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Abstract

Cytidylytransferases in *chol* and wild type strains

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Cytidylyltransferases in cholineless mutant strains ond in the wild type of Neurospora crassa.

It was found by Horowitz et al. (1945 J. Biol. Chem. 159:145) ond Horowitz (1946 J. Biol. Chem. 162:413) that two choline-less mutant strains of Neurospora crassa, chol-1 and chol-2, are deficient in some enzymes of the methylation pathway leading to lecithin biosynthesis. The alternative pathway of lecithin biosynthesis, from CDPcholine and 1,2-diglyceride, was discovered in rot liver by Kennedy ond Weirs (1956 J. Biol. Chem. 222:196). The existence of the same pathway was revealed by in vitro experiments with the wild type strain of N. crassa (Radominska-Pyrek et al. 1969 Acta Biochim. Polon. 16:357), as the synthesis of various cytidine nucleotides from cytidine triphosphates ond appropriate phosphobases was observed. The formation of cytidine nucleotides is connected in N. crassa with phorpholipid biosynthesis, as the phorpholipid fraction become significantly labeled during incubation of a homogenate with radioactive cytidine diphosphote aminoethanols (unpublished results).

The present paper concerns the activities of cytidylyltransferases in the chol-1 and chol-2 strains of N. crassa as compared to those of the wild type strain. The metabolic blocks in the two cholineless mutants and the enzymic reactions studied ore shown in Figure 1.

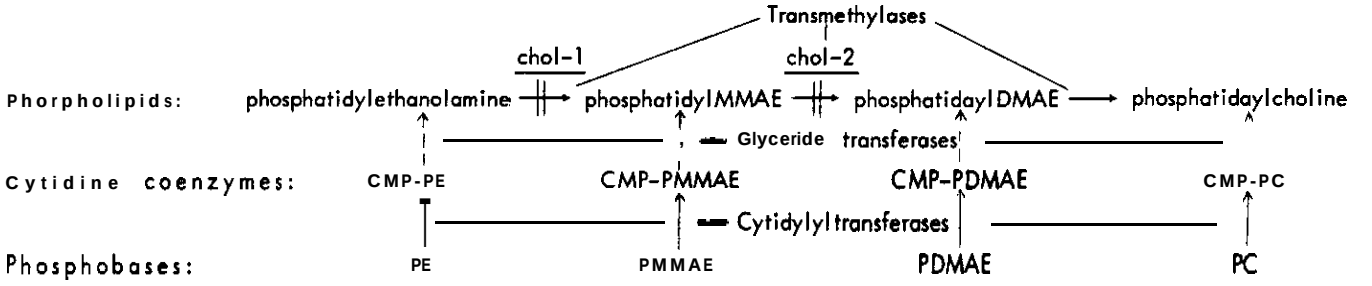

Figure 1: Two alternate pathways of lecithin (phorphotidylcholine) biosynthesis. The dashed arrows symbolize reactions which hove not been studied yet in Neurospora. Abbreviations used: E = ethonolomine; MMAE = monomethylaminoethanol; DMAE = dimethylaminoethanol; C = choline. PE, PMMAE, PDMAE ond PC ore the phosphoric esters of the respective ominoethonols. CMP-PE, CMP-PMMAE, CMP-PDMAE ond CMP-PC are the CMP derivatives of the respective aminoalcohol phosphates.

The following strains were used: wild type 74-OR-23-IA, chol-1 (34484)a, chol-2 (47904)A. Mycelia were grown at 30°C in liquid shake cultures in Vogel's minimal medium plus 1% sucrose. Where indicated (in table 1), this was supplemented with choline chloride (0.2 mmole/1 liter medium) or dimethylaminoethanol (0.1 mmole/1 liter medium). The culture of the chol-2 strain in unsupplemented minimal medium was grown for 3 days. All other cultures were grown for 2 days. The methods of determination of the activity of cytidylyltransferases used in the present report were described previously (Radominska-Pyrek et al. 1969 ibid.)

Table 1. Cyt idylyltransferase specific activities in 105,000 X g supernatants of the wild type, chol-I and chol-2 strains cultivated in different media.

Substrate	wild type minimal	wild type min + chol	chol-I min + DMAE	chol-I min + chol	chol-2 min + chol	chol-2 minimal
PE	7.70	7.05	7.65	6.08	5.96	3.04
PMMAE	2.48	2.41	2.28	2.20	1.80	1.33
PDMAE	1.56	1.68	1.52	1.56	0.97	0.11
PC	2.84	2.92	4.04	4. 12	3.20	0. 17

Each entry in this table stands for nmoles of nucleotide formed per 1 mg protein/15min.

Table 1 shows the specific activities of cytidylyltransferases residing in the 105,000 x g supernatants of mutant strains and the wild type in a typical experiment. It is clear that in the case of the wild type strain no effect of choline in the culture medium was observed. The chol-1 strain grown in the medium supplemented with choline or dimethylethanolamine has PE- and PMMAE-cytidylyltransferase activities similar to those of the wild type, but the PC-cytidylyltransferase activity is slightly but consistently increased. The chol-2 strain grown in unsupplemented medium has about 50% of PE- and PMMAE-cytidylyltransferase activities and only 6% of PDMAE- and PC-cytidylyltronferore activities when compared with the wild type. The chol-2 strain grown in the supplemented medium has PE- ond PMMAE-cytidylyltransferase activities only slightly lowered, while the PC-cytidylyltransferase activity, similarly as in the chol-1 strain, is slightly increased.

The results suggest that the limitation in amount of endogenous choline in the chol-2 strain of N. crassa lowered markedly the activities of all cytidylyltransferases and, in particular, those after the block in the methylation pothwoy. The addition of exogenous choline for cultivation of this strain restored to a large extent the PE- and PMMAE-cytidylyltransferase activities and increased the activity of PC-cytidylyltronsferore. Similar stimulation of PC-cytidylyltronrfemse activity was observed for the chol-1 strain.

The present results indicate that the system studied is not repressed by the choline or DMAE, but on the contrary, that these compounds could be the inducers of K- and PMMAE-cytidylyltransferases. - - - Institute of Biochemistry and Biophysics, Polish Acodcmy of Sciences, Warsaw 12, Rakowiecka 36, Poland.