Phytochemistry, Vol. 27, No. 7, pp. 2011 2016, 1988.
Printed in Great Britain.

0031 9422/88 $3.00 + 0.00

PURIFICATION AND PROPERTIES OF β-CYANO-L-ALANINE SYNTHASE FROM *SPINACIA OLERACEA**

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(*Received* 11 *December* 1987)

Key Word Index—*Spinacia oleracea*; Chenopodiaceae; spinach; β-cyano-L-alanine synthase; cysteine synthase; enzyme purification; amino acid composition; L-cysteine; *O*-acetyl-L-serine; β-cyano-L-alanine; heterocyclic β-substituted alanines.

Abstract—β-Cyano-L-alanine synthase was purified *ca* 6200-fold to homogeneity from the leaves of spinach (*Spinacia oleracea*). The purified enzyme has an apparent M_r of 60 000 and can be dissociated into identical subunits of M_r 30 000. The subunits each contain one molecule of pyridoxal 5′-phosphate. The K_m value is 2.3 mM for L-cysteine and 0.73 mM for cyanide. β-Cyano-L-alanine synthase from *S. oleracea* also catalyses the formation of some *S*-substituted L-cysteines and some heterocyclic β-substituted alanines from L-cysteine or *O*-acetyl-L-serine. The specificity of these additional catalytic activities of the purified enzyme are compared with those of cysteine synthase purified from the same plant, and with those of β-cyano-L-alanine synthase purified from other sources. Some other properties, including the amino acid composition of the purified enzyme, are also described.

INTRODUCTION

β-Cyano-L-alanine (BCA) synthase is widespread in nature. Its metabolic role in plants can be linked to the detoxification of HCN, that arises as a byproduct of ethylene biosynthesis. BCA synthases have been purified from several plant sources [1–5] and also from microorganisms [6–8]. Some BCA synthases purified from plant sources were found to contain one molecule of pyridoxal 5′-phosphate (PLP) per molecule of enzyme, and no subunits; other BCA synthases were found to contain two identical subunits, each subunit containing PLP as a cofactor [5, 8], similar to the cysteine synthases in higher plants [9–15].

In recent years we made comparative studies of some PLP-containing enzymes catalysing the formation of naturally occurring heterocyclic β-substituted alanines [12–17], and we presented evidence that some cysteine synthases from plants, as an additional catalytic activity, can also catalyse the formation of some β-substituted alanines, including BCA, in the presence of *O*-acetyl-L-serine (OAS) and suitable precursors [12–15]. Cysteine synthase purified from spinach (*Spinacia oleracea*) however, could not catalyse the formation of BCA, although high BCA synthase activity was present in extracts of this plant.

In this respect, it seemed of particular interest to extend our studies to BCA synthase which catalyses the reaction between L-cysteine and cyanide to form BCA and hydrogen sulphide in a variety of higher plants, since cyanide serves as a second substrate for the enzyme in the reaction of β-substitution of L-cysteine. Therefore, we have now attempted the purification of BCA synthase from the leaves of *S. oleracea*, which is not a cyanogenic plant, in order to improve our understanding of the biosynthesis of this group of non-protein amino acids.

In this paper we describe the purification and the properties of BCA synthase from *S. oleracea* leaves and its comparison with BCA synthases and cysteine synthases from other sources. Differences between the purified BCA synthase and cysteine synthase present in the same plant are also described.

