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SHORT COMMUNICATION

O-ACETYL-L-SERINE LYASE: A NEW β-ELIMINASE IN EXTRACTS FROM HIGHER PLANTS*

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Abstract—Extracts of *Brassica* species and *Leucaena leucocephala* seedlings contain an enzyme which degrades *O*-acetyl-L-serine. The enzyme has been partially purified from broccoli buds and cleaves *O*-acetyl-L-serine via β-elimination to pyruvic acid, ammonia and acetate. Added pyridoxal phosphate has little effect but the addition of 0·1 mM hydroxylamine and 1 mM NaCN inhibit very strongly. The enzyme appears specific for the L-isomer. L-Serine is not a substrate. *O*-Phospho-DL-serine and *O*-methyl-DL-serine also are not utilized by the enzyme.

INTRODUCTION

IN RECENT years *O*-acetylserine (OAS) has been implicated as an intermediate in the biosynthesis of cysteine by *Salmonella typhimurium* and *Escherichia coli*,[1] yeast and *Neurospora crassa*,[2] and higher plants.[3–6] A very recent report has shown that mimosine and β-pyrazole-alanine, found in the non-protein nitrogen fraction of a number of higher plants[7] can be synthesized by extracts of *Leucaena leucocephala* and watermelon seedlings, respectively, using OAS as an acceptor.[8] The enzymatic formation of OAS from serine and acetyl-CoA has been demonstrated in bacteria[1] and higher plants.[6,9]

The present communication reports the occurrence of a new enzyme, *O*-acetyl-L-serine (L-OAS) lyase in extracts from a number of *Brassica* species. The enzyme cleaves L-OAS to yield pyruvic acid, ammonia, and acetate. Some of the properties of the partially purified enzyme from broccoli buds are described.

RESULTS

A preliminary survey of a number of plant species was carried out by preparing the 0–75% $(NH_4)_2SO_4$ saturated fraction from homogenates and testing these as sources of enzyme. Activity was found in preparations from seedlings of *Leucaena leucocephala*, and in the following *Brassica* species: turnip roots, cabbage leaves, cauliflower buds, and broccoli buds. The latter two were very active, particularly broccoli, and this became the plant of choice.

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TABLE 1. REQUIREMENTS FOR LYASE ACTIVITY

Reaction mixture	Pyruvate produced (μmoles)
Complete	3·2
Complete enzyme heat-inactivated	0
Complete minus PALP	3·0
Complete minus OAS	0·1
Complete minus OAS; plus L-serine	0

Incubation conditions given in text. Heat-inactivated enzyme prepared by heating in boiling water bath for 5 min. 0·2 M substrate and 1 mg enzyme protein were used.

Using the partially purified preparation described in the Experimental the lyase was shown to be quite specific for OAS (Table 1). L-serine was not a substrate. *O*-Phospho-DL-serine, *O*-methyl-DL-serine, and *O*-acetyl-DL-threonine were tested and found to be inactive as substrates.

The activity increased with increasing pH. However, above pH 7·6 there is a rapid *O*- to *N*-acetyl shift in the substrate;[10] therefore the initial pH of the reaction was chosen at pH 7·4–7·5. The K_m for L-OAS was very large. Several determinations gave values from 100–150 mM. Because of the problems of solubility and expense, the reaction was not carried out under substrate-saturating conditions.

The addition of exogenous pyridoxal phosphate (PALP) did not give a large stimulation (Table 1). In a number of experiments the largest stimulation obtained with added PALP was 20%. In the absence of exogenous PALP, hydroxylamine and cyanide gave a large inhibition of lyase activity. For example 0·1 mM hydroxylamine inhibited the reaction 90% and 1 mM NaCN 85%.

The 2,4-dinitrophenylhydrazone (2,4-DNPH) assay, of course, is not specific for pyruvate. The validity of this assay was borne out by comparing the results obtained in a number of reaction mixtures with those obtained using lactic dehydrogenase as an assay. Table 2 shows that the 2,4-DNPH assay and enzymatic determination gave the same result. This proves that all of the 2,4 DNPH-reactive material was an α-keto acid. A large scale reaction was carried out and the 2,4-DNPH product isolated and recrystallized from ethyl acetate. The IR spectrum of this derivative was identical with that of the derivative from known pyruvic acid.

TABLE 2. 2,4-DNPH ASSAY COMPARED TO LACTIC DEHYDROGENASE ASSAY

Pyruvate produced (μmoles)	
2,4-DNPH	Lactic dehydrogenase
0·8	0·7
1·1	1·1
1·6	1·6
2·0	1·8
2·2	2·3

[10] M. FLAVIN and C. SLAUGHTER, *Biochemistry* **4**, 1370 (1965).

To compare the amount of ammonia produced with that of pyruvate formed, it was necessary to dialyse the enzyme solution prior to use so as to remove residual $(NH_4)_2SO_4$. The results of a balance study showed that 4·2 μmoles of ammonia were produced compared to 4·0 μmoles of pyruvate.

Since under the conditions used the K_m for L-OAS was so high and the activity was non-linear with time, the possibility that the enzyme possessed allosteric properties was considered. Adenine nucleotides were found to be quite inhibitory with ATP being the most effective (Table 3). The effect was not due to the pyrophosphate moiety since inorganic pyrophosphate was much less effective than ATP. Some of the effect could be due to the chelating properties of the pyrophosphate since at the same concentration citrate had a similar inhibitory effect. Cyclic AMP was less inhibitory than AMP. Preliminary studies indicate that other nucleoside triphosphates are as inhibitory as ATP. Indoleacetic acid had only a slight inhibitory effect.

