Induction of Capsular Polysaccharide Synthesis by *p*-Fluorophenylalanine in *Escherichia coli* Wild Type and Strains with Altered Phenylalanyl Soluble Ribonucleic Acid Synthetase

SOOSANG KANG AND ALVIN MARKOVITZ

Department of Microbiology and LaRabida-University of Chicago Institute,
University of Chicago, Chicago, Illinois

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ABSTRACT

Escherichia coli K-12 strain AB259 can be induced to form capsular polysaccharide (mucoid clones) by DL-*p*-fluorophenylalanine (FPA; 5×10^{-6} M on agar plates at 37 C or 8×10^{-6} M in liquid medium at 30 C). The change was shown to be phenotypic. An increase in enzymes probably involved in capsular polysaccharide synthesis [phosphomannose isomerase (3.3-fold), uridine diphosphate-D-galactose-4-epimerase (2.5-fold), and guanine diphosphate-L-fucose synthetase] was demonstrated as a result of growth in FPA. These increases appear sufficient to account for the increased synthesis of capsular polysaccharide due to growth in FPA. FPA-resistant derivatives of strain AB259 were obtained by selecting mutants on FPA-containing agar or by transducing in an altered phenylalanyl soluble ribonucleic acid synthetase that activates FPA poorly. Mucoid clones were formed by these strains only in the presence of 30 to 1,000 times as much FPA. Among these strains, there was a close correlation between incorporation of FPA- C^{14} and induction of capsular polysaccharide synthesis. The results are thus consistent with the following model: FPA is incorporated into the protein product of the R_1 gene (repressor) and alters it sufficiently to allow derepression of several enzymes.

Monod, Changeux, and Jacob (27) have suggested that repressors are allosteric proteins. This view is supported, in the case of R_1 and R_2 regulator genes of the alkaline phosphatase system and in the case of the i gene in the β -galactosidase system, by the finding that mutations of these loci can be suppressed by suppressor mutations that act at the level of translation of messenger ribonucleic acid (mRNA) into protein (3, 4, 6, 17, 18, 28, 32, 33). Physiological studies with a temperature-sensitive R_2 phosphatase-constitutive mutant also suggest that the R_2 region specifies a protein (16).

Kinetic studies of β -galactosidase synthesis with temperature-sensitive i mutations in partial diploid strains of *Escherichia coli* K-12 further indicate that the repressor is an allosteric protein composed of subunits (31). Genetic studies of partial diploid strains of *E. coli* K-12 also led to the suggestion that the product of a regulator gene (R_1) that controls several enzymes involved

in capsular polysaccharide synthesis is a protein composed of subunits (26).

Since some amino acid analogues are incorporated into protein (22, 30) and lead to thermolability of certain enzymes (20, 29), it might be possible to alter selectively the activity of repressors by use of low concentrations of amino acid analogues. The system that controls capsular polysaccharide synthesis was chosen for the present study since mucoid clones, an indication of capsular polysaccharide synthesis, are easily observed. Capsular polysaccharide synthesis is controlled by two regulator genes, R_1 and R_2 . The wild-type strain (R_1^+ , R_2^+) produces clones that are nonmucoid, whereas mutations at either R_1 or R_2 give rise to mucoid clones (26). Several enzymes involved in synthesis of capsular polysaccharide are elevated in extracts of R_1 -type mucoid mutants (24). In contrast, none of these enzymes tested [with the possible exception of guanine diphosphate (GDP)-L-fucose synthetase]

is elevated in extracts of R_2 -type mucoid mutants (Markovitz and Lieberman, *unpublished data*). A preliminary report of the present work has been published (S. Kang and A. Markovitz, *Federation Proc.* 35:328, 1966).

MATERIALS AND METHODS

Bacteria. All strains used are derivatives of *E. coli* K-12. Strain AB259 (an Hfr H prototroph obtained from N. Schwartz) is nonmucoid at 37 and 23 C, and contains R_1^+ and R_2^+ . Most wild-type strains are somewhat mucoid at 23 C, in contrast to strain AB259. Strain PFP10 is a DL-*p*-fluorophenylalanine (FPA)-resistant strain and contains an altered phenylalanyl soluble RNA (sRNA) synthetase that activates FPA poorly (11–13). It was obtained from F. C. Neidhardt.