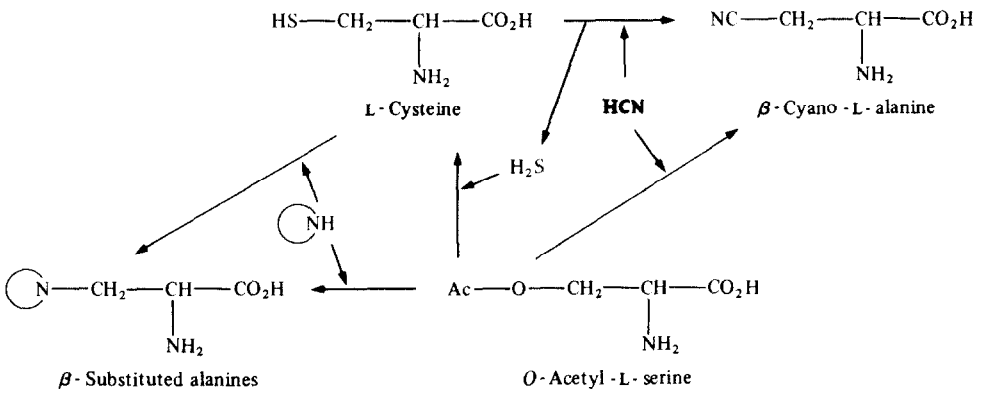
RESULTS

Purification of BCA synthase from spinach leaves

From 8.6 kg of fresh spinach leaves we extracted and purified BCA synthase, mainly using the methods described in previous papers [1, 3, 12–17]. The enzyme was prepared simultaneously with the cysteine synthase activity by a procedure including the preparation of acetonized mitochondria, ammonium sulphate fractionation, ion-exchange chromatography on DEAE-Sephadex A-50, gel filtration on Sephadex G-100 or Ultrogel AcA 44, hydrophobic chromatography on AH-Sepharose 4B and preparative polyacrylamide gel electrophoresis (PAGE) as summarized in Table 1.

The protein demonstrating BCA synthase activity was completely separated from cysteine synthase activity after the first DEAE-Sephadex A-50 column was eluted with a concentration gradient of Tris–HCl buffer. The enzyme activity for BCA synthase was eluted at 230–250 mM and cysteine synthase eluted at 280–300 mM as shown in Fig. 1.

*Parts of this work were reported at the 107th Annual Meeting of the Pharmaceutical Society of Japan at Kyoto, 4 April 1987 (Abstracts, p. 359).



Scheme 1. Biosynthetic pathways for β-cyano-L-alanine, L-cysteine and β-substituted alanines in higher plants.

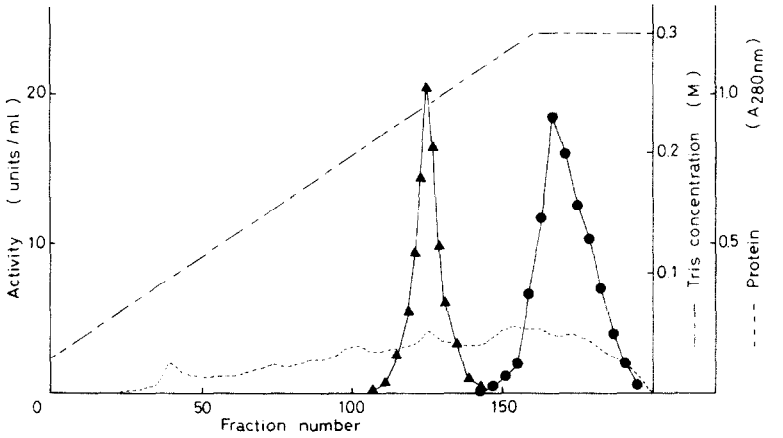


Fig. 1. Elution patterns of β-cyano-L-alanine synthase and cysteine synthase after the first DEAE-Sephadex A-50 column chromatography. β-Cyano-L-alanine synthase activity (▲——▲), cysteine synthase activity (●——●) and protein (A_{280},---) were monitored as shown in the Experimental.

Table 1. Summary of the purification of β-cyano-L-alanine synthase from *Spinacia oleracea*

Purification step	Total activity (units*)	Total protein (mg)	Specific activity (units/mg protein)	Yield (%)	Fold
1. Crude extract†	7030	207000	0.034	100	1
2. Acetonized preparation of mitochondria	4000	12540	0.319	56.9	9.4
3. Ammonium sulphate precipitate‡	3060	2520	1.21	43.5	35.6
4. Sephadex G-100 (peak fractions)	2830	460	6.15	40.3	180
5. 1st DEAE-Sephadex A-50 (230-250 mM)	2080	130	16.0	29.6	470
6. Ultrogel AcA 44 (peak fractions)	1089	33.3	32.7	15.5	962
7. AH-Sepharose 4B (240-275 mM)	571	9.35	59.9	8.1	1760
8. Polyacrylamide-gel electrophoresis	261	1.24	210	3.7	6176
9. 2nd DEAE-Sephadex A-50 (135-145 mM KCl)	235	1.11	212	3.3	6235

*A unit of enzyme activity represents 1 μmol of product formed per min at 30°, in 50 mM Tris–HCl buffer, pH 9.
†Starting from 8.6 kg of the fresh leaves of *Spinacia oleracea*.
‡40–60% saturation and desalted on Sephadex G-25.