Vol. 98, No. 3, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 12, 1981 Pages 693-700

REDUCED LACTOSE OPERON EXPRESSION IN AN *E. coli* MUTANT LACKING LEUCYL-tRNA:PROTEIN TRANSFERASE

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Received December 12,1980

SUMMARY: A mutant of *Escherichia coli* deficient in L-leucyl-tRNA:protein leucyl transferase was found to have reduced specific activities for all three lactose operon proteins when compared to its parent or revertant strains. The effect was most pronounced in cells grown with glucose as the carbon source. Under these conditions, there was an additional polar effect, in which the distal gene product showed the greatest reduction in activity.

Aminoacyl-tRNA:protein transferases are soluble enzymes that catalyze the ribosome-independent addition of single amino acids to the NH_2-terminus of suitable protein or peptide substrates (1). The bacterial enzyme leucyl-tRNA:protein leucyl transferase (EC 2.3.2.6) catalyzes the transfer of leucine, phenylalanine, or methionine from tRNAs to substrates possessing arginine or lysine as the NH_2-terminal residue (2,3). A mutant of *Escherichia coli* K-12 lacking transferase activity has been isolated (4) and shown to exhibit a complex phenotype (5,6). Defects include an abnormal cell morphology, increased production of the iron chelating compound enterochelin, and altered specific activities of proline dehydrogenase, tryptophanase, and phenylalanyl-tRNA synthetase.

Although it was initially predicted that an alteration in specific activity would reflect increased or decreased catalytic activity resulting from lack of modification by the transferase, the three enzymes listed have been found not to be substrates for the transferase (6,7). In the case of proline dehydrogenase, it has been demonstrated by radioimmunoassay that the increased specific activity observed *in vivo* is due to an increased number of proline dehydrogenase molecules (7). These results suggest that

0006-291X/81/030693-08$01.00/0

many of the effects of transferase deficiency are produced indirectly, and that an important substrate for the transfer reaction may be a protein involved in the processes of transcription or translation.

In order to identify this protein, it is necessary to use a system that is well characterized both genetically and biochemically. The lactose (*lac*) operon in *E. coli* is one such system and appears to be particularly suitable for studying the effects of the transferase because the NH_2-terminal sequences of all three proteins coded for by the operon are known. The terminal sequences of β-galactosidase, the *lac* permease, and thiogalactoside transacetylase are Thr-Met-Ile-Thr-Asp- (8), Met-Tyr-Tyr-Leu-Lys- (9), and Asx-Met-Pro-Met-Thr- (10), respectively. None of these are consistent with modification by the transferase. An alteration in the specific activity of one of these proteins would therefore reflect an indirect effect similar to that previously observed.

MATERIALS AND METHODS

Strains and culture conditions. The transferase-deficient mutant (MS845), its parent (W4977), and the revertant R18 have been previously described (4). POR17 is another revertant isolated by Tam *et al.* (11). All strains carry the proA3 deletion and the lac-85 deletion. Pro^+Lac^+ derivatives of each strain were made by introduction of an F' episome (F128) carrying both the *pro* and *lac* genes. The donor strain (E5014) was obtained from Dr. Barbara Bachmann (Coli Genetics Stock Center) and mating was carried out as described by Miller (12). Cells were grown in Medium A minimal salts (12) supplemented with carbon sources as specified below. Where required, isopropyl-β-D-thiogalactoside (IPTG, Sigma Chemical Co.) was added as an inducer of the *lac* operon.

Assays. All assays were performed on cells grown to mid-exponential phase. β-galactosidase activity was determined in whole cells made permeable to the substrate o-nitrophenyl galactoside (Sigma Chemical Co.) by treatment with chloroform and sodium dodecyl sulfate. Units of activity are expressed as described in Miller (12). *Lac* permease activity was measured by the uptake of (D-glucose-1-^{14}C)lactose (Amersham Corporation, 1μCi/μmole). Exponential phase cells were suspended in Medium A containing 150μg/ml chloramphenicol at a density of 50 Klett Units (red filter). Cells (100μl) and (^{14}C) lactose (100μl) were mixed and incubated at 37°C for 15-120 seconds. After dilution with 5 ml of warm Medium A containing 150μg/ml chloramphenicol, the cells were collected on nitrocellulose filters (Gelman GN-6, 0.45μm pores). Radioactivity was determined in a liquid scintillation counter and *lac* permease activity expressed as nmoles lactose taken up per min. per Klett unit of cells. Thiogalactoside transacetylase activity was measured in

