A REGULATORY MUTATION IN TYROSINE BIOSYNTHESIS*

E. Gollub and D. B. Sprinson+

Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Received April 4, 1969

SUMMARY: Mutants of Salmonella with an altered control of the tyrosine repressible enzymes were obtained by selection for resistance to 4-fluorophenylalanine. The tyrosine repressible 3-deoxy-D-arabino-heptulosonate 7-phosphate synthetase and prephenate dehydrogenase (specified by aroF, and tyr, respectively) were constitutively and co-ordinately derepressed. The mutation to resistance was co-transducible with aroF and tyr. A unit of regulation in tyrosine biosynthesis in indicated.

Control of aromatic amino acid biosynthesis in Salmonella is effected by tyrosine, phenylalanine, and tryptophan through repression and feedback inhibition of the three isoenzymes catalyzing the initial reaction in the pathway (DAHP‡ synthesis), and of the branch point enzymes following chorismate (1, 2). The tyrosine, phenylalanine, and tryptophan repressible DAHP synthetases are specified by unlinked genes aroF, aroG, and aroH, respectively (2).

In the present study, selection for resistance to 4-fluorophenylalanine yielded mutants with altered control of the tyrosine repressible enzymes. DAHP synthetase (tyr) and prephenate dehydrogenase (specified by aroF and tyr, respectively) were constitutively and co-ordinately derepressed. The mutation to resistance was co-transducible with aroF, tyr, and phe which were previously shown to be linked (2, 3). A unit of regulation in tyrosine biosynthesis is indicated.

* This work was supported by grants from the American Cancer Society, the American Heart Association, the National Institutes of Health of the U. S. Public Health Service, and The National Science Foundation.

+ Career Investigator of the American Heart Association.

‡ Abbreviation used: DAHP, 3-deoxy-D-arabino-heptulosonate-7-P.

Experimental

Approximately 10^8 washed cells of an overnight broth culture of Salmonella typhimurium LT2 were spread on gradient plates containing 0-20 mM DL-4-fluorophenylalanine. Confluent growth occurred at low concentrations of analogue. Several minute colonies with "haloes" around them appeared after 24-48 hours at high concentrations, and were purified by reisolation on plates containing varying concentrations of fluorophenylalanine. These organisms were resistant to 7 mM fluorophenylalanine, and fed tyrosine auxotrophs when plated on analogue media. The properties of two such mutants (fpr-1 and fpr-2), isolated independently, are described in the present report.

Tyrosine and phenylalanine auxotrophs of S. typhimurium tyr 11, 19, 25, and 33, and phe 20 were a kind gift from Dr. Y Nishioka. These strains lack prephenate dehydrogenase and prephenate dehydratase, respectively (4, 5). AroF and aroG were isolated by A. DeLeo in our laboratory, and lack the tyrosine sensitive and phenylalanine sensitive DAHP synthetase, respectively. PLT22 phage lysates were prepared as described by Adams (6).

Growth of cells (O. D. $_{660}$ 1.0, late log), preparation of extracts and assays for DAHP synthetase, dehydroshikimate reductase, and dehydroquinase, have been described previously (1). Prephenate dehydrogenase was assayed by an unpublished method (5). Prephenate dehydratase was measured according to the procedure of Cotton and Gibson (7). Specific activity is expressed as μmoles of substrate used or product formed per hour per mg of protein. Barium prephenate was isolated from accumulation media of tyr 19 (5). DL-4-Fluorophenylalanine was purchased from Calbiochem, and 4-hydroxyphenylpyruvate from Sigma.

Results and Discussion

The fpr and wild type strains had identical doubling times of 60 minutes

TABLE I

Derepression of Tyrosine Specific Enzymes in fpr Mutants*

Organism	DAHP synthetase tyr	DAHP synthetase phe	5-Dehydro-shikimate reductase	5-Dehydro-quinase	Prephenate dehydro-genase	Prephenate dehydra-tase
fpr-1	8.2	1.9	0.80	1.8	5.5	0.45
LT2	0.45	1.3	0.73	1.7	0.5	0.51

* Methods and definition of units are given in the text. Assays of prephenate dehydrogenase were carried out on freshly prepared extracts.

