INDUCTION OF ASPARTIC TRANSCARBAMYLASE BY CARBON DIOXIDE

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Carbon dioxide is involved in the biosynthesis of arginine and the pyrimidines as a precursor of carbamyl phosphate (CP), which in turn donates carbamyl residues to the above pathways in reactions catalyzed respectively by ornithine transcarbamylase (OTC) and aspartic transcarbamylase (ATC). This scheme holds for a variety of organisms, including Neurospora (Reichard, 1959; Davis, 1962). In addition, some responses to carbon dioxide observed in Neurospora mutants deficient in the synthesis of arginine or pyrimidines (Reissig and Nazario, 1962; Charles, 1962) are not explainable in terms of the role of carbon dioxide as CP precursor, suggesting that carbon dioxide may also have a regulatory role in these pathways. In this communication we shall report on a further instance of carbon dioxide response in Neurospora, resulting from the induction of ATC.

Among the pyr3 mutants affecting ATC, two types can be recognized: pyrN mutants, having very little or no ATC and therefore pyrimidine auxotrophs; and $pyr^{su\text{-}arg}$ mutants, having levels of ATC which are low, but still compatible with prototrophic growth (Reissig, 1963b). Both types are selected taking advantage of their ability to suppress the arginine requirement of mutant arg2 (Reissig, 1963a). In this fashion, the $pyr^{su\text{-}arg}$ alleles were obtained on strains carrying arg2, and their pyrimidine requirement was investigated in the presence of excess arginine. It was found that while $pyr^{su\text{-}arg}$ mutants grow prototrophically under air, they become markedly pyrimidine dependent if all carbon dioxide is withdrawn (Table I).

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TABLE I

Stimulation of pyr^{su-arg} by uridine in the absence of CO_2

genotype	Strain number	Effect of adding 100 mg/l uridine (% difference in growth index) under air	under CO_2-free air
arg2, pyr^{su-arg}	KØ492-29a	-8	+37
	KØ492-33a	-6	+196
	KØ492-39a	-8	+343
	KØ492-40a	-4	+90
	KØ493-41a	+11	+39
	KØ493-59a	+69	+276
	KØ493-97a	+14	+96
	KØ495-105a	+21	+54
	KØ495-107a	+11	+102
$arg2, pyr3^+$ (control)	ED416-1a	-6	+10
$arg2^+, pyr3^+$ (controls)	KØ493-27a	-13	+12
	KØ493-56a	-9	+15
	KØ493-68a	-7	0
	KØ493-124a	-6	+15

Microconidia were inoculated on plates containing solid "minimal" medium supplemented with 20 mg/l arginine, and uridine as indicated. For the CO_2-free series, the plates were placed in vacuum desiccators over concentrated KOH solutions. Growth was measured at convenient intervals, and the growth index equals the time taken for the mycelial front to travel 40 mm, minus 40 hours (the shortest lag period). This index was chosen because Neurospora mutants which respond to carbon dioxide eventually grow at maximal rate even under deficient conditions, probably because of endogenous carbon dioxide formation; therefore growth responses are displayed only at the initial stages (Reissig, 1963b). ATC specific activity in 4 of the above pyr^{su-arg} strains ranged from 20-50% of that in the controls (A. J. Jobbágy, personal communication).

All strains tested were derived from ED416-1a by one mutational step. Therefore, the differences between pyr^{su-arg} and the controls can be traced to allelic differences at pyr. Since these differences in turn involved ATC deficiencies, the results presented strongly suggest that either the synthesis or the activity of ATC is stimulated by carbon dioxide, this effect being detectable as a growth response only in those strains in which ATC is limiting.

Direct testing of the above hypothesis failed to show an effect of bicarbonate on ATC activity in vitro, but confirmed that cultures grown under higher tensions of carbon dioxide synthesize more ATC (Table II). The concomitant reduction in the partial pressure of nitrogen is not responsible for the effect observed: cultures under 70% air plus either 30% argon or 30% oxygen displayed the same ATC specific activities as under air (1.2, 1.1 and 1.3 respectively, but 5.9 for 30% carbon dioxide in the same experiment).

TABLE II

Induction of ATC by carbon dioxide

Composition of the gas phase	ATC specific activity in cultures		
	4 days old on minimal	4 days old on uridine	8 days old on minimal
Air	1.2	0.9	1.8
99% air + 1% CO_2	1.9	1.6	2.9
90% air + 10% CO_2	3.5	2.5	2.2
70% air + 30% CO_2	8.4	7.6	3.2
64% air + 30% CO_2 + 6% O_2			3.1
40% air + 50% CO_2 + 10% O_2			4.2
16% air + 70% CO_2 + 14% O_2			8.7

