On the glutamic family and lysine in *Neurospora*: enzymic formation of glutamic γ-semialdehyde and α-aminoadipic δ-semialdehyde from penta- and hexahomoserine*

In examining the pyrroline-5-carboxylate reductase[1] of *N. crassa*, another pyridine nucleotide-dependent enzymic reaction was noted, which has now been found to involve the open-chain form of PC**, namely GSA. The enzyme catalyzing this reaction appears to be an ω-hydroxy-α-amino acid dehydrogenase, since it also acts on PHS*** (the alcohol corresponding to GSA) and on HHS.

Enzyme extracts were prepared from lyophilized mycelium of mutant strain 21863a[3] (which is deficient[4] in PC reductase) of *N. crassa*, grown on minimal medium plus L-proline at 30° C for 3 to 5 days with aeration. Mycelial extracts in cold 0.1 *M* phosphate buffer (pH 7) were clarified by centrifugation at about 80,000 × *g*, and fractionated with ammonium sulfate at 0° C. The fractions between 45 and 55% of saturation were collected and dissolved in and dialyzed against the same buffer.

Dehydrogenation of PHS or HHS depends on the presence of DPN or TPN and was followed by observing the increase in optical density at 340 mμ (see Table I). Only the L-forms of the two ω-hydroxy-α-amino acids are active. GSA was recognized as a dehydrogenation product of PHS by the characteristic yellow color given with *o*-aminobenzaldehyde[5]. Approximately one mole of L-GSA (determined by bioassay[5]) was formed for each mole of nucleotide reduced. The dehydrogenation product of HHS is α-aminoadipic δ-semialdehyde (presumably in L-form), as shown by the color reaction with *o*-aminobenzaldehyde. The orange-yellow color obtained probably is due to a dihydroquinazolinium compound[6,5] derived from the cyclic form of α-aminoadipic δ-semialdehyde, Δ^1-piperideine-6-carboxylic acid.

TABLE I

ENZYMIC DEHYDROGENATIONS AND REDUCTIONS

Substrate used	*Nucleotide used*	*Initial change in O.D. per minute*
PHS	DPN	+ 0.080
HHS	DPN	+ 0.080
None	DPN	+ 0.006
PHS	TPN	+ 0.016
HHS	TPN	+ 0.015
None	TPN	+ 0.005
GSA	DPNH	— 0.075
None	DPNH	— 0.005
GSA	TPNH	— 0.010
None	TPNH	— 0.004

The complete system (pH 9.8) for the dehydrogenation of PHS or HHS contained: diethanolamine, 280 μmoles; DL-PHS or DL-HHS, 30 μmoles; nucleotide, 3 μmoles; enzyme (see text), 2 mg protein with 20 μmoles phosphate; water to 3 ml. The complete system (pH 6.0) for the reduction of GSA contained: phosphate, 300 μmoles; DL-GSA (calculated as the open-chain form), 6 μmoles; nucleotide, 0.6 μmole; enzyme, 2 mg protein; water to 3 ml. For both systems, the temperature was 25° C, and optical density was measured at 340 mμ in a Beckman spectrophotometer (light path, 1 cm). No appreciable optical density changes occurred with boiled enzyme preparations or in the absence of nucleotide.

The DPNH- or TPNH-dependent reduction of GSA (prepared as previously described[5]) is also presented in Table I. The reaction product, PHS, was identified by two-dimensional co-chromatography with authentic PHS and by chemical conversion to proline[7]. A one to one molar relationship was observed between L-GSA consumed and L-PHS (determined as L-proline[5], corrected for incomplete conversion) produced.

The enzymic reactions reported thus appear to be in accord with the following schematic equation:

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** The following abbreviations are used: PC, Δ^1-pyrroline-5-carboxylic acid; GSA, glutamic γ-semialdehyde; PHS, pentahomoserine (α-amino-δ-hydroxyvaleric acid); HHS, hexahomoserine (α-amino-ε-hydroxycaproic acid); DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; DPNH and TPNH, the reduced forms of the respective nucleotides; O.D., optical density.

*** DL-PHS was kindly provided by Dr. L. BERLINGUET and by Dr. E. E. SNELL. DL-HHS and optical enantiomorphs[2] of PHS and of HHS were also obtained through the generosity of Dr. BERLINGUET. The homoserine, serine, and threonine used were in the DL-form.

$$HOCH_2\cdot(CH_2)_n\cdot\underset{\displaystyle NH_2}{\underset{|}{C}}H\cdot COOH + DPN^+ \rightleftharpoons OCH\cdot(CH_2)_n\cdot\underset{\displaystyle NH_2}{\underset{|}{C}}H\cdot COOH + DPNH + H^+$$
(or TPN^+) (or TPNH)

where $n = 2$ or 3.

Presumably all the reactions described by this equation are catalyzed by a single enzyme, since an enzyme preparation that was more than 90% inactivated through heating at 60° C showed approximately the same relative activity with the respective substrates and nucleotides.

The fresh and heat-treated preparations also catalyze the reduction of DPN or TPN in the presence of a lower homologue of PHS, homoserine. In this case, with fresh preparations, TPN is reduced considerably faster than is DPN, whereas the reverse is true with heat-treated preparations (under similar test conditions). It is thus possible that the fresh preparations contain more than one enzyme capable of acting on homoserine. Accordingly, the relationship of the PHS- or HHS-dehydrogenating enzyme to the homoserine dehydrogenase[8] of yeast is uncertain. Serine, threonine, and acetaldehyde did not appear to be substrates for the enzyme preparations.

Very probably, the dehydrogenase that acts on PHS or HHS is involved in the growth response of certain *N. crassa* mutant strains to these ω-hydroxy-α-amino acids. PHS is utilized by mutants that respond alternatively to proline or to ornithine[9] and HHS by some mutants that respond to lysine[10]. The partial replacement of arginine by PHS in the diet of chicks[11] may well depend in part on a similar enzymic reaction. The growth-promoting activities of PHS and HHS are thus ascribed to the formation of the respective ω-semialdehydes. While it is thought likely that the ω-semialdehydes, but not the corresponding hydroxy compounds, are actual biosynthetic intermediates, the possibility that the hydroxy compounds have some "normal" metabolic function is not excluded. The key position of GSA in the glutamic family of *N. crassa* has been reported[12,13]. The previously considered possible role of α-aminoadipic δ-semialdehyde as an intermediate in lysine synthesis[14–17] is supported by the present finding that this semialdehyde is the product of an enzymic reaction whose substrate is known to satisfy a lysine requirement.

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The structure of the prosthetic group of bovine submaxillary gland mucoprotein

Bovine submaxillary gland mucoprotein (BSM) contains about 17% sialic acid and 9.2% N-acetylgalactosamine; in addition, N-acetylglucosamine, galactose, mannose, and fucose are present in very small amounts[1,2,3]. It was shown previously[4] that the reducing group of sialic acid is joined in a glycosidic linkage to the rest of the mucoprotein and that, at pH 1.0 and 80°, 78% sialic acid, and sialic acid only, is released from BSM. *Vibrio cholerae* neuraminidase[5] splits off up to 64%

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