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Characterization of O-Acetylserine (Thiol) Lyase from Spinach Chloroplasts

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S u m m a r y

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O-acetylserine (thiol) lyase (EC 4.2.99.8) in green and non green tissues from higher plants has been located predominantly in the plastids, the cytosol, but is also present in the mitochondria. The chloroplastic isoform of O-acetylserine (thiol) lyase from plant tissue has been purified to homogeneity. We have isolated and characterized a cDNA from a λgt 11 spinach library encoding the complete chloroplastic O-acetylserine (thiol) lyase. The deduced primary sequence revealed that the 331 amino acid polypeptide is cytoplasmically synthesized with a 52 amino acid targetting peptide. We also report the presence, in chloroplasts, of a multifunctional protein complex which links serine to cysteine synthesis as previously characterized in bacteria.

I n t r o d u c t i o n

Both higher plants and microorganisms use inorganic sulfate for the synthesis of sulfur containing amino acids (cysteine, methionine), coenzymes and for iron sulfur clusters of enzymes (ANDERSON 1980). Cysteine synthesis represents the first step of incorporation of the reduced sulfur into an activated carbon source, O-acetylserine (KREDRICH & TOMKINS 1966). This reaction (1) is catalyzed by O-acetylserine (thiol) lyase:

(1) O-Acetylserine + "sulfide" --> cysteine + acetate

O-acetylserine is the product of the reaction catalyzed by serine acetyl-

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transferase from serine and acetylCoA (2):

(2) L-serine + acetylCoA --> O-acetylserine + CoA

O-acetylserine (thiol) lyase was detected in the plastids of green and non-green tissues (SCHMIDT 1986), the cell compartment involved in the reduction of sulfate to sulfide (SCHWENN & al. 1976). O-acetylserine (thiol) lyase is also present outside the plastids, although the exact localization of the extraplastidial enzyme is unknown (SCHMIDT 1986). Therefore, we decided to reexamine the subcellular localization of O-acetylserine (thiol) lyase in green tissue (spinach leaves) and non-green tissue (cauliflower buds). For this purpose, we used Percoll purified organelles, and fractions obtained by differential centrifugation of protoplast lysates. Anion exchange chromatography was used as a probe to characterize the compartment specific isoform of O-acetylserine (thiol) lyase (LUNN & al. 1990, ROLLAND & al. 1992).

We also initiated the purification and characterization of one specific isoform, the chloroplastic O-acetylserine (thiol) lyase linked to sulfur assimilation (DROUX & al. 1992). Polyclonal antibodies against the purified enzyme were prepared, and used to screen a λgt11 spinach leaf cDNA library. A complete cDNA encoding O-acetylserine (thiol) lyase precursor was isolated (ROLLAND & al. 1993).

Materials and Methods

Chloroplasts and mitochondria were prepared from spinach leaves and cauliflower buds using Percoll gradient as previously described (LUNN & al. 1990, ROLLAND & al. 1992a). Crude extracts were obtained by homogenization of the tissues in presence of Triton X100. Protoplasts were prepared and fractionated from spinach leaves and from inflorescence buds as described previously (ROBINSON 1987, ROLLAND & al. 1992a). All the different isolated compartments were tested for respective cross-contamination. Fractionation of spinach leaf and cauliflower buds extracts (soluble proteins from a total homogenate, plastids, mitochondria and cytosol) was performed by anion exchange chromatography on a Mono Q HR5/5 column (LUNN & al. 1990; ROLLAND & al. 1992, DROUX & al. 1992). Stromal extract was also fractionated by gel filtration chromatography on a Superdex 200 (DROUX & al. 1992). Purification of O-acetylserine (thiol) lyase was carried out with percoll-purified chloroplasts. In short, the procedure involved a heat treatment, a fractionation with ammonium sulfate, gel filtration chromatography, hydrophobic interaction, anion-exchange chromatography and finally chromatography on hydroxyapatite (DROUX & al. 1992). Serine acetyltransferase was partially purified from stromal extract by ammonium sulfate fractionation, gel filtration chromatography and anion-exchange chromatography. Antibodies against purified chloroplastic O-acetylserine (thiol) lyase were prepared in rabbits. These antibodies were used for a screening of a cDNA (λgt11) library synthesized from spinach leaf poly (A^+) mRNA (ROLLAND & al. 1993). One positive clone was obtained, and the insert extracted from the lambda DNA was subcloned into pUC 19 plasmid, further amplified in competent *E. coli* strain DH 5α. DNA sequencing analysis was carried out on both strands by the dideoxy chain termination method using T7 DNA polymerase. O-acetylserine (thiol) lyase assay was performed as described previously (DROUX & al. 1992). Serine acetyltransferase was assayed by measuring its capacity to promote cysteine synthesis in presence of O-acetylserine (thiol) lyase when needed (DROUX & al. 1992).

Results and Discussion

In green (spinach leaves) and non green tissues (cauliflower buds), O-acetylserine (thiol) lyase is located primarily in the plastids and the cytosol (Table 1). But O-acetylserine (thiol) lyase activity is also present in the mitochondria. Three forms of O-acetylserine (thiol) lyase from the different subcellular compartments were separated by anion exchange chromatography, and characterized by the salt concentration for their specific elution from the column (Table 1). Each activity was found to be specific to one intracellular compartment. The presence of O-acetylserine (thiol) lyase in each of the three compartments may be linked to protein synthesis.

