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On the biosynthesis of carnitine in Neurospora crassa lys-1 (33933)

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Abstract

On the biosynthesis of carnitine in *lys-1* (33933)

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The isolation of 6-N-trimethylamino-2-oxohexanoic acid (TMCO) from N. crassa (Villanueva and Lederer 1975 Neurospora Newsl. 22:7; 1975 FEBS Lett. 52:308) and the purification of the enzyme forming this compound (Villanueva and Le 1976 Phytochemistry 15:1762) from 6-N-trimethyllysine (TML) prompted us to test TMCO as a possible precursor of carnitine which is known to originate from TML via butyrobetaine (Horne and Broquist 1973 J. Biol. Chem. 248:2170).

Two kinds of incubations in duplicate were carried out as follows: First, 6-N-trimethyl($^{14}CH_3$)lysine was incubated in four separate tubes in 0.1 M phosphate buffer, pH 8.9 with 6-N-trimethyllysine aminooxydase isolated from N.crassa (1500 fold purification, Lê and Villanueva, to be published) at 30° C for 4 hours. This was sufficient for more than 99 % conversion of TML into TMCO. Then the supernatant of a homogenate of N. crassa lys-1 (33933) in 0.1 M phosphate buffer, pH 7.5, was added to each of the four previous incubations containing labeled TMCO. Ascorbate, KCl, 2-oxoglutarate, ferrous sulfate and catalase (cofactors needed for butyrobetaine hydroxylation) were added to two of the tubes and then all of the incubations were continued for an additional five hours. The reactions were terminated with TCA and, after centrifugation, each supernatant was passed through a column of Dowex 1X-8, OH^- to purify the quaternary compounds (Cox and Hoppel 1973 Biochem. J. 136:1083). The eluates were dried, dissolved in citrate buffer, pH 2.2, with 1 micromole of authentic L-carnitine and analysed by automatic ion exchange column chromatography to check the conversion of 6-N-trimethyl($^{14}CH_3$)amino, 2-oxohexanoic acid to labeled carnitine.

The incubations with or without cofactors gave rise to five unknown labeled areas with varied radioactivity. None of these, however, corresponded either to carnitine or to butyrobetaine. Furthermore, cultures of N. crassa grown in a medium containing TM($^{14}CH_3$)CO failed to incorporate significant radioactivity into carnitine. These results demonstrate, at least under our experimental conditions, that 6-N-trimethylamino, 2-oxohexanoic acid is not a precursor of carnitine in N. crassa. - - - Institut de Chimie des Substances Naturelles, CNRS, 91190 Gif sur Yvette, France.