

## E220

Partial purification and characterization of ornithine carbamoyltransferase from the chloroplasts of *Canavalia lineata*

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Ornithine carbamoyltransferase was partially purified from the chloroplasts of *Canavalia lineata* leaves. The enzyme activity of chloroplast was 14 - 37% of the total activity of the leaves. The enzyme had a MW of 107 KD and a pH optimum at 8.0. Kinetic analysis gave Michaelis constants of 5.7 mM for L-ornithine and 0.48 mM for carbamoylphosphate. Ornithine carbamoyltransferase also used canaline as for substrate to produce ureidohomoserine. The profiles of DEAE-Sephacel ion exchange chromatography and Sephacryl S-300 gel filtration showed that ornithine dependent activity and canaline dependent activity were overlapped each other. The Michaelis constants for L-canaline and carbamoylphosphate were 3.5 mM and 0.030 mM respectively.

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Purification and characteration of four Carboxypeptidases from cotyledons of *Canavalia lineata*.

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The activity of carboxypeptidases was measured in cotyledons of *Canavalia lineata* during germination. 2 fold increase occurred in 2 days after germination. The four fractions of carboxypeptidases(CP1, CP2, CP3, CP4) were separated by CM-cellulose, Sephacryl S-300 and Procion red dye chromatography. The enzymes were inhibited by phenylmethan-sulphonyl fluoride and N-carbobenzoxy-phenylalaline-chloromethyl ketone, but unaffected by Iodoacetate, 1,10-phenanthlorine, pepstatin A, dithiothreitol, N-tosyl-phenylalaline chloromethyl ketone and N-tosyl-lysine chloromethyl ketone. The susceptibility of different N-carbobenzoxy-dipeptide was determined by penultimate amino acid. The highest reaction rate was usually obtained substrates having phenylalaline in the penultimate position, whereas substrates having glycine, proline were hydrolyzed slowly, or not at all. The values of  $K_m$  for N-carbobenzoxy-Phe-Ala were 0.5, 0.65, 1.30 and 1.35 mM respectively and the values of  $V_{max}$  were 7.7, 16.2, 31.4 and 12.4  $\mu\text{mol}/\text{min}/\text{mg}$  protein respectively.