TABLE II

Repression of Tyrosine Specific Enzymes by Exogenous Tyrosine

Concentration of L-tyrosine in growth medium	DAHP synthetase (tyr)		Prephenate dehydrogenase	
	fpr-2	LT2	fpr-2	LT2
mM				
0.0	6.5	0.40	6.1	0.70
0.055	6.1	0.27	5.3	0.12
0.110	4.0		3.4	
0.137	2.6	0.0	2.5	0.0
0.192	0.42		0.85	
0.275	0.23	0.0	0.77	0.0
0.550	0.0		0.58	

on minimal media. Activities of several aromatic enzymes in the two strains are compared in Table I. DAHP synthetase activity of the mutant is 5-10 fold higher than in wild type, owing entirely to 10-20 fold derepression of the tyrosine sensitive isoenzyme. While two other common pathway enzymes showed normal levels, prephenate dehydrogenase, which is specific for tyrosine biosynthesis, was increased 10 fold over wild type. Prephenate dehydratase levels were unaffected. The two tyrosine specific enzymes are thus constitutively and co-ordinately derepressed, though they are still repressible by exogenous tyrosine (Table II). It is of interest, however, that chorismate mutase-T, which is closely associated with prephenate dehydrogenase (4, 7), is not significantly

affected by the fpr mutation (unpublished results). The reasons for this surprising observation are not clear. Non-coordinate derepression of chorismate mutase and prephenate dehydrogenase has been reported by Zalkin (8) in an aromatic auxotroph under derepressed conditions.

AroF, tyr, and phe, which specify DAHP synthetase (tyr), chorismate mutase-prephenate dehydrogenase, and chorismate mutase-prephenate dehydratase, respectively, have been shown to be co-transducible (2). Hence, it was thought worthwhile to test whether the mutation to resistance was linked to these loci. AroF, tyr, and phe mutants were transduced by phage prepared from fpr-2. Transductants were selected on minimal media when tyr or phe mutants were the recipients, and on phenylalanine and tryptophan when aroF was the recipient. (AroF cannot grow on phenylalanine plus tryptophan unless tyrosine is supplied). After 36 hours when the recombinant colonies were still very small, the plates were replicated to 7 mM fluorophenylalanine, since direct plating on analogue media did not give reliable results. It may be seen from Table III that 80-90% of the prototrophic recombinants had simultaneously acquired resistance and were able to grow on the analogue. When the same recipients were transduced with wild type phage, or when an unlinked leucine auxotroph was transduced with phage from fpr-2, all of the resulting prototrophic transductants remained sensitive to the analogue. These results indicate close linkage between the fpr mutation and tyr, phe, and aroF genes.

Several of the transductants from the fpr-1 versus tyr 33 cross were purified. In all strains, the levels of DAHP synthetase (tyr) and prephenate dehydrogenase were derepressed, while prephenate dehydratase, the product of the phe gene, remained at wild type levels (Table IV). The mutation to fluorophenylalanine resistance has apparently affected only the two genes involved in tyrosine biosynthesis. The phe gene, while linked by transduction

TABLE III

Transductions with fpr as Donor

An overnight culture of the recipient in nutrient broth (0.1 ml, 2 x 10^8 cells), and phage prepared from fpr-2 (0.1 ml, 10^9 particles) were spread on a minimal agar plate. The selecting medium for aroF and aroG contained 40 μg/ml each of phenylalanine and tryptophan, or of tyrosine and tryptophan, respectively. Replication to fluorophenylalanine medium was carried out at 36-40 hr when colonies had not yet reached full size. Numerical values refer to wild type recombinants per plate (average of two experiments).

Recipient strain	Donor strain			
	fpr-2		Wild type	
	Minimal	7 mM fluoro-phenylalanine	Minimal	7 mM fluoro-phenylalanine
tyr 11	150	140	200	0
tyr 19	130	130	210	0
tyr 25	150	120	140	0
tyr 33	160	150	290	0
phe 20	150	150	180	0
aroF-1	77	68	70	0
aroF-3	48	38	50	0
aroG-1	60	0	60	0
leu-A	130	0	190	0

TABLE IV

Derepression of Tyrosine Specific Enzymes in Transductants of fpr x tyr Crosses*

Strain	DAHP synthetase		Prephenate dehydro-genase	Prephenate dehydratase
	tyr	phe		
fpr-1	9.0	3.0	5.0	0.54
fpr-2	10.1	2.0	6.8	0.71
transductant-1	4.2	1.4	5.5	0.31
transductant-2	4.3	2.1	5.7	0.30
LT2	0.49	1.8	0.60	0.50

* Activities are average values from assays of three independently grown batches of cells.

to the fpr mutation, codes for an enzyme which is not on the tyrosine pathway and would not be expected to be regulated by the same operator or repressor.

