated enzyme appears on the right.

Properties of the Chloroplast and Cytosolic Spinach Leaf Aldolases—The two aldolase isoenzymes were isolated as described under “Experimental Procedures.” Greater than 50% recoveries of the two isoenzymes were consistently observed during the purification procedures, and the enzyme preparations were found to be greater than 97% pure on the basis of electrophoretic criteria. The cytosolic and chloroplast aldolases had specific catalytic activities of 10–12 units/mg and 7–8 units/mg, respectively. These specific activities are similar to those exhibited by the well characterized mammalian and avian aldolases A (about 15 units/mg) and C (about 8 units/mg). The two spinach leaf aldolases differed considerably in their sensitivities to thermal denaturation. As shown in Fig. 3, the

Fig. 2. Comparisons between the aldolase isoenzymes present in total leaf extracts and in isolated spinach leaf chloroplasts. Chloroplasts were isolated as described under “Experimental procedures.” Aldolase activity in chloroplast and in total leaf extracts was concentrated and partially purified by adsorption to DEAE-cellulose followed by elution with 0.5 M NaCl. Aliquots of these preparations were electrophoresed on cellulose polyacetate strips and the strips were stained for aldolase activity. As shown in Fig. 2, the acidic isoenzyme was detected in the chloroplast preparation, while both aldolases were detected in total leaf homogenates. Thus, in the intact leaf, the acidic and basic aldolases appear to be present in the chloroplast and cytosol, respectively.

Fig. 3. Thermal stability of chicken muscle, spinach cytosolic (cyto.), and spinach chloroplast (chlo.) aldolases at 50°C. The three enzymes in 10 mM Tris-HCl, 1 mM EDTA, 2-mercaptoethanol (pH 7.2 at 50°C) were diluted to 0.1 mg/ml and were incubated at 50°C in conical plastic tubes (100 μl total volume). At the indicated times, aliquots were assayed for aldolase activity.

Fig. 4. Subunit molecular weights of the chloroplast and cytosolic spinach leaf aldolases. The purified enzymes were electrophoresed in a sodium dodecyl sulfate-polyacrylamide slab gel using proteins of known molecular weights as standards. The gel was stained for protein with Coomassie blue. Lane 1, cytosolic isoenzyme; lane 2, chloroplast isoenzyme; lane 3, (1 + 2), mixture of the two isoenzymes. Protein standards used were: 1) muscle enolase (M_r = 50,000); 2) muscle creatine kinase (M_r = 43,000); 3) muscle aldolase (M_r = 40,900); 4) muscle glyceraldehyde-3-P dehydrogenase (M_r = 37,000).

Fig. 5. Inactivation of chicken muscle, spinach cytosolic (cyto.), and spinach chloroplast (chlo.) aldolases by digestion with carboxypeptidase A. The three aldolases were diluted to 0.4 mg/ml in 10 mM Tris-HCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, pH 7.6, and were adjusted to 1% lithium chloride. Bovine pancreatic carboxypeptidase A (Sigma, type II) was added at a concentration of 1 μg/ml, and the solutions were incubated at 25°C. At the times indicated, aliquots were assayed for aldolase activity. After incubation for 3 h, other aliquots were subjected to electrophoresis in a sodium dodecyl sulfate-polyacrylamide gel, and the gel was stained for protein (see inset). In each comparison the control sample appears on the left and the carboxypeptidase A-treated enzyme appears on the right.
Characterization of Cytosolic and Chloroplast Aldolases

The two spinach leaf aldolases differ in primary structure. As shown by the results of Ouchterlony double diffusion tests presented in Fig. 6, the two isoenzymes were readily resolved by immunological criteria. Also, the two enzymes were found to have distinct amino acid compositions (Table I). Further, the two enzymes generated different tryptic peptide patterns when tryptic digests were resolved by high performance liquid chromatography (Fig. 7). Finally, the two isoenzymes were distinguished on the basis of Edman degradative analysis (Table II). The cytosolic isoenzyme was not degraded with the Edman reagent suggesting that this aldolase had a derivatized (presumably acetylated) NH₂ terminus. In contrast, the chloroplast aldolase was readily degraded; the sequence of the first 18 amino acid residues of the chloroplast aldolase is presented in Table II.

