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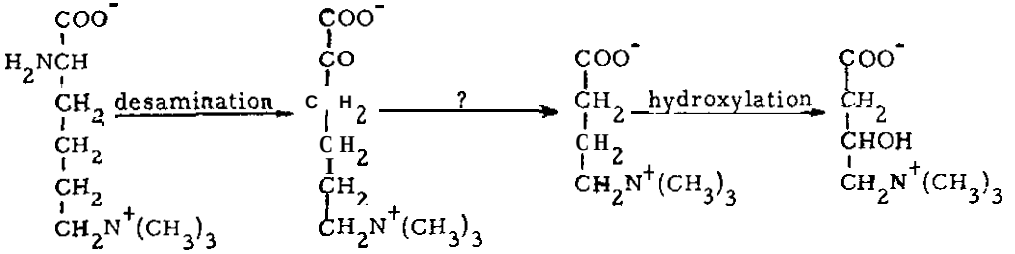
6-N-trimethyllysine metabolism and carnitine biosynthesis in N. crassa.

Villanueva and Lederer (1974, Phytochemirtry 13: 2157) hove recently reported the presence of free and protein-bound 6-N-mono-, di- and tri-methylated lysines in Neurospora crassa. Horne and Broquist (1973 J. Biol. Chem. 248: 2170) hove shown that 6-N-trimethyllysine (I) is a highly efficient precursor of carnitine (IV) via butyrobetaine (II') in Neurospora crassa. Although the same relationship among there metabolites has been found in the rat, (Tanphaichitr and Broquist 1973 J. Biol. Chem. 248: 2176; Cox and Hoppel 1973 Biochem. J. 136: 1083), no intermediates of this metabolic pathway hove been isolated nor identified as yet. Neither Lindsted and Lindsted (1965 J. Biol. Chem. 240: 316) nor Cox and Hoppel ('974 Biochem. Biophys. Acta 362: 403) could obtain incorporation of radioactivity from 5-N-($^{14}CH_3$)trimethylaminopentanoate or 6-N($^{14}CH_3$)trimethylaminohexanoate into carnitine. We hove now examined the possibility of a lysine decarboxylating pathway for 6-N-trimethyllysine (Me_3Lys). The expected intermediate would be N-trimethylcadaverine (Me_3Cad). Extracts of cultures of N. crassa strain lys-1 (33933) (FGSC #74), growing in a medium containing $Me_3(^{14}CH_3)$Lys or $Me_3(^{14}CH_3)$ Cod, were analysed by appropriate automatic ion exchange column chromatography and checked for the eventual conversion of $Me_3(^{14}CH_3)$ Lys into $Me_3(^{14}CH_3)$Cad, as well as for the conversion of the latter into butyrobetaine and carnitine. Neither conversion of $Me_3(^{14}CH_3)$lys into $Me_3(^{14}CH_3)$Cad nor conversion of $Me_3(^{14}CH_3)$Cad into butyrobetaine and carnitine was observed. Unexpectedly, analysis of the extracts labelled with $Me_3(^{14}CH_3)$Lys gave rise to three mean radioactive peaks: the first one the unknown was excluded form the column; the second eluted at the position corresponding to that of butyrobetaine-carnitine and the last one, to that of Me_3Lys. The unknown radioactive product gave a positive reaction with 2,4-dinitrophenylhydrazine, indicating the presence of a keto-group. We thought that the unknown compound could be 6-N-trimethylamino, 2-oxohexanoate. In order to check this hypothesis we subsequently transformed the isolated radioactive unknown into its 2,4-dinitrophenylhydrazone derivative and submitted it to hydrogenolysis in a Parr bomb. Analysis of the reaction products by TLC and ion exchange column chromatography (4 different systems) showed a positive ninhydrine-reacting substance at the same Rf and with the same elution time as that of authentic Me_3Lys and containing more than 85% of the radioactivity of the 2,4-dinitrophenylhydrazone before hydrogenolysis. This result strongly suggests that the unknown radioactive product is indeed 6-N-trimethylamino, 2-oxohexanoic acid (II).

Experiments with extracts of N. crassa using $Me_3(^{14}CH_3)$Lys led to the same results as "in vivo" experiments, demonstrating that an enzymate system exists which con convert Me_3Lys (I) into the corresponding ketoacid (II).

Work to verify that this ketoacid is an intermediate in the biosynthesis of carnitine (IV) is in progress.



(I) (II) (III) (IV)

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