Media. Penassay Broth (Difco) was used as a complex liquid medium. M9 minimal medium (1) supplemented with 2.5×10^{-3} M CaCl_2 , 10 $\mu\text{g}/\text{ml}$ of thiamine HCl, and 0.6% glucose was used for liquid cultures and was solidified with 1.5% agar for plating experiments.

Chemicals and enzymes. Glucose-6-phosphate (G6P), fructose-6-phosphate, GDP-D-mannose, uridine diphosphate (UDP)-D-galactose, nicotinamide adenine dinucleotide phosphate (NADP), reduced NADP (NADPH₂), nicotinamide adenine dinucleotide (NAD), G6P dehydrogenase, phosphoglucose isomerase (PGI), and UDP-D-glucose dehydrogenase were purchased from Sigma Chemical Co., St. Louis, Mo. G6P dehydrogenase from Boehringer und Soehne GmbH, Mannheim, Germany, was also used. Mannose-6-phosphate was purchased from British Drug House Ltd., Poole, Dorset, England. GDP-4-keto-D-rhamnose (a gift from N. Winkler) was prepared from GDP-D-mannose with an enzyme preparation from strain GS (25) by a modified procedure (N. Winkler, *unpublished data*). P^{32} -labeled sodium pyrophosphate was purchased from the Radiochemical Centre, Amersham, England. FPA and FPA-3- C^{14} (specific activity, 3.5 mc/mmmole) were purchased from Calbiochem, Los Angeles, Calif. L-Leucine-4,5- H^3 (specific activity, 5.0 c/mmmole) was purchased from New England Nuclear Corp., Boston, Mass.

Transduction of FPA resistance. Transduction was carried out with bacteriophage P1kc as described by Lennox (21) except that, after treatment with the transducing phage, the bacteria were allowed to grow in liquid L-broth overnight to permit expression of FPA resistance before plating on selective medium.

Mutation to FPA resistance. Strain AB259 (approximately 5×10^8 cells) was spread on minimal agar that contained 5×10^{-3} M FPA, and a spontaneous resistant mutant (strain AB259-PFP-3) was purified from the clones that appeared.

Release and determination of capsular polysaccharide. Liquid cultures were placed in a boiling-water bath for 15 min. This procedure releases essentially all the capsular polysaccharide from the cells. Cells were removed by centrifugation and the supernatant liquid was dialyzed overnight versus running tap water. Methylpentose was determined by the method of Dische (9) and uronic acid by the method of Dische

and Shettles (10). Most of the capsular polysaccharide is released into the culture medium during growth and, for purposes of qualitative analysis by paper chromatography, was isolated from the medium by dialysis followed by alcohol precipitation.

Qualitative analysis of capsular polysaccharide. Polysaccharide was hydrolyzed for 3.5 hr at 100 C in 1 N HCl. The HCl was neutralized with Dowex 1- HCO_3^- . Paper chromatography was performed in pyridine-ethyl acetate-glacial acetic acid-water (5:5:1:3).

Antisera and immunodiffusion assay of polysaccharides. Antisera to the capsular polysaccharide of mucoid strain 17-9 (26) were prepared with formaldehyde-killed cells (2). Reactions of polysaccharide with antisera were performed by use of agar plates (7). A central well containing antiserum was surrounded by 12 wells (6 mm diameter) at a distance of 10 mm to which 0.05 to 0.1% polysaccharide preparations were added.

Incorporation of FPA-3- C^{14} and leucine-4,5- H^3 into protein. FPA-3- C^{14} and leucine-4,5- H^3 were added to exponentially growing cultures in minimal media at 30 C to a final concentration of 2×10^{-4} and 1×10^{-3} M, respectively. An equal volume of 10% trichloroacetic acid was added to each sample, and the mixture was heated at 90 C for 15 min. The precipitate deposited quantitatively on a membrane (Millipore Filter Corp., Bedford, Mass.) by filtration and washing with 5 ml of 5% trichloroacetic acid. Radioactivity was determined in a Tri-Carb liquid scintillation spectrometer with 0.65% 2,5-diphenyloxazole (PPO)-0.0105% 1,4-bis-2-(5-phenyl-oxazolyl)-benzene (POPOP) in a total volume of 14 ml of toluene.

