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The Purification and Characterication of Cystathionine-ß-lyase from *Echinochloa colonum*

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Summary

TURNER W. L., LEA P. J. & PALLETT K. E. 1992. The purification and characterization of cystathionine-ß-lyase from *Echinochloa colonum*. - Phyton (Horn, Austria) 32 (3): (143)-(146).

Cystathionine-ß-lyase (EC 4.4.1.8), the second enzyme unique to methionine biosynthesis, has been purified 933 fold from *Echinochloa colonum* to give a specific activity of 1213 nmol min^{-1} mg $protein^{-1}$. Native PAGE indicated the presence of nine protein bands, one of which stained for cystathionine-ß-lyase activity. The enzyme was shown to have a molecular weight of 160 kDa. The substrate specificity was limited to L-(+)cystathionine and L-djenkolate with K_m values of 0.13 and 0.46 mM respectively.

Introduction

In higher plants the pathway of methionine synthesis is known to be derived from aspartate (GIOVANELLI & al. 1989). The synthesis and regulation are complicated due to the fact that two other amino acids - lysine and threonine - share common biosynthetic enzymes.

Cystathionine-ß-lyase is the second enzyme unique to methionine biosynthesis. Purification and characterization of this enzyme will lead to a deeper understanding of this important area of amino acid biosynthesis.

Recently STATON & MAZELIS (1991) purified cystathionine-ß-lyase from spinach to apparent homogeneity with a specific activity of 1226 nmol min^{-1} mg $protein^{-1}$. This paper describes the purification of the enzyme from a liquid cell suspension culture of *Echinochloa colonum*.

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Materials and Methods

Cystathionine-ß-lyase catalyses the following reaction:

CYSTATHIONINE + H_2O --> PYRUVATE + HOMOCYSTEINE + NH_3

Enzyme activity was assayed according to the method of GIOVANELLI & MUDD (1971), whereby pyruvate production was coupled to NADH oxidation via lactate dehydrogenase. The resultant decrease in absorption could be followed spectrophotometrically at 340 nm. The assay was carried out in Tris/HCl buffer at pH 8.6.

The current purification scheme that produced the highest specific activity is shown in Figure 1.

Results and Discussion

A typical purification of cystathionine-ß-lyase from *Echinochloa colonum* cells (150 g frozen weight) is shown in Table 1.

The specific activity of cystathionine-ß-lyase from the final fraction was 1213 nmol min^{-1}1 mg $protein^{-1}$ with a fold purification of 933 and an 18 % yield. After dialysis and concentration against polyethylene glycol this fraction was subjected to native PAGE. Staining with Coomassie blue revealed nine protein bands whereas an enzyme activity stain, based on tetraphenyl boron, detected only one band of activity. Although the specific activity reached in this purification procedure is equivalent to the homogenous preparation obtained by STATON & MAZELIS (1991) from spinach leaves, it is clear that there are still a significant number of contaminating proteins present in the *Echinochloa colonum* purified cystathionine-ß-lyase.

The affinities of cystathionine-ß-lyase for cystathionine and djenkolate were determined by varying the substrate concentration between 1.0 mM and 0.05 mM. Apparent K_m and V_{max} values are shown in Table 2.

Aminooxyacetic acid (AOA), an inhibitor of pyridoxal phosphate enzymes, and L-aminoethoxyvinylglycine (AVG), a known inhibitor of cystathionine-ß-lyase (DATKO & MUDD 1982), were both shown to be active. I_{50} values were shown to be 35 and 120 µM for AOA and AVG respectively.

The molecular weight of cystathionine-ß-lyase was determined using a Sephacryl S-200 gel filtration column that had been calibrated using standard marker proteins. The molecular weight was found to be 160 kDa, as compared to the 210 kDa obtained for the spinach leaf enzyme by STATON & MAZELIS (1991).

Acknowledgments

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Table 1. Purification of cystathionine-ß-lyase from *Echinochloa colonum* tissue culture cells. See Fig. 1 for full details of purification.

Stage	Total Protein (mg)	Total Units (nmol min^{-1})	Specific Activity (units mg $protein^{-1}$)	Purification	Yield (%)
Crude	2112	2752	1.2	1.0	100
A.S./G.25	391.3	3462	8.9	6.8	126
Heath	276.1	3319	12.0	9.2	121
F.F.Q.	100.0	2445	24.2	19	89
Octyl	47.8	2286	47.8	37	83
S-200	1.7	608	358	275	22
Yellow 1	0.4	485	1213	933	18

Table 2. The K_m and V_{max} for cystathionine and djenkolate.

Substrate	Km (mM)	Vmax (nmol min^{-1})
L-(+)Cystathionine	0.13	2.22
L-Djenkolate	0.46	2.38

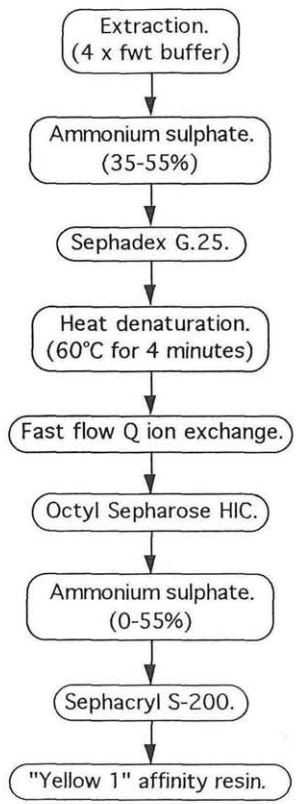

Fig. 1. Purification scheme for cystathionine-ß-lyase from the cell suspension culture *Echinochloa colonum*.