BIOSYNTHESIS IN VITRO OF 2-(3-AMINO-3-CARBOXYPROPYL)-ISOXAZOLIN-5-ONE, THE NEUROTOXIC AMINO ACID IN LATHYRUS ODORATUS

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2-(3-Amino-3-carboxypropyl)-isoxazolin-5-one (ACI), a neurotoxic amino acid from Lathyrus odoratus, was confirmed to be derived enzymatically from S-adenosyl-L-methionine (SAM) and isoxazolin-5-one. Some properties of an enzyme in the biosynthesis of ACI are described.

KEYWORDS biosynthesis ; 2-(3-amino-3-carboxypropyl)-isoxazolin-5-one ; Lathyrus odoratus ; Leguminosae ; S-adenosyl-L-methionine ; neurotoxic amino acid

Several isoxazolinedione derivatives have been found in the genera Lathyrus, Lens and Pisum.1) In sweet pea (Lathyrus odoratus L.) β-(isoxazolin-5-on-2-y1)-alanine (BIA) and 2-(3-amino-3-carboxypropyl)-isoxazolin-5-one (ACI) are prominent metabolites during the seedling stage.2) ACI has been shown to cause neurotoxic symptoms similar to 2,4-diaminobutyric acid, the neurotoxin from Lathyrus sylvestris.3) Recently, it was proven that BIA is enzymatically synthesized from O-acetyl-L-serine (OAS) and isoxazolin-5-one by cysteine synthases purified from pea (Pisum sativum) and grass pea (Lathyrus sativus)4) seedlings by a mechanism similar to the biosynthesis of other heterocyclic β-substituted alanines in higher plants.5-8)

Since ACI is the higher homologue of BIA with one more carbon in the side chain, we presumed that the enzymatic synthesis of ACI might be similar to that of BIA. However, during in vivo experiments, Callebaut et al. found much more incorporation from [14C]-labelled S-adenosyl-L-methionine (SAM) than from homoserine,9) and we also found that the 14C-label of SAM was incorporated in the side chain of ACI.10) Other natural occurring 3-amino-3-carboxypropyl-substituted heterocycles like discadenine in Dictyostelium discoideum11) and a modified nucleotide from Escherichia coli tRNA12) were found to derive from SAM as the donor of the 3-amino-3-carboxypropyl side chain.

This paper reports the biosynthesis in vitro of ACI from SAM and the isoxazolin-5-one ring by an enzyme partially purified from sweet pea (L. odoratus) seedlings (Chart 1).

![Chart 1. Biosynthetic Pathway for ACI in Lathyrus odoratus](image-url)

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The enzyme was purified from the seedlings (cotyledons removed) of sweet pea (L. odoratus) grown in moistened vermiculite in the dark for 5-6 days at 26-28°C by methods described in our previous papers. After the purification step with Sephacryl S-200 gel filtration, the enzyme preparation was used as the source of enzyme activity for the formation of ACI. isoxazolin-5-one was chemically synthesized according to the method of De Sarlo et al. and the structure was confirmed by spectroscopic methods.

Reaction mixtures used to demonstrate the formation of ACI contained 6 mM of SAM, 18 mM of isoxazolin-5-one, 2 mM of ATP, 0.5 mM of Mg2+ and 0.4 ml enzyme preparation containing 0.5-1 mg of protein and were incubated at 30°C for 60 min in a final volume of 0.7 ml. The incubation mixtures normally contained 50 mM Tris-HCl buffer, pH 7.5. Reactions were terminated by acidification to pH 1.7-1.8 with 6 N HCl and then analyzed by using an automatic amino acid analyzer (Hitachi 835-10) coupled to a UV-detector (265 nm) in addition to post-column ninhydrin reaction; under standard operating conditions ACI eluted from the column after about 66 min. This method clearly confirms the formation of a product that reacts with ninhydrin, showing UV absorption at 265 nm, and that was inseparable from added authentic ACI. The product was not formed when the reaction mixture was lacking either SAM, isoxazolin-5-one, ATP or Mg2+, or when the enzyme preparation was pretreated at 100°C for 15 min. The formed ACI could be estimated quantitatively by using the automatic amino acid analyzer.

Under standard assay conditions, the rate of synthesis of ACI was constant for at least 90 min but then decreased. The optimum pH for the enzymatic formation of ACI was 7.5-7.6 using 50 mM Tris-HCl buffer. The synthase activity for ACI was dependent upon the concentrations of SAM and isoxazolin-5-one used. A relatively low concentration of ca 18 mM isoxazolin-5-one was sufficient to give a maximum rate of ACI formation in the presence of a fixed concentration of SAM (6 mM). ACI formation was also dependent upon the concentrations of ATP and Mg2+. The enzyme showed no activity when SAM was substituted for O-acetyl-L-homoserine, O-phospho-L-homoserine or L-methionine. The enzyme was reasonably stable: when stored at 0°C for 25 h the remaining activity was about 75% of the activity associated with a freshly prepared extract. Enzyme preparations from the pea (P. sativum) seedlings could not catalyze the formation of ACI.

This is the first evidence for the presence in higher plants of an enzyme transferring the 3-amino-3-carboxypropyl moiety of SAM onto a heterocyclic ring and confirms the pathway proposed by Callebaut et al. ACI is also present in the shoots of L. sativus, which are a popular vegetable in Bangladesh, and may contribute to the occurrence of the crippling human lathyris. Further study of the enzyme involved in the biosynthesis of the neurotoxin ACI is of importance in this area of human lathyris and nutrition.

Nicotianamine and nicotianamine are other non-protein amino acids bearing the same 3-amino-3-carboxypropyl moiety occurring in tobacco plants (Nicotiana tabacum) and in beechnut seeds (Fagus silvatica). Although the side chain of nicotianamine could be chemically derived from azetidine-2-carboxylic acid, its biochemical origin is unknown, but, from the above results, it might be suggested that SAM may play a role in its biosynthesis as a general mechanism for the biosynthesis of γ-substituted α-aminobutyric acids. Studies on this problem are now in progress.
REFERENCES


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