Protein. Protein was measured by the method of Lowry et al. (23).

Extracts of *E. coli* K-12. Extracts for all enzymatic analyses, except aminoacyl sRNA synthetase, were prepared from cells washed and suspended in 0.05 M $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ (pH 7.0) by sonic disruption in an MSE sonic generator (Measuring & Scientific Equipment, Ltd., London, England) for from 10 to 90 sec followed by centrifugation at $34,000 \times g$ for 20 min. For assay of GDP-L-fucose synthetase (GDP-4-keto-D-rhamnose stimulated NADPH₂ oxidation), 2 mM MgCl_2 and 1 mM ethylenediaminetetraacetate (EDTA) were added to the buffer for washing and suspending the cells prior to sonic disruption. The supernatant fractions were assayed the same day. Extracts for the preparation of crude aminoacyl sRNA synthetases were prepared from cells washed and suspended in 0.1 M tris(hydroxymethyl)aminomethane (Tris) containing 6×10^{-2} M K^+ and 7×10^{-3} M Mg^{++} (pH 7.5) by sonic disruption in a Raytheon 9-kc sonic disintegrator for 20 min followed by centrifugation at $105,000 \times g$ for 60 min.

Enzymatic analyses of *E. coli* K-12. All assays were performed at room temperature (23 C) unless otherwise indicated. Spectrophotometric assays of G6P dehydrogenase, PGI, and phosphomannose isomerase (PMI) all contained 34 mM Tris (pH 7.6), 6 mM MgCl_2 , 0.2 mM NADP, 1 to 10 mM $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$, 0.01 to 0.1 ml of supernatant enzyme, and the indicated additions in final concentrations: for G6P dehydrogenase, 0.12 mM G6P; for PGI, 0.10 mM fructose-6-

phosphate and 0.48 units of G6P dehydrogenase per ml; for PMI, 0.4 mM D-mannose-6-phosphate, 0.48 units of G6P dehydrogenase per ml, and 0.65 units of PGI per ml. NADPH₂ formation was measured at 340 m μ in a Gilford model 2,000 Multiple Sample Absorbance Recorder attached to a Beckman DU monochromator. GDP-D-mannose hydro-lyase and GDP-L-fucose synthetase (GDP-4-keto-D-rhamnose stimulated NADPH₂ oxidation) were measured as described previously (24) except that MgCl₂ concentration was 0.2 mM. UDP-D-galactose-4-epimerase was determined by the two-step procedure (19). Aminoacyl sRNA synthetase was measured by the amino acid-dependent incorporation of pyrophosphate into adenosine triphosphate (ATP; 8, 12). The reaction mixture contained the following components in a total volume of 1 ml: 100 mM Tris (pH 7.5), 10 mM MgCl₂, 10 mM KCl, 2 mM KF, 5 mM ATP, 1 mM L-phenylalanine or 2 mM FPA, 1 mM P³²-pyrophosphate (10⁵ counts/min), and 0.1 ml (0.5 mg of protein) of supernatant enzyme. The mixture was incubated at 37 C for 10 min, and the reaction was terminated by the addition of an equal volume of 10% trichloroacetic acid. Radioactivity was measured in a windowless gas-flow counter (Nuclear Chicago Corp., Des Plaines, Ill.).

RESULTS

Induction of mucoid clones in the presence of FPA. Strain AB259 (R_1^+ , R_2^+) is nonmucoid on minimal agar plates at 37 and 23 C. In contrast, many wild-type strains (R_1^+ , R_2^+) are nonmucoid at 37 C, but are somewhat mucoid at 23 C, whereas mucoid mutants (R_1^- , R_1^e , or R_2 -mucoid) are mucoid on minimal agar plates at 37 or 23 C (24, 26). A plating experiment (Fig. 1 and Table 1) demonstrates that 2.5×10^{-6} M FPA induces the synthesis of capsular polysaccharide in strain AB259 at 37 C with little or no reduc-

tion in number of clones. *o*-Fluorophenylalanine is not effective, whereas *meta*-fluorophenylalanine is slightly effective in inducing polysaccharide synthesis. At lower concentrations of FPA than shown in Table 1 (5×10^{-7} to 1×10^{-6} M), the clones appeared mucoid at the earliest time that they could be observed, but then grew out as nonmucoid clones. The latter result suggests depletion of the medium with respect to FPA.