Structural Similarity between the Cytosolic Aldolases Derived from Plant and Animal Origin—As shown in the top of Fig. 8, the cytosolic spinach leaf aldolase was found to cross-react with apparent immunological identity with the single aldolase present in spinach seeds. This observation together with the fact that both of these aldolases are composed of 40-kDa subunits (present studies and Ref. 12) suggest that these two enzymes are very similar, if not identical, molecules. Further, as shown in the bottom of Fig. 8, antisera raised against cytosolic spinach aldolase recognized the single aldolase present in wheat germ, an aldolase also composed of 40-kDa subunits (12). The generation of a predominant “spur” be-

### Table I

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Chloroplast*</th>
<th>Cytosolic*</th>
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<td>72 h</td>
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<td>17.2</td>
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<tr>
<td>Isoleucine</td>
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</tr>
<tr>
<td>Leucine</td>
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<tr>
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<td>10.3</td>
</tr>
<tr>
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</tr>
<tr>
<td>Histidine</td>
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<tr>
<td>Lysine</td>
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<tr>
<td>Arginine</td>
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</tr>
<tr>
<td>Tryptophan</td>
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</tr>
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</table>

*Expressed as residues/38,000 Da.

**Expressed as residues/40,000 Da.

Value obtained by extrapolation to zero time.

Determined from performic acid oxidation.

72-h value used.

ND, not determined.

The cytosolic isoenzyme was similar to chicken muscle aldolase A, in that it was quite stable to prolonged heating at 50 °C. In contrast, the chloroplast aldolase was inactivated with a first order half-life of under 3 min at this elevated temperature (Fig. 3).

The two spinach leaf aldolases have different subunit molecular weights as judged by electrophoretic analysis of the enzymes in sodium dodecyl sulfate-polyacrylamide gels (Fig. 4). The cytosolic and chloroplast aldolases were found to be composed of subunits of about 40,000 and 38,000, respectively, and these subunits were easily resolved by co-electrophoresis of a mixture of the two enzymes (Fig. 4).

As shown in Fig. 5, both leaf aldolases were similar to chicken aldolase A in that they were severely (90% or more) inactivated by treatment with very low levels of bovine pancreatic carboxypeptidase A. In all cases, the inactivation of enzyme activity was associated with very limited proteolysis at the COOH-terminal ends of the enzyme subunits, since no detectable differences in subunit molecular weights of treated and untreated enzymes were detected (Fig. 5, inset). It has been well documented that inactivation of aldolase molecules derived from both animal (23, 25) and plant (26) origin by digestion with carboxypeptidase A directly reflects proteolytic removal of functional COOH-terminal tyrosine residues from the enzymes; thus, the data in Fig. 5 suggest that both spinach leaf aldolases contain functional COOH-terminal tyrosine residues.

Cytosolic and chloroplast aldolases at concentrations of 0.48, 0.24, and 0.12 mg/ml. Only samples containing the cytosolic aldolase formed visually apparent precipitin lines with the antisera. Bottom, antisera prepared against the chloroplastic aldolase was placed in the center well. Outer wells contained clockwise from the top, alternating serial dilutions of cytosolic and chloroplast aldolases at concentrations of 0.48, 0.24, and 0.12 mg/ml. Only samples containing chloroplast aldolase formed visually apparent precipitin lines with the antisera.
Figure 7. Tryptic peptide patterns of the cytosolic (top) and chloroplast (bottom) spinach leaf aldolases as revealed by high performance liquid chromatography of peptide digest. Approximately 1 mg of the two aldolases was carboxymethylated and treated with trypsin as described under “Experimental Procedures.” Resulting digests were resolved by high performance liquid chromatography (see under “Experimental Procedures”). Isozyme 1, cytosolic aldolase; isozyme 2, chloroplast aldolase.

Table II

NH2-terminal sequence of spinach chloroplast fructose-P, aldolase

<table>
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<tr>
<th>Cycle</th>
<th>Residue</th>
<th>Yield*</th>
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</tr>
<tr>
<td>2</td>
<td>Serine</td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>Tyrosine</td>
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<tr>
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<td>Alanine</td>
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</tr>
<tr>
<td>5</td>
<td>Aspartic acid</td>
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</tr>
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<td>Glutamic acid</td>
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</tr>
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<td>7</td>
<td>Leucine</td>
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<tr>
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<td>Valine</td>
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<td>11</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>18</td>
<td>Glycine</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Value given in nanomoles. Approximately 20 nmol of protein was analyzed.

Although the cytosolic spinach leaf aldolase did not cross-react immunologically with chicken aldolase A, the structural homology between these aldolases was demonstrated by the ability of the two enzymes to hybridize in vitro (Fig. 9). The fact that a five-membered set of tetramers formed following reversible dissociation of the enzymes demonstrates that all possible tetrameric combinations of spinach and chicken aldolase subunits were produced during the renaturation process.

