On the biosynthesis of proline in *Neurospora crassa*: enzymic reduction of Δ^1-pyrroline-5-carboxylate*

Mycelial-pad experiments with *Neurospora crassa* have provided evidence for the reduction of glutamate to glutamic γ-semialdehyde and the further reduction of the spontaneously cyclized form of the semialdehyde (PC**) to proline[1,2]. Recently, oxidative enzymic relationships involving proline, glutamic γ-semialdehyde, and glutamate in mammalian liver were studied by STRECKER AND MELA[3]. The present communication is concerned with a soluble enzyme system that catalyzes the reductive formation of proline.

Extracts were prepared by grinding mycelial pads of wild-type strain 74 A of *N. crassa* (grown on minimal medium at 30°) with 0.1 *M* phosphate buffer (pH 7) and centrifuging the resulting mixtures at 100,000 × *g*. The extracts obtained were partially purified by fractionation with solid ammonium sulfate at 0°; the fractions between 26 and 36% of saturation were collected, dissolved in buffer (pH 7), and dialyzed against the same buffer in the presence of 10^{-3} *M* glutathione. The substrate, PC, was synthesized as previously described[4].

The reduction of PC was found to depend on the presence of reduced pyridine nucleotide, as shown in Table I. The reaction proceeded with both TPNH and DPNH and was followed by observing the decrease in optical density at 340 mμ. TPNH was the more active nucleotide under the conditions used. Presumably, TPNH and DPNH are cofactors for the same enzyme, since an enzyme preparation that was 95% inactivated through dialysis and storage in the absence of glutathione showed the same relative activity with the two nucleotides as did the usual preparations. The reduction of PC is partly inhibited by 10^{-3} *M* cyanide. This inhibition suggests that the enzyme system may have a metal component.

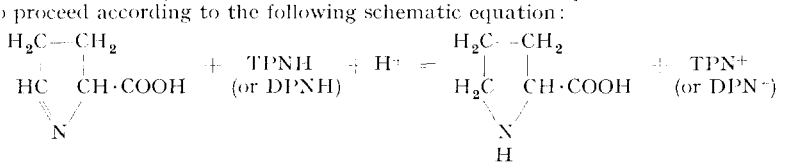
TABLE I

ENZYMIC REDUCTION OF PC

The complete system contained: phosphate buffer (pH 7), 300 μmoles; PC, 6 μmoles; TPNH (or DPNH), 0.6 μmole; enzyme (see text), 150 μg protein; water to 3 ml. The temperature was 25°. Optical density was measured at 340 mμ in a Beckman spectrophotometer (light path, 1 cm).

Modification of complete system	*Nucleotide used*	*Initial decrease in O.D. per minute*
None	TPNH	0.250
PC omitted	TPNH	0.000
Enzyme boiled	TPNH	0.000
None	DPNH	0.030
PC omitted	DPNH	0.000
Enzyme boiled	DPNH	0.000
Nucleotide omitted	—	0.000

The formation of proline was recognized by the response of a proline-requiring mutant of *Escherichia coli*, by bioautography, and by the characteristic yellow color obtained with ninhydrin. It was further shown that for each mole of nucleotide oxidized (as determined spectrophotometrically) about one mole of L-proline (as determined by bioassay with the *E. coli* mutant) was produced. Therefore, the reaction appears to proceed according to the following schematic equation:



The name pyrroline-5-carboxylate reductase might be suitable for the enzyme described.

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** The following abbreviations are used: PC, Δ^1-pyrroline-5-carboxylate; TPNH, reduced triphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; TPN^+ and DPN^+, the oxidized forms of the respective nucleotides; O.D., optical density.

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