Replica plating of strain AB259 cells from minimal agar, where they are nonmucoid, to minimal agar with 2.5×10^{-5} M FPA resulted in replication of all clones and all clones were mucoid. Replication back to media without FPA again yielded all nonmucoid clones.

Prevention of the effect of FPA by phenylalanine and tyrosine. Equal concentrations of either L-phenylalanine or L-tyrosine, with respect to FPA, when incorporated into the medium, prevent growth inhibition and induction of mucoidness due to FPA at all concentrations tested (5×10^{-6} to 3.2×10^{-4} M). That is, all clones grow and are nonmucoid. In 10^{-3} M FPA and half that concentration of L-phenylalanine, all clones survive and are nonmucoid. However, in 10^{-3} M FPA and half that concentration of L-tyrosine, all clones survive and are mucoid.

Synthesis of capsular polysaccharide in liquid media. The capsular polysaccharide is composed of D-glucose, D-galactose, D-glucuronic acid, and L-fucose (24). The methylpentose, L-fucose, is readily measured by the cysteine-sulfuric acid method (10), and is a representative structural unit of the polysaccharide. The results presented in Table 2 demonstrate the induction of poly-

FIG. 1. Colony morphology of AB259 in the presence (right) and absence (left) of 10^{-5} M FPA.

TABLE 1. Viability and clone morphology of strain AB259 in the presence of various concentrations of fluorophenylalanines^a

Concn of analogue	No. and type of clones		
	DL- <i>p</i> -Fluorophenylalanine	DL- <i>meta</i> -Fluorophenylalanine	DL- <i>o</i> -Fluorophenylalanine
<i>M</i>			
0	159 NM ^b	—	—
1.25×10^{-6}	—	—	110 NM
2.5×10^{-6}	120 M	—	135 NM
5×10^{-6}	156 M	146 NM	82 NM
1×10^{-5}	129 M	137 M ^c	0 (2 NM)
2×10^{-5}	0 (83 M)	133 NM/M	—
4×10^{-5}	0	158 NM, 5M	—

^a Strain AB259 was grown in Penassay medium overnight, diluted in M9 minimal medium, and plated. Observations were recorded after 60 hr of incubation at 37 C except for those in parentheses, which were recorded after 100 hr.

^b NM, M, and NM/M refer to nonmucoid clones, mucoid clones, and nonmucoid clones with small mucoid centers, respectively.

^c Mucoid appearance was not typical.

TABLE 2. Effect of FPA on the synthesis of capsular polysaccharide in liquid cultures of strain AB259^a

Expt	Type of strain	Concn of FPA	Final optical density at 600 m μ	Production of polysaccharide ^b
I	Nonmucoid type	<i>M</i>		
		0	0.8	9.4
		2.5×10^{-6}	0.7	5.7
		1×10^{-5}	0.66	65.0
II	Nonmucoid type <i>R</i> ₁ -type mucoid mutant	8×10^{-5}	0.47	133.0
		0	0.43	180.4
		0	0.53	550.0
		0	0.53	550.0

^a Cells were grown aerobically in minimal medium with various concentrations of FPA at 30 C. Optical density at 600 m μ was adjusted to 0.01 at the beginning of growth, and nondialyzable methylpentose and final optical density were determined after 22 hr of incubation.

^b Expressed as micrograms of nondialyzable methylpentose per milliliter of medium per unit of optical density.

saccharide synthesis by FPA in liquid cultures growing at 30 C on a reciprocal shaker. The increase in the *R*₁⁺ strain due to growth in FPA is 14-fold, and approximately one-third as much polysaccharide is synthesized by the wild type (*R*₁⁺) in the presence of FPA as in an *R*₁-type

mucoid mutant of the same strain. Figure 2 shows that polysaccharide synthesis begins several hours after cells are placed in medium that contains FPA, but growth starts at an earlier time.