The Chloroplast Spinach Leaf Aldolase Does Not Refold into an Enzymatically Active Conformation following Denaturation in Vitro—During our attempts to construct heterotetramers composed of chicken and spinach chloroplast aldolase subunits, we observed that the chloroplast aldolase did not regain catalytic activity following treatment at pH 2.3 or with 5.8 M urea. The striking differences in the abilities of cytosolic and chloroplast aldolases to regain catalytic activity following
denaturation are illustrated in Table III. Both chicken and spinach cytosolic aldolases regained greater than 80% of their initial activities following treatment at pH 2.3 and 50% or more of the initial activity following treatment with 5.8 M urea. In contrast, the chloroplast spinach leaf aldolase did not regain detectable activity following these treatments (Table III). Further, these chloroplast aldolase subunits did not hybridize with chicken aldolase A subunits following reversible dissociation of a mixture of these enzymes at pH 2.3. As shown in the top of Fig. 10 chicken aldolase A₄ was the only enzymatically active species produced during the reassociation process. More importantly, chicken aldolase A₄ was the only protein species detected in the reassociation mixture by polyacrylamide gel electrophoresis under nondenaturing conditions (Fig. 10, bottom). These observations suggest that, once unfolded, the chloroplast aldolase subunits do not have the ability to refold into a specific conformation; it is possible that the chloroplast aldolase failed to enter the gel at all.

DISCUSSION

The present work confirms a number of observations that green plant leaves contain both chloroplast and cytosolic forms of fructose-P₇ aldolase. As shown here, the two forms derived from spinach leaves have similar specific catalytic activities, and both forms appear to possess functional COOH-terminal tyrosine residues. However, the two aldolases are encoded for by different genes as evidenced by their distinctive immunological properties, amino acid compositions, and tryptic peptide patterns. Schnarrenberger and associates (8, 9) have also recently reported that these aldolases can be distinguished immunologically.

The cytosolic spinach leaf aldolase was found to be similar to the well characterized chicken aldolase A₄ in terms of the following characteristics: 1) its specific catalytic activity; 2) its subunit molecular weight (Mr = 40,000); 3) its derivatized NH₂-terminal structure; 4) its resistance to thermal denaturation at 50 °C; and 5) its ability to refold into the enzymatically active conformation following reversible denaturation at pH 2.3 and in 5.8 M urea. Further, this cytosolic aldolase of dicot plant origin cross-reacted immunologically with the cytosolic aldolase found in wheat, a monocot plant, and readily hybridized with chick aldolase A₄ in vitro. These observa-
tions, together with our previous demonstration of the formation of heterotetramers composed of rabbit C and wheat germ aldolase subunits (12), emphasize the close evolutionary homology between the cytosolic aldolases derived from plant and animal species.

The cytosolic and chloroplast forms of pea leaf aldolase are encoded for by the nuclear genome (27), as is the case with other “Calvin cycle” enzymes (3). Thus, this nuclear encoded chloroplast-localized enzyme should be synthesized as a higher molecular weight precursor on cytosolic polysomes. This precursor would be envisioned to contain an NH₂-terminal extension (transit peptide) (28) which would be removed during uptake of the precursor into the chloroplast organelle. The fact that mature chloroplast aldolase is composed of “small” subunits, has undervatized NH₂ termini, and is unable to refold following denaturation in vitro may be a reflection of these posttranslational processing events. We are currently investigating the synthesis and posttranslational processing of the spinach leaf chloroplast aldolase.

A final question raised by the present work concerns the evolutionary relationship between the cytosolic and chloroplast plant leaf aldolases. A popular hypothesis proposes that present-day plastids (and mitochondria) are descendants of prokaryotic symbionts which took up residence in primitive eucaryotic cells (29, 30). During evolution, it is envisioned that the genetic information which codes for many of the chloroplast and mitochondrial proteins, including the “Calvin cycle” enzymes, was transferred from the prokaryotic to the nuclear genome of the eucaryotic host (29, 30). Thus it would be predicted that the chloroplast and mitochondrial proteins of present-day eucaryotes should share a common procaryotic gene. However, more recent observations by ourselves (35, 36) and others (37–45) demonstrated that some prokaryotic cells do contain Class I aldolases. Consequently, there may be a close evolutionary relationship between Class I aldolases of plant chloroplasts and those of certain prokaryotic cells. This possibility is currently being investigated in our laboratories.

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