The close linkage observed between fpr, aroF, and tyr may indicate

that fpr is a mutation at an operator site which has resulted in a lowered affinity for repressor. However, mutation of a regulator type gene resulting in a repressor with altered binding for tyrosine is not ruled out, and the higher tyrosine concentration required for repression of the tyrosine pathway enzymes in fpr (Table II) favors a partial loss in effectiveness of the repressor. Tests for dominance are in progress in order to differentiate between these two possibilities. A third explanation could be based on the hypothesis that aminoacyl-tRNA acts as a corepressor, as indicated in the regulation of histidine (9, 10) or valine (11) biosynthesis. An alteration of either tyrosine tRNA or tyrosyl-tRNA synthetase would therefore result in derepression of both

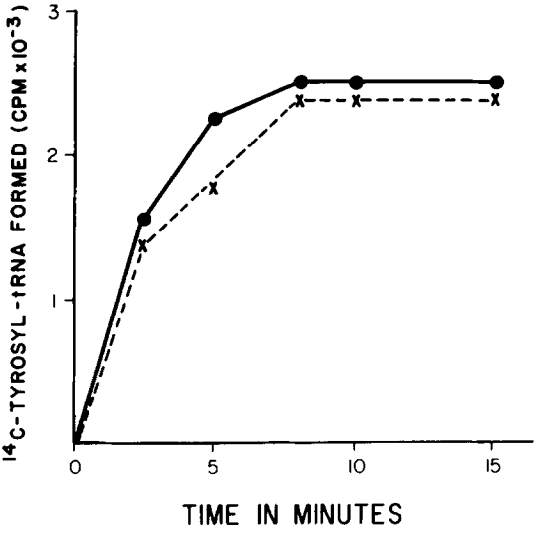

Fig. 1. Tyrosine acceptor activity of tRNA from wild type (●—●) and fpr-2 (x---x) cells. tRNA was extracted from whole cells, freed of esterified amino acids, and assayed by a modification of the method of von Ehrenstein (13). The source of tyrosyl-tRNA synthetase was an extract of wild type cells treated with streptomycin. The supernatant solution was dialyzed, and preserved in 50% glycerol at -15°. The assay mixture contained 0.05 mg of tRNA, 0.02 μmoles of ^{14}C-tyrosine (2.8 x 10^5 cpm), extract (100 μg of protein), and the other reagents (13), in a volume of 0.5 ml. Each point represents the average of three determinations.

tyrosine repressible enzymes. However, tyrosine analogues are known to repress DAHP synthetase in Escherichia coli, though they are not activated by tyrosyl-tRNA synthetase (12). With extracts of wild type cells as a source of tyrosyl-tRNA synthetase, tRNA from fpr and wild type was charged by ^{14}C-tyrosine to essentially the same extent (Fig. 1). Studies are in progress to determine whether there has been any alteration in the tyrosyl-tRNA synthetase of fpr strains.

The linkage of the structural genes, and the finding of co-ordinate derepression of the tyrosine specific enzymes, suggest a functional unit of control comprising the genes aroF and tyr. This constitutes the first unit of regulation, besides the tryptophan operon, in aromatic biosynthesis.

Acknowledgements

We thank Mrs. Gabrielle Hanoune and Mrs. Laird Pylkas for excellent technical assistance.

References

1. Gollub, E., Zalkin, H., and Sprinson, D. B., J. Biol. Chem., 242, 5323 (1967).
2. DeLeo, A. B., and Sprinson, D. B., in preparation.
3. Nishioka, Y., Demerec, M., and Eisenstark, A., Genetics, 56, 341 (1967).
4. Dayan, J., and Sprinson, D. B., Fed. Proc., 27, 290 (1968).
5. Dayan, J., and Sprinson, D. B., in preparation.
6. Adams, M. H., Bacteriophages, Interscience Publishers, Inc., New York 1959.
7. Cotton, R. G. H., and Gibson, F., Biochim. Biophys. Acta, 100, 76 (1965).
8. Zalkin, H., Biochim. Biophys. Acta, 148, 609 (1967).
9. Schlesinger, S., and Magasanik, B., J. Mol. Biol., 9, 670 (1964).
10. Roth, J. R., and Ames, B. N., J. Mol. Biol., 22, 325 (1966).
11. Eidlic, L., and Neidhardt, F. C., Proc. Nat. Acad. Sci., U.S.A., 53 539 (1965).
12. Ravel, J. M., White, M. N., and Shive, W., Biochem. Biophys. Res. Commun., 20, 352 (1965).
13. von Ehrenstein, G., in S. P. Colowick and N. O. Kaplan (Editors), Methods in Enzymology, Vol. XII, Academic Press, New York, 1967, p. 588.