Chemical and immunological identity of FPA-induced polysaccharide and polysaccharide from a mucoid mutant. Paper chromatography of acid hydrolysates showed that the polysaccharide induced by PFP, and that from a mucoid mutant, contained glucose, galactose, and fucose in similar proportions. Uronic acid was present in similar amounts in both, as determined by the molar ratio of methylpentose to uronic acid (2:1). Agar diffusion studies, performed with antisera against a vaccine of a mucoid strain, demonstrated that the polysaccharide induced by FPA and that from a mucoid mutant behaved as a single antigen and were indistinguishable.

Effect of FPA on synthesis of enzymes in strain AB259. The following enzymatic reactions were studied:

- (1) glucose-6-phosphate + NADP $\rightarrow$ 6-phosphogluconolactone + NADPH₂
- (2) fructose-6-phosphate $\rightarrow$ glucose-6-phosphate
- (3) mannose-6-phosphate $\rightarrow$ fructose-6-phosphate
- (4) GDP-D-mannose $\rightarrow$ GDP-4-keto-D-rhamnose + water
- (5) GDP-4-keto-D-rhamnose + NADPH₂ $\rightarrow$ GDP-L-fucose + NADP
- (6) UDP-D-galactose $\rightarrow$ UDP-D-glucose

Reaction 1 is catalyzed by G6P dehydrogenase; reaction 2, by PGI; reaction 3, by PMI; reaction 4, by GDP-D-mannose hydro-lyase; reaction 5, by GDP-L-fucose synthetase (GDP-4-keto-D-rhamnose stimulated NADPH₂ oxidation); and reac-

FIG. 2. Production of capsular polysaccharide by strain AB259 in the presence of 8×10^{-5} M FPA. Experimental procedures are the same as in Table 2.

tion 6, by UDP-D-galactose-4-epimerase. Previous results (24; Markovitz, *unpublished data*) demonstrated that the enzymes catalyzing reaction (3 through 6) were increased in specific enzymatic activity 7- to 10-fold in crude extracts of R_1 -type mucoid mutants as compared with the nonmucoid strains. In contrast, enzymes catalyzing reactions 1 and 2 were present in similar quantities in the mucoid and nonmucoid strains (24). Table 3 presents the results of specific enzymatic activity measurements on these six reactions in freshly prepared supernatant enzyme fraction of strain AB259 grown in the presence or absence of 8×10^{-5} M FPA. The increase in specific enzymatic activity due to growth in FPA is 2.5 for UDP-D-galactose-4-epimerase and 3.3 for PMI (Table 3). The results with GDP-L-fucose synthetase have been variable. In our best experiment (Table 3, experiment II, in which the highest activities were found) there was a 12-fold increase in specific enzymatic activity due to growth in FPA. In experiment I (Table 3), enzymatic activity was found in cells grown with but not without FPA. GDP-D-mannose hydro-lyase, G6P dehydrogenase, and PGI do not appear to be affected by growth with FPA (Table 3). Thus, three of the four enzymes that are elevated in R_1 -type mucoid mutants are elevated by growth in FPA.

Induction of capsular polysaccharide and metabolism of FPA in FPA-resistant derivatives of strain AB259. Strains AB259-PFP-1 and AB259-PFP-2 (obtained by transduction to FPA resist-

ance with strain PFP-10 as a donor and AB259 as a recipient) and strain AB259-PFP-3 (a spontaneous FPA-resistant mutant of AB259) require high concentrations of FPA to convert them to mucoid clones (Table 4, from 1.6×10^{-4} to 5×10^{-3} M).

The resistant derivatives of strain AB259 obtained by transduction with PFP-10 as a donor were expected to contain the altered phenylalanyl sRNA synthetase that activates FPA poorly, since this alteration appears to be the mechanism of resistance to FPA in strain FPA-10 (11-13). The results of L-phenylalanine and FPA-dependent pyrophosphate-ATP exchange studies indicate that this is true (Table 5). However, the resistant mutant (strain AB259-PFP-3) appears to contain a normal phenylalanyl sRNA synthetase as measured by the pyrophosphate-ATP exchange reaction (Table 5).

