# AMINO ACID INHIBITION OF A $\Delta^1$-PYRROLINE-5-CARBOXYLATE DEHYDROGENASE PREPARATION FROM BEEF KIDNEY MITOCHONDRIA[1]

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We have recently demonstrated that beef kidney mitochondria exhibit $\Delta^1$-pyrroline-5-carboxylate dehydrogenase activity which is inhibited by some common amino acids in cumulative fashion as defined by Stadtman et al., (1968). We have previously reported (Lundgren, Ogur and Ogur, 1969; Lundgren and Ogur, 1970) that the cumulative amino acid inhibition of this enzyme in yeast could be correlated with the inhibition of growth of a glutamate auxotroph on proline, arginine or $\Delta^1$-pyrroline-5-carboxylate as growth substitutes for glutamate, and of the growth of prototrophic strains on these substrates serving as principal nitrogen source. The amino acid inhibition is thus not limited to yeast but of more general significance.

A beef kidney was removed immediately after slaughter, placed in a 0.25 M sucrose solution containing $10^{-4}$ M EDTA and adjusted to pH 7.8 with KOH. The solution was maintained at 0-5° in ice until used. Fifty grams of cortex tissue were removed from the main body of the kidney, avoiding adipose capsule and cortical substance, and minced. Mitochondria were prepared by the method of Weinback (1961). The pellet obtained by centrifuging the homogenate at 8500 x g was resuspended in an equal volume of 0.25 M sucrose, pH 7.8, rapidly frozen to -20° and after three hours rapidly thawed to 5°. The thawed suspension was dialyzed against 0.01 M TAPS buffer pH 7.8, containing $10^{-4}$ M EDTA for twenty hours with intermittent buffer changes. The dialyzate was centrifuged at

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TABLE I

INHIBITION OF BEEF KIDNEY
$\Delta^1$-PYRROLINE-5-CARBOXYLATE DEHYDROGENASE

| Amino Acid | Observed | Calculated for Cumulative Inhibition |
|---|---|---|
| L-Serine (A) | 11.4 ± 0.5 | |
| L-Leucine (B) | 24.2 ± 0.8 | |
| Glycine (C) | 31.6 ± 0.7 | |
| L-Valine (D) | 60.3 ± 0.7 | |
| L-Alanine (E) | 70.9 ± 0.8 | |
| (A)+(B) | 47.1 ± 0.1 | 32.8 |
| (A)+(B)+(C) | 63.8 ± 0.8 | 63.4 |
| (A)+(B)+(C)+(D) | 80.7 ± 0.6 | 85.4 |
| (A)+(B)+(C)+(D)+(E) | 91.5 ± 0.6 | 95.9 |

Enzyme activity was estimated by monitoring $NAD^+$ reduction with synthetic $\Delta^1$-pyrroline-5-carboxylate as substrate according to Strecker (1960). Components of the incubation mixture were: 9.9 µmoles $NAD^+$; 1.05 $\Delta^1$-pyrroline-5-carboxylate; 40 µmoles of each amino acid; 150 µmoles TAPS buffer, pH 7.7; and enzyme (1.9 mg protein) in 3.0 ml. Results are expressed as percent inhibition ± S.D. of the mean. Cumulative inhibition was calculated according to Stadtman et. al., (1968).

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10,000 x g for 20 min. The supernatant, containing $\Delta^1$-pyrroline-5-carboxylate dehydrogenase activity was decanted and used to study the inhibition by the common amino acids. Results are summarized in Table I.

Although a difference in the degree of inhibition by individual amino acids was observed when the yeast and mammalian enzyme preparations were compared, as might be expected, the similarities in the characteristics of the two enzymes

are impressive. In both cases the inhibition is cumulative and rapidly approaches 100% as the number of amino acids increases.

Similar but more limited inhibition by amino acids of glutamine catabolism in yeast, localized in part at glutaminase, has been reported from our laboratory (Woscinski and Ogur, 1972).

These observations suggest that the inhibition of the catabolic pathways to glutamate by other amino acids may have general significance as a regulatory mechanism.

End product inhibition or repression of the biosynthesis of carbon structures leading to specific amino acids seems to be well suited to the regulation of carbon flow. Cumulative amino acid inhibition of the catabolic pathways to glutamate from arginine, proline and glutamine may be well-suited to the regulation of nitrogen flow through non-specific control of the central donor by the level of the amino acid pool.

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