URACIL BIOSYNTHESIS IN SERRATIA MARINORUBRA

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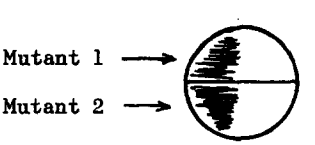
The correspondence between gene mutations and altered biosynthetic capacity has been amply demonstrated. (Bonner, 1952) During the course of experiments designed to yield biochemically deficient auxotrophs of Serratia marinorubra, nine independently occurring mutants were obtained which respond to uracil. The first of these mutants prompted interest in the group, which has been borne out by subsequent investigations. This mutant, when grown on defined medium supplemented with limiting concentrations of uracil, accumulates a precursor in sufficient quantity that it crystallizes out in the agar medium. Some of the crystalline material was isolated and chromatographed using an n-propanol: NH_4OH solvent system. The UV absorbing component of the crystalline material had the same Rf value as orotic acid in this solvent system. Since orotic acid is a known intermediate in uracil synthesis, its accumulation behind the genetic block suggests a precursor role in this organism also. These findings lead to the possibility that the other mutants might also accumulate precursors behind the genetic blocks, which would make it possible to examine the biosynthesis of uracil in this organism.

To test the hypothesis, the mutants were streaked in pairs on defined medium lacking uracil. If precursors accumulate, cross feeding should occur by diffusion of precursors. (Davis, 1950)

The plates were streaked in this fashion:



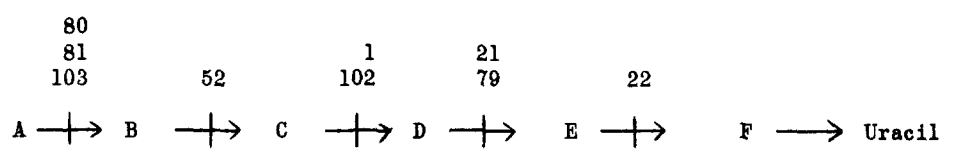
When the nine mutants were checked in this way, cross feeding did occur, and the results are presented in Table 1.

TABLE 1

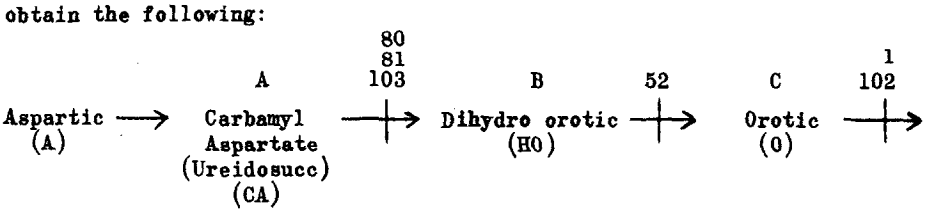
Cross Feeding Pattern of Uracil Mutants
Streaked on Defined Medium Lacking Uracil.

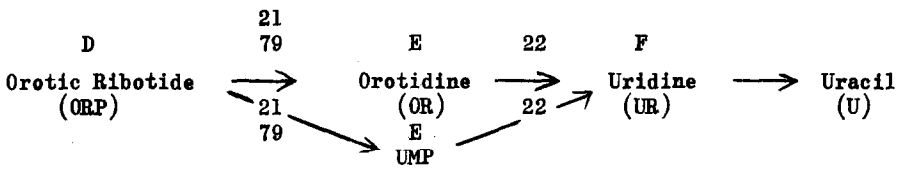
Mutant Fed.	Mutant Feeding								
	80	81	103	52	1	102	21	79	22
80	-	-	-	+	+	+	+	+	+
81	-	-	-	+	+	+	+	+	+
103	-	-	-	+	+	+	+	+	+
52	-	-	-	-	+	+	+	+	+
1	-	-	-	-	-	-	+	+	+
102	-	-	-	-	-	-	?	?	?
21	-	-	-	-	-	-	-	-	+
79	-	-	-	-	-	-	-	-	?
22	-	-	-	-	-	-	-	-	-

On the basis of these results, it was possible to construct a tentative biosynthetic sequence of the mutants as follows:



As shown by chromatography, Compound C is apparently orotic acid. Comparing this sequence with the biochemical pathway described by Carter (1956), we obtain the following:





If this sequence of biochemical events represents the mechanism for uracil biosynthesis in Serratia marinorubra, each mutant should respond to those compounds which follow the blocked step, but not to those preceding the genetic block. Most of these intermediates were obtained* and checked for their ability to support growth of the mutants. Orotic ribotide and orotidine were not available for test.

A dense suspension of each mutant was prepared in physiological saline. 0.1 ml aliquots were delivered to the surface of defined medium plates lacking uracil. The inoculum was spread until dry, and wax pencil identifying numbers placed on the bottom of the dish. Crystals of the intermediates were dropped onto the agar surface above the appropriate mark. The plates were incubated at 29° C. Those intermediates which satisfied the requirement of the mutant were surrounded by an area of dense growth within 48 hours. Table II shows the results of this test.

TABLE II

Response of Uracil Mutants to known intermediates in Uracil Biosynthesis.

Mutant No.	Uracil Intermediates (A)	(CA)	(HO)	(O)	(UMP)	(UR)	(U)
80	0	0	+	+	+	+	+
81	0	0	+	+	+	+	+
103	0	0	+	+	+	+	+
52	0	0	0	+	+	+	+
1	0	0	0	0	0	0	+
102	0	0	0	0	0	0	+
21	0	0	0	0	+	+	+
79	0	0	0	0	+	+	+
22	0	0	0	0	+	+	+

*California Corp. for Biochemical Research.

The results of this test confirm, in part, the tentative placement of the mutants in the uracil pathway as previously described. The response of mutants 80, 81 and 103 to all intermediates after ureido succinic (CA), and the failure to grow on ureido succinic suggests that these three lack the enzymatic capacity for ring closure, but do possess all of the remaining enzymes in the pathway. Mutant 52 is apparently blocked in the conversion of di hydro-orotic acid to orotic acid, and consistent with the previous tests, numbers 1 and 102 are apparently unable to add ribose to the orotic acid base. Not having either orotidine or orotic acid ribotide, the placing of mutants 21, 79 and 22 is still in doubt. The response of all three mutants to UMP suggests that they may all be blocked in the same place, namely conversion of orotic acid ribotide to orotidine or UMP. The cross-feeding test on the other hand suggests that 22 accumulates a precursor capable of feeding 21 and possibly 79, and therefore may be blocked at a later step. Confirmation of the relative positions of these three mutants must await further biochemical tests.

The failure of mutants 1 and 102 to respond to UMP and uridine may reside in the nature of the biosynthetic mechanism. The accumulation of orotic acid in these mutants would place the site of the block at the conversion of orotic acid to orotic ribotide. The inability of the mutants to respond to intermediates subsequent to orotic acid such as UMP and uridine, however, indicates the possibility that these mutants may also lack the ability to remove ribose to produce uracil.

Both 1 and 102 were derived as single step mutants and are apparently altered in only one enzyme. If this is true, then this may be a single enzyme with a dual role. An alternative possibility is that these mutants arose by deletion and thus represent multiple site mutants. This seems unlikely since reversions have been observed.

If the same enzyme is responsible both for adding ribose and for its subsequent removal, then the specific response to uracil which was observed would result.

Isolation and study of this enzyme, and comparison with gene fine structure should provide further information on the relationship between gene and enzyme.

ACKNOWLEDGEMENT

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