The resistant strains and strain AB259 were tested for incorporation of FPA- C^{14} into hot trichloroacetic acid-insoluble material (protein and cell wall fraction) by use of leucine- H^3 to measure "normal" synthesis. The results (Fig. 3) show that FPA incorporation is reduced in all resistant strains. Strain AB259-PFP-2 requires the highest FPA concentration to convert it to mucoid (Table 4) and incorporates FPA least readily (Fig. 3); strains AB259-PFP-1 and -PFP-3 become mucoid at lower FPA concentrations (Table 4) and incorporate FPA more readily than strain AB259-PFP-2 (Fig. 3). Cross-feeding

TABLE 3. Effect of *p*-fluorophenylalanine on synthesis of enzymes in *Escherichia coli* strain AB259

Enzyme	Specific enzymatic activity ^a					
	Expt I		Expt II		Expt III	
	-FPA	+FPA	-FPA	FPA+	-FPA	+ FPA
Glucose-6-phosphate dehydrogenase	6.5	6.7			8.0	6.2
Phosphoglucose isomerase	10	15			9.5	5.5
Phosphomannose isomerase	1.0	2.6	0.71	3.0		
GDP-D-mannose hydro-lyase	0	0.03			0.02	0.015
GDP-L-fucose synthetase	0	0.061	0.021	0.26		
UDP-galactose-4-epimerase	0.5 ^b	1.3 ^b			0.6	1.5
Polysaccharide ^c	3.9	210	75	430	22	200
Final growth (optical density at 600 $m\mu$) ^d	0.28	0.39	0.68	0.25	0.63	0.28

^a Expressed as micromoles of substrate disappearing or product appearing per hour per milligram of protein.

^b Data are from a separate experiment in which PMI, but not G6P dehydrogenase and PGI, was also elevated.

^c As micrograms of nondialyzable methylpentose per milliliter per unit of optical density at 600 $m\mu$.

^d Cells from an overnight culture were inoculated (initial optical density at 600 $m\mu$ = 0.01) and grown shaking at 30 C with or without 8×10^{-5} M FPA.

TABLE 4. Induction of mucoidness by FPA in strain AB259 and its FPA-resistant derivatives^a

Strain	Minimal FPA for mucoidness	Viability (% survivors)
AB259.....	5×10^{-6}	85
AB259-PFP-1		
Clone 1.....	1.6×10^{-4}	61
Clone 2.....	6.4×10^{-4}	28
AB259-PFP-2		
Clone 1.....	$>2.5 \times 10^{-3}$	22
Clone 2.....	$>2.5 \times 10^{-3}$	47
AB259-PFP-3.....	6.4×10^{-4}	87

^a Bacteria, grown overnight in minimal medium, were diluted and plated (160 cells) on minimal agar containing varying concentrations of FPA. Plates were incubated at 37 C, and results are recorded after 60 to 80 hr of incubation.

TABLE 5. Phenylalanine- and FPA-dependent exchange of pyrophosphate into ATP in enzymes of AB259 and its FPA-resistant derivatives

Strain	Supplement	Radio-activity in ATP ^a	Ratio (FPA/phenylalanine)
		counts/min	
AB259	Phenylalanine	780	0.23
	FPA	180	
AB259-PFP-1	Phenylalanine	580	<0.01
	FPA	0	
AB259-PFP-2	Phenylalanine	540	0.057
	FPA	31	
AB259-PFP-3	Phenylalanine	810	0.16
	FPA	130	

^a Data were corrected by subtracting the exact counts per minute incorporated into ATP in the absence of added L-phenylalanine or FPA (approximately 150 counts/min). Reported counts per minute represent 5% of the total ATP, i.e., the sample counted.

studies indicate that none of the resistant strains excretes phenylalanine. The difference in the kinetics and extent of FPA-¹⁴C incorporation between the two transductants that contain the altered phenylalanyl sRNA synthetase (AB259-PFP-1 and -PFP-2) is worth noting. It may be caused by a difference in endogenous synthesis of phenylalanine in the presence of FPA (due to repression or feedback inhibition by FPA), or to a difference in concentration of the altered phenylalanyl sRNA synthetase.

