THE APPARENT CONSERVATION OF THE INTERNAL LOW EFFICIENCY PROMOTER OF THE TRYPTOPHAN OPERONS OF SEVERAL SPECIES OF *ENTEROBACTERIACEAE*¹

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ABSTRACT

The specific activities of the tryptophan biosynthetic enzymes were determined for eleven different species of *Enterobacteriaceae* grown under repressing and non-repressing conditions. In all the bacteria examined the multiplicity of derepression for the first two enzymes of the pathway was at least twofold greater than the multiplicity of derepression for the last three enzymes.

IN the enteric bacteria, *Escherichia coli* and *Salmonella typhimurium*, the genes specifying the enzymes of the tryptophan biosynthetic pathway are closely linked on the chromosome (YANOFSKY and LENNOX 1959; BLUME and BALBINDER 1966) and are transcribed into a single polycistronic mRNA (YANOFSKY 1967). The synthesis of the enzymes is repressed by the end product L-tryptophan, and upon derepression all the enzymes are produced coordinately (ITO and CRAWFORD 1965; BAUERLE and MARGOLIN 1966a). These and other data are consistent with the JACOB-MONOD model of the operon (JACOB and MONOD 1961). In *S. typhimurium* it was found that a derepressed tryptophan auxotroph had activity levels of the products of the first two genes of the operon (anthranilate synthetase (ASase) and phosphoribosylanthranilate transferase (PRTase), which indicated a derepression almost twice as great for these two enzymes as for the products of the last three genes: indoleglycerol phosphate synthetase (InGPase) and tryptophan synthetase (TSase) BAUERLE and MARGOLIN 1966a). These and other findings led to the hypothesis that the basis for this phenomenon, termed semi-coordinate regulation (BAUERLE and MARGOLIN 1966a), was the presence of a second promoter (P2) between the second and third structural genes of the operon. According to the hypothesis the non-repressed levels of the first two enzymes would depend upon initiations at the operator-associated promoter (P1). The non-repressed levels of the last three enzymes would be determined by initiations at both P1 and P2. But under conditions of repression most initiations at P1 would be blocked and initiations at P2 would result in the higher repressed levels of the last three enzymes. This hypothesis would explain the discrepancy in the multiplicity of derepression for the products of the first two genes as compared to the products of the last three

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genes. The multiplicity of derepression, the ratio of the specific activity of each enzyme under non-repressed conditions to that under repressed conditions, is about twofold greater for the first two enzymes than for the last three enzymes (BAUERLE and MARGOLIN 1966a). The existence of the second promoter has been inferred from similar data for *E. coli*, and in this case, the first two activities are derepressed about five times as much as the last three activities (MORSE and YANOFSKY 1968). The coordinate derepression of the tryptophan enzymes in a wide variety of enteric bacteria has been established (LARGEN 1972). Accordingly, the multiplicity of derepression of the tryptophan enzymes was examined as evidence for the existence of a second promoter.

MATERIALS AND METHODS

The bacteria used in this study and their sources were: from the National Collection of Type Cultures, London, England, *Enterobacter cloacae* NCTC 9394, *Enterobacter hafniae* NCTC 9540, *Citrobacter freundii* NCTC 9750, *Citrobacter ballerupensis* NCTC 6021, and *Proteus morganii* NCTC 235; from Dr. MORTIMER P. STARR, *Erwinia carotovora* ICPB EC153 and *Erwinia dissolvens* ICPB ED106; from the University of California, Riverside, *Proteus vulgaris*, *Serratia marcinorubra*, and *Serratia marcescens* ATCC 9195; and from the American Type Culture Collection *Enterobacter liquifaciens* ATCC 14460.

Cells were grown to approximately 200 Klett units, measured using a Klett-Summerson colorimeter (filter #66, red), in Vogel and Bonner salts (1956) supplemented with 1% (w/v) acid hydrolyzed casein and 0.5% (w/v) D-glucose. For repressing conditions the above medium was supplemented with 50 μ g/ml L-tryptophan. Cells were harvested, extracts prepared and enzyme activities measured as described previously (HUTCHINSON and BELSER 1969).

RESULTS

The specific activities of the tryptophan enzymes for several enteric bacteria grown under non-repressing and repressing conditions, together with the multiplicities of derepression are presented in Table 1. The differences in the multiplicity of derepression for the first two enzymes compared to that for the last three enzymes were in some cases as much as ten- to fifteen-fold. These data are consistent with the conservation of a low efficiency internal promoter in the tryptophan operon of these enteric bacteria.