Table 1

Specific activities of β-galactosidase in *E. coli* strains

carbon source	strain			
	W4977/F128	MS845/F128	R18/F128	POR17/F128
glycerol	6355	5911	7192	6998
gluconate	2737	2202	2630	2687
glucose	2311	996	2525	2522
glucose-6-phosphate	1402	1389	1375	1201
glucose + gluconate	968	580	793	929

Cultures were grown to mid-exponential phase in Minimal Medium A containing 0.5% (W/V) of the carbon source(s) indicated and 10^{-3}M IPTG. β-galactosidase activity was measured in permeabilized whole cells. Data are the averages of two to three separate experiments.

alumina extracts as described by Alpers, *et al.* (13). Protein concentration was determined by the method of Lowry, *et al.* (14), using bovine serum albumin as the standard, and specific activity expressed as μmoles of Coenzyme A consumed per min. per mg protein.

RESULTS

The parental (W4977), mutant (MS845), and revertant strains (R18, POR17) used in these studies have a complete deletion of the lactose operon. An F' episome carrying the *lac* genes (F128) therefore was introduced by conjugation into each strain. The specific activity of β-galactosidase was determined first after growth in the presence of the gratuitous inducer IPTG and various carbon sources (Table 1). For each strain, maximal activity was observed with glycerol as the carbon source and the β-galactosidase level decreased with growth conditions producing varying degrees of catabolite repression (15). The greatest difference between the transferase-deficient mutant and its parent was observed with glucose as the carbon source. Here the mutant exhibited only 40% of the wild type activity.

Activity was restored to normal in the two revertants that have reacquired transferase activity. Smaller differences between the mutant and its parent were found with gluconate (82%) or gluconate and glucose (65%) as carbon sources. No difference was observed after growth on glucose-6-phosphate.

To determine if these differences in the fully-induced levels of β-galactosidase were related to the rate of enzyme synthesis, the kinetics of β-galactosidase induction were examined with glucose or glycerol as carbon sources (Figure 1). Both strains exhibited a low basal activity and rapidly initiated synthesis of β-galactosidase upon exposure to the inducer IPTG. The time required for the appearance of the enzyme was 2.5 minutes with either carbon source. The rate of β-galactosidase synthesis in the mutant during growth on glucose was 23% of that in the parental strain. The rate of synthesis was also reduced (31%) with glycerol as the carbon source.

The specific activities of the other proteins of the *lac* operon then were determined after growth in the presence of IPTG and either glucose or glycerol (Table 2). With glycerol as the carbon source, the specific activities of all three *lac* proteins in the transferase-deficient mutant were about 90% of those found in the parental or revertant strains. A much greater reduction in activity was found with glucose as the carbon source, and this reduction exhibited a polarity related to the order of the genes within the *lac* operon. While the specific activity of β-galactosidase (the lacZ product) in the mutant was about 40% of the wild-type level, the specific activity of the *lac* permease (the lacY product) was only 25% of that in the parental or revertant strains. The level of thiogalactoside transacetylase (the lacA product) was reduced further to 16% of the wild type activity.

DISCUSSION

The data in this paper indicate that *lac* operon expression was reduced in the transferase-deficient mutant of *E. coli*. All three *lac* proteins

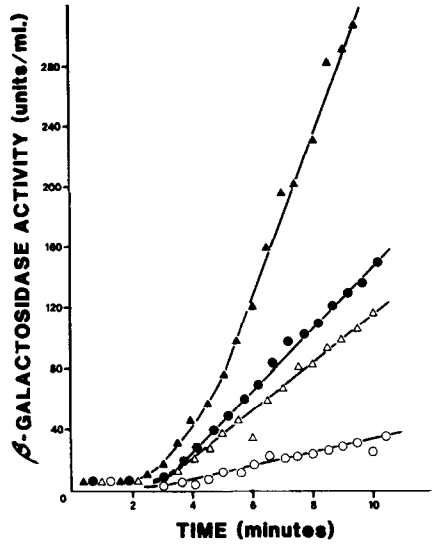

Figure 1. Kinetics of β-galactosidase synthesis in *E. coli* strains. Cultures were grown to mid-exponential phase in minimal Medium A containing either 0.5% glucose or 0.5% glycerol. Induction was initiated by addition of 10^{-3}M IPTG and 0.5ml aliquots removed at 30 second intervals for determination of β-galactosidase activity. W4977/F128 grown in glycerol (▲), MS845/F128 grown in glycerol (△), W4977/F128 grown in glucose (●), MS845/F128 grown in glucose (○).

were affected, particularly with glucose as the carbon source, even though the susceptibility of the system to induction by IPTG or catabolite repression was essentially normal. The relatively small differences in specific activities between the mutant and the wild types were similar to those previously observed for other enzymes (5,6). Since all three *lac* proteins are synthesized from a common polycistronic mRNA (16), and cannot be substrates for the transferase themselves, the results suggest that the mutant has a defect in the transcription, stability, or translation of messages.