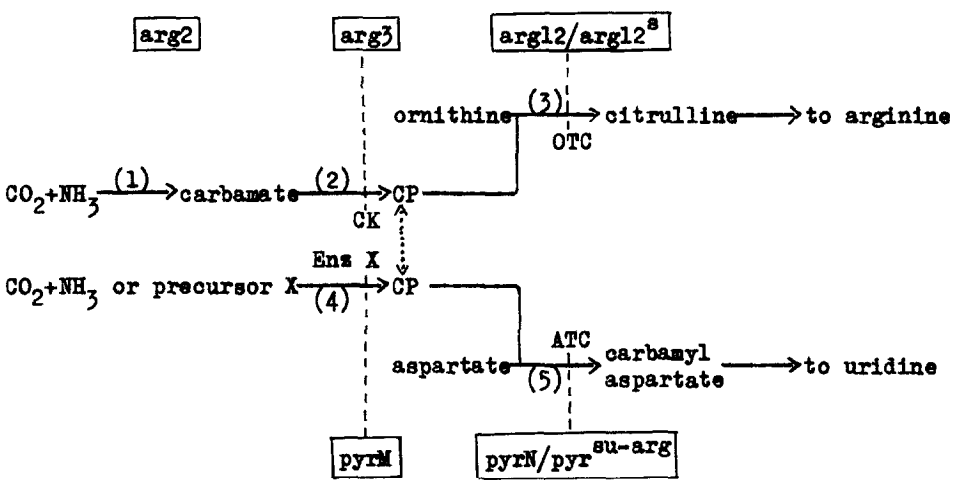
Strain KØ493-56a was cultured on solid medium (Table I) overlayered with cellophane to prevent anaerobic growth, in desiccators with the indicated gas mixtures. Uridine was added at 100 mg/l. The aerial mycelium was lyophilized, extracted by suspending in cold 0.018 M Tris acetate, pH 8.2, and assayed for ATC by the method of Lowenstein and Cohen (1956), slightly modified. Protein was measured according to Lowry et al. (1951). Specific activity is expressed as units (micromoles of carbamyl aspartate formed per hour at 35°) per mg protein.

No depression of ATC was observed in cultures grown in a desiccator over KOH; a finding which probably means that endogenous carbon dioxide cannot be easily removed exhaustively. Neither carbon dioxide, nor bicarbonate raised the ATC level of liquid cultures; suggesting that submerged cultures may be already saturated with carbon dioxide. This interpretation is in accordance with the high ATC content of such cultures (4 to 5 units/mg protein).

Since carbon dioxide is a precursor of CP, the possibility was considered that induction be mediated by CP, after the fashion of the "simultaneous adaptation" hypothesis (Stanier, 1951). This possibility was ruled out because a culture blocked in the conversion of carbon dioxide to CP (arg3,pyrM: 30300-37301; kindly provided by R. H. Davis) displayed the usual ATC increment in the presence of high carbon dioxide.

Discussion: The data presented indicate that carbon dioxide, or a product of its metabolism different from CP, induces ATC. Although growth experiments (Table I) and enzyme assays (Table II) indicate responses at different ranges, this only implies a methodological difference. The effects on growth could only be detected with flux controlling levels of enzyme (Kacser, 1963) and this is achieved only in the early stages of growth (see legend of Table I), when endogenous carbon dioxide is not a significant factor. Enzyme assays, on the other hand, are performed on fully grown cultures.

Scheme I



Step (1) is probably spontaneous (Jones and Lipmann, 1960); the enzyme catalyzing step (4), Enz X, has not been identified. CK stands for carbamate kinase. Intermediate mutant alleles ($pyr^{su\text{-}arg}$ and $arg12^{s}$) have also been indicated. Mutants in the pyr3 region have been classified as pyrN or pyrM according to whether they affect ATC or not. Arg2 has CK but appears to affect in some way steps (1) or (2) in vivo. Steps (4) and (5) are regulated by uridine and carbon dioxide (see text). The dotted arrow indicates that the two CP pools, normaly utilized respectively for arginine and pyrimidine synthesis, may feed into each other under special circumstances.

Scheme I, emerging from the work of several laboratories (Davis, 1962; Davis and Woodward, 1962; Fairley and Adams, 1961; Reissig and Nazario, 1962; Charles, 1962; Woodward, 1962) has been elegantly confirmed by Davis (1963).

Evidence that step (4) might be subjected to the same regulatory regime as step (5) is provided by the fact that arg2 grows slowly in the absence of arginine if carbon dioxide is provided, and that this slow growth is antagonized by uridine (Reissig and Nazario, 1962). More compelling evidence is provided by the fact that pyrN and pyrM are contiguous on the chromosome. This proximity could hardly be coincidental, and suggests either the existence of a bifunctional enzyme catalyzing steps (4) and (5) (Woodward and Davis, 1963) or of two enzymes coordinated in an operon. The adaptive significance of the induction of steps (4) and (5) by carbon dioxide should be considered in connection with the general phenomen of "derepression by the first substrate" (Gorini, 1963).

References

Charles, H. P., Nature 195 (1962) 359.
Davis, R. H., Genetics 47 (1962) 351.
Davis, R. H., Science 142 (1963) 1652.
Davis, R. H. and Woodward, V. W., Genetics 47 (1962) 1075.
Fairley, J. L. and Adams, A. B., Science 134 (1961) 471.
Gorini, L., Bact. Revs. 27 (1963) 182.
Jones, M. E. and Lipmann, F., Proc. nat. Acad. Sci. (Wash.) 46 (1960) 1194.
Kacser, H., in Biological Organization at the Cellular and Supercellular Level, Academic Press (1963) 25.
Lowenstein, J. M. and Cohen, P. P., J. Biol. Chem. 220 (1956) 57.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., J. Biol. Chem. 193 (1951) 265.
Reichard, P., Adv. Enzymol. 21 (1959) 263.
Reissig, J. L., J. gen. Microbiol. 30 (1963a) 317.
Reissig, J. L., J. gen. Microbiol. 30 (1963b) 327.
Reissig, J. L. and Nazario, M., Resumen de Comunicaciones, II Sesiones Científicas de Biología (Córdoba), p. 76 (1962).
Stanier, R. Y., Ann. Rev. Microbiol. 5 (1951) 35.
Woodward, V. W., Proc. nat. Acad. Sci. (Wash.) 48 (1962) 348.
Woodward, V. W. and Davis, R. H., Heredity 18 (1963) 21.