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Chloroplast and Cytoplasmic Enzymes

VIII. AMINO ACID COMPOSITION OF THE PEA LEAF ALDOLASES[1]

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LOUISE E. ANDERSON[2]
Department of Biological Sciences, University of Illinois at Chicago Circle, Box 4348, Chicago, Illinois 60680

ROBERT L. HEINRIKSON
Department of Biochemistry and Franklin McLean Memorial Institute, University of Chicago, Chicago, Illinois 60637

ABSTRACT

Pea leaf chloroplast aldolase contains seven more aspartyl (asx) residues and four fewer leucine and isoleucine residues than the cytoplasmic enzyme. The two forms are therefore primary isoenzymes, differing in amino acid sequence.

Although the pea (*Pisum sativum*, L.) leaf chloroplast and cytoplasmic fructose-1,6-diP aldolases (ketose-1-P aldehyde-lyase, EC 4.1.2.7) have distinct isoelectric points (1, 3), it has seemed possible that the two forms might simply be conformers because both forms have the same subunit mol wt and identical terminal sequences (2). We now report that the chloroplast aldolase contains seven more aspartyl (asx) residues than the cytoplasmic enzyme. The isoelectric points, therefore, do reflect differences in primary structure.

MATERIALS AND METHODS

The chloroplast and cytoplasmic forms of aldolase were isolated from pea (*Pisum sativum* L., cv. Little Marvel) shoots as described previously (2). Dialyzed, lyophilized samples were hydrolyzed *in vacuo* in 6 M HCl (24 h, 110 C) and the hydrolysates were analyzed for amino acid content by automated ion exchange chromatography on a Durrum D-500 analyzer. Despite extensive dialysis against deionized H_2O the aldolase samples contained large amounts of nonproteinaceous material, possibly inorganic salts. In calculating amino acid composition we assumed that 326 residues were recovered per 37,000 dalton subunit. No corrections were made for decompositional losses of serine and threonine or for the possibly slower rate of liberation of valine and isoleucine during the course of hydrolysis. Cystine and tryptophan were not quantitated in this study.

RESULTS AND DISCUSSION

The amino acid compositions of the chloroplast and cytoplasmic aldolases are given in Table I. There are seven more aspartyl (asx) residues in the chloroplastic form. The only other major differences in composition are in leucine and isoleucine, with four more residues of each of these neutral amino acids being found in the cytoplasmic enzyme. The distinct isoelectric points of the two forms (1, 3) then do reflect differences in the primary structure of the isoenzymes and not simply differences in conformation.

Divergence in amino acid content can be used to make a crude approximation of the relatedness of proteins. We have estimated the divergence in the amino acid content of the pea leaf aldolases, rabbit muscle aldolases A and C, and two Class I aldolases from procaryotes using the calculation of Harris and Teller (7) (Table II). The divergence value is the square root of the sum of the squared differences in mole fractions of each amino acid. For identical proteins the divergence value is 0; for nonrelated proteins the divergence value is usually greater than 0.1. It can be seen that the chloroplast and cytoplasmic pea leaf aldolases are apparently about as closely related to rabbit type C aldolase as to one another. Cytoplasmic aldolase is about as closely related to rabbit type A aldolase as is rabbit type C aldolase, while chloroplast aldolase is apparently less closely related. None of the eucaryotic aldolases is at all closely related to the bacterial Class I aldolases. It will be recalled that the pea leaf aldolases and rabbit type C aldolase are slightly smaller (37,000 subunit mol wt) than rabbit type A aldolase (40,000 subunit mol wt) (2). We have suggested previously that both of the higher plant aldolases were derived

Table I. *Amino Acid Composition of Pea Leaf Chloroplast and Cytoplasmic Aldolases*

Values shown represent mean values for two preparations of each enzyme. In calculating amino acid composition we assumed that 326 residues were recovered per subunit.

Amino Acid	Chloroplast Aldolase	Cytoplasmic Aldolase	Difference (Chloroplast − Cytoplasmic)
Asp	33.5	26.5	+7.0
Thr	18.7	17.6	+1.1
Ser	23.8	21.5	+2.3
Glu	34.4	36.2	−1.8
Pro	17.7	16.0	+1.7
Gly	32.4	32.2	+0.2
Ala	33.2	33.7	−0.5
Val	25.8	25.7	+0.1
Met	4.4	3.9	+0.5
Ile	14.2	17.8	−3.6
Leu	25.7	30.2	−4.5
Tyr	7.4	8.9	−1.5
Phe	9.9	10.9	−1.0
His	5.8	5.8	0.0
Lys	24.3	21.6	+2.7
Arg	15.2	16.4	−1.2

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[2] Address correspondence to this author.

Table II. *Divergence in Amino Acid Composition of Some Class I Aldolases*

Composition divergence was calculated using the formula of Harris and Teller (7). Amino acid composition data used were: pea leaf enzymes, Table I; rabbit C, Lee and Horecker (11); rabbit A, Anderson *et al.* (4); *Escherichia coli*, Baldwin and Perham (5); *Micrococcus aerogenes*, Lebherz *et al.* (10).

Source	Chloroplast	Cytoplasmic	Rabbit C	Rabbit A	*E. coli*	*M. aerogenes*
Chloroplast	0	0.032	0.036	0.048	0.11	0.11
Cytoplasmic	0.032	0	0.033	0.037	0.11	0.10
Rabbit C			0	0.036	0.11	
Rabbit A				0	0.12	

from a common ancestral enzyme which was the prototype of the type C aldolases (2). The present data are consistent with this hypothesis. Clearly the pea leaf chloroplast aldolase is a eucaryotic Class I aldolase no more like the microbial Class I aldolases than is the rabbit muscle enzyme. While the chloroplast may have had a procaryotic origin (12) chloroplast aldolase did not.

Heil and Lebherz (8) have been able to hybridize several plant aldolases with rabbit C and A aldolases. Since the pea leaf cytoplasmic aldolase and the rabbit A and C aldolases show the same divergence in amino acid composition it is probably not surprising that all three can hybridize. Their results suggest conservation of basic structure and conformation of aldolase during evolution.

The transport of only one protein into the chloroplast has been studied: an N-terminal piece is cleaved from a 20,000 dalton precursor of the small subunit of ribulose-bisP carboxylase either during or after transport into the chloroplast (6, 9). It seems possible that the additional seven asx residues in chloroplast aldolase are involved in some way in transport across the chloroplast envelope.

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