TABLE 3. INHIBITION BY ADENINE NUCLEOTIDES

Inhibitor	mM	Relative activity
None		100
ATP	5	15
	2	22
ADP	5	29
AMP	5	64
	2	74
Cyclic AMP	2	95
Adenosine	5	103
Inorganic pyrophosphate	5	66
Citrate	5	77

The activity of D-OAS as a substrate was compared to that of L-OAS (Table 4). The enzyme clearly appears to be specific for the L-isomer. The D-isomer had at the most only a slight inhibitory effect.

TABLE 4. THE STEREOSPECIFICITY OF THE LYASE REACTION

Reaction mixture	Substrate	Concentration (mM)	Pyruvate produced (μmoles)
1	L-OAS	200	2·7
2	D-OAS	200	0·0
3	L-OAS + D-OAS	200 200	2·3

Reaction conditions as described in the text using 1·6 mg enzyme per reaction.

DISCUSSION

The biosynthesis of OAS[6,9] and its utilization in further syntheses[3–6,8] by extracts from higher plants has been demonstrated previously. The present report completes the story by describing the biodegradation of OAS by plant preparations by the following reaction:

$$\text{L-OAS} + H_2O \rightarrow \text{acetate} + \text{pyruvate} + NH_4^+ + H^+.$$

Pyruvate and ammonia have been identified as products, but the production of acetate has only been inferred by logic. Thus, plants have the complete series of enzymes for the production, utilization, and degradation of this compound. Although this amino acid has not been found as a natural constituent, it appears that it ought to be. The recent findings of Murakoshi *et al.*[8] raise the possibility that the large number of β-substituted alanines found in the non-protein nitrogen fraction in higher plants may reflect the ability of the particular substituent in the organism concerned to interact with OAS.

The cystathionine γ-synthase of *Salmonella typhimurium* can cleave *O*-succinylhomoserine by an analogous reaction to succinic acid, α-keto-butyrate, and ammonia.[11] Recently it has been shown that the same enzyme can cleave *O*-succinylserine to succinate, pyruvate, and ammonia[12] but OAS is not a substrate.[13] The enzyme described in the present communication appears to be unique in this regard.

EXPERIMENTAL

Enzyme preparation. Broccoli purchased at local markets was washed and chilled and the buds removed. All the following operations were carried out in the cold. The buds were homogenized with 0·2 M HEPES pH 7·5 in a Waring blendor using 1·5 ml buffer for each gram of buds. The homogenate was strained through cheesecloth and centrifuged for 30 min at 30,000 *g*. The supernatant solution was treated with 0·15 ml of 1% protamine sulfate per ml. The precipitate was removed by centrifugation and the solution fractionated with $(NH_4)_2SO_4$. The fraction precipitating between 45 and 70% saturation was dissolved in 0·5 M HEPES pH 7·3. This fraction was used as the enzyme source. It was purified 5-fold over the centrifuged homogenate with a recovery of greater than 80% of the total activity. A unit of enzyme activity is defined as that which produces 1 nmole of pyruvate per minute.

Assay procedures. The standard incubation mixture consisted of the following components at the given final concentrations: 0·2 M HEPES (pH 7·5); 50 μM pyridoxal phosphate (PALP); 0·05–0·2 M L-OAS and 1–4 mg enzyme in a final volume of 1 ml. Usually the substrate was added to start the reaction. After 10 min at room temp. the reaction was terminated by the addition of 3 ml 10% trichloroacetic acid. The precipitate was removed by centrifugation and aliquots of the clarified solution were assayed for pyruvate by the total keto acid method of Friedmann and Haugen.[14] In some instances pyruvate was determined by the use of lactate dehydrogenase. In balance studies the ammonia produced was measured by microdiffusion followed by nesslerization.

A large scale preparation for isolation of the 2,4-DNPH derivative of the product consisted of 2 mmoles L-OAS, 1 mmole HEPES, 0·5 μmole PALP, 307 mg enzyme having a specific activity of 47 in a final volume of 10 ml. The final pH was 7·4. After 1 hr the pH had dropped to 6·8. The reaction mixture was adjusted to pH 7·3 by the addition of 2·5 N NaOH. At the end of the next hour 2 N HCl was added until the pH was 4·0 and the precipitate that formed was removed and discarded. 35 ml of saturated 2,4-dinitrophenylhydrazine in 2 N HCl was added and the mixture placed at 10° for 2 days. The product was filtered and dissolved in warm EtOAc. Any insoluble material was removed by filtration and light petroleum added to the filtrate until a precipitate was produced. After 24 hr at 10° the precipitate was collected, washed with light petroleum and dried.

Protein was determined by the spectrophotometric method described by Layne.[15]

Chemicals. L-OAS was purchased from Miles Laboratories and Cyclo Chemical Co. D-OAS was synthesized by the method described in Greenstein and Winitz.[16] L-OAS but not D-OAS had a small contamination of serine when examined by paper chromatography with *n*-BuOH–HOAc–H_2O (52:13:35).

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