The O-acetylserine (thiol) lyase from the chloroplast fraction was purified to homogeneity. The protein was purified 800-fold from the stroma, with a specific activity of 96 units per mg of protein. O-acetylserine (thiol) lyase had a molecular mass of 68,000 and consisted in two identical subunits. The enzyme exhibited an absorbance in the visible with a maximum at 407 nm (pH 7.5), due to the presence of pyridoxal phosphate. The pyridoxal phosphate content was 1.1 per subunit. The chromophore of the enzyme was displaced from the polypeptide upon addition of O-acetylserine. The chloroplastic enzyme shares some biochemical homology with its bacterial counterpart (BORONAT & al. 1984, BECKER & al. 1969). At the level of the amino-acid composition, the enzyme differs from that one purified from crude extract (MURAKOSHI & al. 1985).

Table 1. Subcellular localization of O-acetylserine (thiol) lyase from spinach leaves and cauliflower buds. Enzyme activity (nmoles mg protein^{-1} min^{-1}) determined from a crude extract, and percoll purified organelles (*). Subcellular distribution (%) obtained from protoplasts fractionation, corrected from respective contamination (°). Salt concentration (mM NaCl) for elution of O-acetylserine (thiol) lyase activity, from crude extract, Percoll purified organelles and cytosol obtained from protoplasts fractionation, by chromatography on Mono Q HR (5/5) (§).

	Crude Extract	Plastids	Mitochondria	Cytosol
Spinach leaves				
Enzyme activity (*)	90.2	58.5	18.6	
Distribution (°)		42	14	44
NaCl/MonoQ HR5/5 (§)	130-200	200	130	155
Cauliflower buds				
Enzyme activity (*)	590	1240	950	
Distribution (°)		42	16	42
NaCl/MonoQ HR5/5 (§)	70-200	110	80	170

Table 2. Biochemical characteristics of O-acetylserine (thiol) lyase and serine acetyltransferase/O-acetylserine (thiol) lyase complex.

	O-Acetylserine (thiol) lyase	Serine acetyltransferase/ O-acetylserine (thiol) lyase complex
Km O-acetylserine (mM)	1.3	
Km sulfide (mM)	0.25	
pH optimum	7.5 - 8.5	8.0
Km serine (mM)		1.8
Km Acetyl CoA (mM)		0.2
Molecular mass (kDa)	68	310

Upon gel-filtration chromatography on Superdex 200, part of chloroplastic O-acetylserine (thiol) lyase activity was eluted in association with serine acetyltransferase activity at a position corresponding to a molecular mass of 310,000. Partial purification of this complex was obtained by gel filtration chromatography and anion exchange chromatography. In these two steps both activities co-eluted, suggesting the existence in the chloroplast of a supra-molecular complex. A similar complex has been observed in bacteria (KREDRICH & al. 1969). The characteristics of purified O-acetylserine (thiol) lyase and of serine acetyltransferase are presented in Table 2. Two points emerge from these results: O-acetylserine (thiol) lyase exhibits a relatively poor affinity for the substrate O-acetylserine; upon gel filtration chromatography, all the serine acetyltransferase was eluted in association with O-acetylserine (thiol) lyase (cysteine synthase complex). From these results, together with the fact that O-acetylserine is unstable at alkaline pH (pH of chloroplast stroma is above 8.0), it is difficult to conceive that *in vivo* the enzymes involved in cysteine synthesis, namely serine acetyltransferase and O-acetylserine (thiol) lyase, behave as separate components in the stromal space. One consequence of a supramolecular complex (cysteine synthase) would be the channeling of O-acetylserine without equilibration with the bulk phase, so that high concentration of O-acetylserine can be maintained at the active site of O-acetylserine (thiol) lyase.

A polyclonal antibody prepared against the chloroplastic O-acetylserine (thiol) lyase showed a very low cross-reactivity (on an activity basis) with a preparation of mitochondrial matrix and cytosolic proteins. This result suggests that the chloroplastic isoform is distinct from the mitochondrial and cytosolic counterparts.

By using this antibody preparation, we have isolated, for the first time, a cDNa encoding the complete O-acetylserine (thiol) lyase precursor from spinach leaf chloroplast. The deduced primary structure revealed that the 331 amino polypeptide is cytoplasmically synthesized with a 52 amino acid targetting peptide. Sequence comparisons reveal 40 to 60 % homology with prokaryotic O-acetylserine (thiol) lyase (BYRNE & al. 1988, LEVY & DANCHIN 1988). From the sequence comparison with several pyridoxal phosphate bearing proteins the pyridoxal-phosphate binding site showed to be lysine 108. Southern blot analysis of chloroplast and total spinach DNA have demonstrated that O-acetylserine (thiol)lyase was encoded by more than one gene in the spinach leaf genomic DNA.

A synthetic cDNA was constructed, coding for the entire 331-amino-acid mature O-acetylserine (thiol)lyase and for an initiating methionine. A high level of expression of the active mature chloroplast isoform was achieved in an *Escherichia coli* strain carrying the T7 RNA polymerase system (ROLLAND al. 1993).

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