DISCUSSION

In *S. typhimurium* the second promoter was located between the ends of two deletions, one ending late in the PRTase gene and one ending early in the InGPase structural gene (BAUERLE and MARGOLIN 1967). Recently P2 has been mapped in *E. coli* by analysis of short internal deletions in the tryptophan operon (JACKSON and YANOFSKY 1972). This study has indicated that a nucleotide sequence within the PRTase structural gene functions to code for the carboxy-terminal portion of the PRTase molecule and also functions as a transcription initiator. These workers suggested that P2 may have no physiological function as a promoter, but is simply a consequence of the nucleotide sequence of P2 coding for a sequence of amino acids required for a functional PRTase. No specific functional role has been suggested for P2 by any other workers.

TABLE 1. Steady state and repressed activities of tryptophan enzymes

Organism	Trp	ASase	PRTase	PRAIase	InGPase	TSaseB
<i>E. cloacae</i>	+	.09 (40)	.13 (23)	7.53 (5)	.52 (8)	.15 (7)
	-	3.64	3.02	38.20	3.93	1.12
<i>E. dissolvens</i>	+	.33 (29)	.23 (27)	5.00 (9)	.46 (12)	.08 (11)
	-	9.71	6.13	43.00	5.32	.86
<i>C. freundii</i>	+	.17 (42)	.07 (39)	2.24 (17)	.16 (23)	.20 (17)
	-	7.17	2.65	38.50	3.66	3.45
<i>E. carotovora</i>	+	.20 (63)	.06 (53)	4.93 (14)	.29 (16)	.28 (31)
	-	12.50	3.18	68.50	4.74	8.55
<i>C. ballerupensis</i>	+	.35 (11)	.22 (11)	6.97 (5)	.54 (5)	.43 (8)
	-	3.67	2.33	37.20	2.91	3.49
<i>E. hafniae</i>	+	.08 (70)	.10 (55)	8.54 (5)	.77 (4)	.28 (13)
	-	5.61	5.47	40.70	3.27	3.54
<i>S. marcescens</i>	+	.13 (246)	.10 (237)	1.40 (22)	.50 (22)	.19 (28)
	-	32.00	23.70	30.20	11.23	5.40
<i>S. marinorubra</i>	+	.14 (100)	.09 (96)	1.90 (14)	.44 (8)	.23 (11)
	-	14.00	8.60	26.40	3.23	2.60
<i>E. liquifaciens</i>	+	.14 (226)	.14 (124)	1.84 (27)	.69 (21)	.08 (10)
	-	31.70	17.30	50.00	14.70	.80
<i>P. vulgaris</i>	+	.03 (330)	.03 (56)	1.00 (20)	*	.24 (39)
	-	10.00	1.67	20.00	*	9.30
<i>P. morganii</i>	+	.04 (500)	.03 (316)	3.00 (19)	*	.08 (20)
	-	17.50	9.50	56.00	*	1.60

Activities are expressed as nanomoles substrate consumed or product produced/min/mg protein. "+" or "-" under the heading "Trp" indicates cells grown under repressing and non-repressing conditions respectively. Multiplicity of derepression is indicated in parentheses. * indicates not determined because of presence of glycerol in extract.

In the bacteria described here the PRTase molecules are quite different (LARGEN 1972). In *C. ballerupensis*, *C. freundii*, *E. cloacae*, and *E. dissolvens* the PRTase is part of macromolecular complex with ASase, as in the case for *E. coli* (ITO and YANOFSKY 1969), and for *S. typhimurium* (BAUERLE and MARGOLIN 1966b). In the two *Proteus* species, *E. hafniae* and *E. carotovora*, the PRTase activity is not associated with any other enzyme of the pathway and has a molecular weight of 67,000. In *S. marcescens*, *S. marinorubra*, and *E. liquifaciens* the PRTase is not associated with any other activity and it has a molecular weight of 45,000. If the nucleotide sequence of P2 has been conserved solely because it codes for a sequence of amino acids critical to PRTase function (JACKSON and YANOFSKY 1972) then this sequence has been very strongly conserved in diverse PRTase molecules. This argument must be considered in the context of several considerations. It has been shown for several operons that promoters can be

created (WUESTHOFF and BAUERLE 1970) or destroyed (SCAIFE and BECKWITH 1966) by single nucleotide changes. On the other hand the known degeneracy of the genetic code would make it possible to substitute one base for another and still code for the same amino acid (CRICK 1966). In this case it may be that there are nucleotide changes which neither abolish P2 function nor alter the amino acid sequence of the PRTase. Another possibility which should be considered, of course, is that the second promoter is not located in the same position in each of these organisms. If this is the case then P2 has probably been conserved because of a yet unknown physiological function.

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