FIG. 3. Incorporation of FPA-¹⁴C and leucine-³H into protein in strain AB259 and its FPA-resistant derivatives. FPA-¹⁴C (2×10^{-4} M, 1 μ C/ μ mole) and leucine-³H (10^{-3} M, 1 μ C/ μ mole) were added to bacteria growing exponentially at 30 C in 5 ml of minimal medium (optical density at 600 m μ = 0.1). Samples were taken at intervals for 2 hr, and protein (+ cell wall fraction) was isolated as described in Materials and Methods.

DISCUSSION

Induction of capsular polysaccharide synthesis in *E. coli* K-12 can result from mutation of either of two loci (*R*₁ or *R*₂) (24, 26) but enzymes of polysaccharide synthesis (PMI, UDP-D-galactose-4-epimerase, GDP-D-mannose hydro-lyase and GDP-L-fucose synthetase) are elevated 7- to 10-fold only in *R*₁-type mucoid mutants (24; Markovitz, unpublished data). *R*₂-type mucoid mutants may have elevated levels of GDP-L-fucose synthetase (Markovitz and Lieberman, unpublished data). Growth in medium that contains appropriate concentrations of FPA also derepresses capsular polysaccharide synthesis. This change is phenotypic, since it occurs in all cells, is reversed upon growth without FPA, and is prevented by the simultaneous presence of phenylalanine or tyrosine.

Four enzymes are elevated in *R*₁-type mucoid mutants and three of these were elevated (UDP-D-galactose-4-epimerase, PMI, and GDP-L-fucose synthetase) due to growth of nonmucoid strain AB259 in FPA (Table 3). These increases appear sufficient to account for the increased synthesis of capsular polysaccharide. One complicating factor in attempts to demonstrate increased enzyme synthesis may be that incorporation of FPA into these proteins may affect their specific enzymatic activity or their stability. Regulator genes for UDP-D-galactose-4-epimerase have been described that are located in the *Gal* region and the

lysine region of the chromosome (5), but no effect of these regulators on polysaccharide synthesis or on other enzymes involved in polysaccharide synthesis was reported. Therefore, it seems likely that derepression of capsular polysaccharide synthesis by FPA is due to its effect on the product of the R_1 gene that controls the synthesis of enzymes involved in capsular polysaccharide synthesis.

The strains that are resistant to FPA because of an altered phenylalanyl sRNA synthetase (strains AB259-PFP-1 and -PFP-2) are not mucoid in low concentrations of FPA, activate FPA poorly, and incorporate little FPA into the protein fraction. They do become mucoid at high concentrations of FPA. These results suggest that formation of FPA-sRNA or some subsequent step in protein synthesis is necessary for derepression by FPA. Strain AB259-PFP-3 is FPA-resistant, is permeable to FPA, is not mucoid at low concentrations of FPA, and incorporates FPA poorly, but it does activate FPA. Therefore, activation of FPA is not sufficient for derepression of polysaccharide synthesis, and some subsequent step in protein synthesis may be involved (i.e., the incorporation of the analogue into protein). It is well demonstrated that enzymes are altered by the incorporation of various amino acid analogues. β -Galactosidase that has incorporated fluorotyrosine is thermolabile (20, 29). Similarly, induction of capsular polysaccharide synthesis by FPA is greater at 30 to 37 C on agar than at 23 C with strain AB259. The results are thus consistent with the following model. FPA is incorporated into the protein product of the R_1 locus (repressor) and alters it sufficiently to allow derepression of some enzymes. Recent experiments demonstrated that FPA also derepresses alkaline phosphatase synthesis 100-fold in *E. coli* strain PR1 (Kang and Markovitz, *to be published*), a strain with a temperature-sensitive alkaline phosphatase R_2 regulator gene (15). Taken together, these results support the view, obtained from studies with suppressors (see introduction), that the products of regulator genes (repressors) are proteins.

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