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Proteolytic Clipping of Arginine Decarboxylase

We isolated a cDNA clone for the plant ARGdc by the strategy of purifying the protein from oat leaves (the richest source), obtaining some peptide sequence, constructing an oligonucleotide probe based on the amino acid sequence, and then identifying the clone in an oat leaf cDNA library. By expressing separate regions of the cDNA individually in E. coli, we could produce polypeptides, and then generate antibodies specific to amino and carboxyl regions of the precursor protein.

The cDNA clone for ARGdc from oats contains an open reading frame encoding a 66,000 Da polypeptide. On western blots of plant extracts we could not find a 66,000 Da polypeptide in oat extracts. Instead, we detected a 42,000 Da polypeptide derived from the amino 2/3 of the full length protein, and a 24,000 Da polypeptide derived from the carboxyl 1/3 of the full length protein. Additional western blots, immunoprecipitation analysis, and reconstruction experiments, were performed to show that the two polypeptides detected were actually present in vivo, and were not an artifact of the extraction method (Malmberg, Smith, Bell, Cellino). This data allowed us construct the following hypothesis for arginine decarboxylase structure: ARGdc is translated as a 66,000 da preprotein that has little or no enzyme activity. The preprotein is activated by proteolytic clipping into 42,000 da and 24,000 da products that remain together in the final enzyme. Experiments with radiolabeled inhibitor, DFMA, showed that it bound to a site on the 24,000 Da fragment. Additional experiments showed that the 24,000 Da fragment was also phosphorylated in vivo.

The next problem is to determine the mechanism by which the proteolytic processing occurred, and to find out if the processing was a required activation step. To study this we have developed an in vitro clipping reaction that reproduced the 42,000 Da and 24,000 Da products seen in vivo. Since we could not find the full length precursor protein in oat plants, we tried several methods of generating it, eventually settling on the following method:
- in vitro transcription of the oat arginine decarboxylase cDNA clone
- in vitro translation of the mRNA via rabbit reticulocyte lysates
- test the translation product in various assays for in vitro processing / activation
Using this method we have been able to show that the processing of arginine decarboxylase requires an additional plant protease. Arginine decarboxylase does not process itself.

**Purification of tobacco ornithine decarboxylase**

The original clone that we isolated that we thought might be ornithine decarboxylase turned out to not be it. We therefore have begun using the fall back strategy we proposed, to purify the plant ornithine decarboxylase protein, obtain amino acid sequence, and then use this information to obtain a cDNA clone. We began with 40 liters of exponential phase tobacco cell culture, from which we obtained about 2 gm of protein in the crude extract, and then purified ornithine decarboxylase activity conventionally until we had a 200 fold enrichment of specific activity. At this point we could radiolabel the ornithine decarboxylase using $^3$H-DFMO, essentially as described by for the mammalian enzyme. We then identified 2 labeled spots on 2 dimensional gel electrophoresis of this protein sample. The 2 labeled spots differed a small amount in the isoelectric focusing dimension, suggesting they are both different forms of ORNdc.

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