Perhaps the most interesting observation here is the increased polarity in gene expression found in the mutant during growth on glucose. This polarity might reflect premature termination during transcription, increased

Table 2

Specific activities of *lac* operon proteins

carbon source	strain	β-galactosidase	*lac* permease	thiogalactoside transacetylase
glycerol	W4977/F128	6355	0.76	16.7
	MS845/F128	5991	0.68	15.6
	R18/F128	7192	0.68	16.7
	POR17/F128	6998	1.01	28.0
glucose	W4977/F128	2311	0.083	5.9
	MS845/F128	996	0.020	0.9
	R18/F128	2325	0.095	6.3
	POR17/F128	2522	0.100	8.0

Cultures were grown to mid-exponential phase in minimal Medium A containing 0.5% of the carbon source indicated and 10^{-3}M IPTG. Activites were determined as described in Methods.

degradation of the distal portions of the mRNA, or diminished translation of the lacY and lacA gene transcripts. Ullman, Joseph, and Dauchin (17) have presented evidence that much of the natural polarity of the *lac* operon is dependent on cyclic AMP, the catabolite activator protein (CAP), and the transcription termination protein rho. The cAMP-CAP complex is thought to have an anti-termination effect on rho, and the increased polarity found in the transferase-deficient mutant might be related to one of these factors. Although the NH_2-terminal sequence of the CAP is inconsistent with its being a substrate for the transferase (15), the NH_2-terminus of rho has not yet been determined. It is possible that failure to modify this latter protein in the mutant results in an increased frequency of termination. Alternatively, the mutant might be partially defective in the synthesis of cAMP. This could explain why polarity is accentuated during growth with a

carbon source such as glucose. Under these conditions, intracellular cAMP levels would be reduced, further diminishing the anti-termination effect and increasing polarity.

ACKNOWLEDGEMENT

This work was supported by a grant from the Kalamazoo College Faculty Development Committee.

REFERENCES

1. Deutch, C.E., Scarpulla, R.C., and Soffer, R.L. (1978) Curr. Topics Cell. Regula. 13, 1-26.

2. Leibowitz, M.J., and Soffer, R.L. (1970) J. Biol. Chem. 245, 2066-2073.

3. Scarpulla, R.C., Deutch, C.E., and Soffer, R.L. (1976) Biochem. Biophys. Res. Commun. 71, 584-589.

4. Soffer, R.L., and Savage, M. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 1004-1007.

5. Deutch, C.E., and Soffer, R.L. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 405-408.

6. Deutch, C.E., Scarpulla, R.C., Sonnenblick, E.B., and Soffer, R.L. (1976) J. Bacteriol. 129, 544-546.

7. Scarpulla, R.C., and Soffer, R.L. (1979) J. Biol. Chem. 254, 1724-1725.

8. Zabin, I., and Fowler, A.V. (1972) J. Biol. Chem. 247, 5432-5435.

9. Büchel, D., Gronenborn, B., and Müller-Hill, B. (1980) Nature 283, 542-545.

10. Zabin, I., and Fowler, A.V. (1978), in The Operon (Miller, J.H., and Reznikoff, W.S., eds.), Cold Spring Harbor Laboratory, New York, pp. 89-121

11. Tam, A., Herrington, M.B., Kapoor, V. and Newman, E.B. (1978) J. Bacteriol. 135, 1154-1155.

12. Miller, J.H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, New York.

13. Alpers, D.H., Appel, S.H., and Tompkins, G.H. (1965) J. Biol. Chem. 240, 10-16.

14. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem., 193, 265-275.

15. Pastan, I., and Adhya, A. (1976) Bacteriol. Rev. 40, 527-551.

16. Contesse, G., Crepin, M., and Gros, F. (1970), in The Lactose Operon (Beckwith, J.R. and Zipser, D., eds.), Cold Spring Harbor Laboratory, New York, pp. 111-137.

17. Ullman, A., Joseph, E., and Danchin, A. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 3194-3197.