THE RELATIONSHIPS IN BIOSYNTHESIS OF THE β-GALACTOSIDASE- AND Pz-PROTEINS IN *ESCHERICHIA COLI**

by

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INTRODUCTION

It has been shown that normal strains of *E. coli* synthesize the enzyme β-galactosidase in response to the presence of a specific inducer (certain galactosides with intact ring structure)[1]. Extensive investigations have established that the appearance of β-galactosidase activity corresponds to the formation of a new protein quantitatively identifiable with the enzyme[2,3,4,5]. Following the purification and immunochemical characterization of β-galactosidase, it was demonstrated that the formation of this enzyme involves the synthesis of a distinct antigenic component (Gz) absent from cells that have not been grown in the presence of an inducing galactoside.

However, extracts from non-induced cells, which contain no Gz, possess a component (Pz) which cross-reacts specifically with antibody to the enzyme. This cross-reacting protein is also present in extracts from induced cells containing Gz. The large degree of the cross reaction and the fact that the solubility properties of the Pz and Gz proteins are extremely similar, indicates that they are structurally very closely related, so much so that these two proteins have been difficult to differentiate. However, the Pz protein is devoid of β-galactosidase activity and the two proteins have been found to be physically separable either by specific precipitation with antibody under certain conditions, or on the basis of differential solubility at certain temperatures[6]. Moreover, the Pz protein is not antigenic, while Gz is a very active antigen, at least in the rabbit; Pz is destroyed by trypsin under conditions where Gz is resistant.

The very close similarity of the physical and immunochemical properties of these two proteins suggests that they are related in their biosynthesis. If so, interaction and/or interdependence in their synthesis by the cells should be expected. The observations reported here bear on this point. They have been carried out with the aid of special immunological methods, (described in preceding publications) which permit the simultaneous titration of Gz and Pz in bacterial extracts[2-4].

MATERIALS AND METHODS

Strains

The ML strain of *E. coli* (MONOD ET AUDUREAU[7]) and a series of spontaneous or induced mutants

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References p. 289.

derived from it, and differing from it by loss or gain of the capacity to utilize specific sugars, were used.

The K-12 strain of *E. coli* was used in certain experiments. Hereafter the mutant strains of ML will be designated by a number referring to a given clone and by symbols referring to their capacity for utilizing specific sugars as carbon and energy source. The symbols are: M for maltose, L for lactose and G for galactose. The (+) sign indicates utilization, and a (—) sign non-utilization of the sugar.

Media

All cultures were grown in the synthetic medium 56 already described[2]. The carbon source was added separately, and is specified for each experiment.

Method of culture

The technic of continuous culture described by MONOD[8] was employed. The theoretical and practical reasons for the selection of this method are discussed in the reference cited.

In general the following conditions were employed. 500 ml of culture at cell concentrations of approximately 10^9 bacteria per ml (0.7 mg dry weight/ml) were placed in a revolving 2 liter flask and stabilized in continuous culture for periods between 3–5 hours. At various times after stabilization, samples of the culture were taken for analysis. When L^- or G^- mutants were used, the cultures were analyzed for their content in reversion mutants L^+ and G^+ to be certain that there had been no significant populational change during the course of the experiment. The specific conditions for each experiment are indicated separately with the tables and diagrams.

Preparation of extracts for analysis

The bacterial suspension was drawn out of the continuous culture apparatus on to finely cracked ice. The temperature fell to 0° C almost immediately. The culture was centrifuged, washed once with *M*/10 triethanolamine phosphate buffer (pH 7.0), and the cells, resuspended in the same buffer, were broken up by grinding with sand in a rapid shaker (9). The extracts were centrifuged for 1 hour at 12,000 r.p.m. in the Sorvall SS-1 centrifuge, to give clear extracts. All operations were carried out in the cold. Extracts from induced organisms, *i.e.*, which contained more β-galactosidase than the trace amount (about 0.5 μM/h/mg N niphegalase (see below) are referred to as, *active extracts*. Conversely, extracts containing less than a trace of niphegalase (from non-induced organisms, *i.e.* grown on glucose, maltose, fructose, etc. . . . from mutants incapable of synthesizing β-galactosidase) will be referred to as *inactive extracts*.

The protein nitrogen in these extracts was determined after precipitation with 5% trichloracetic acid overnight. A micro-Kjeldhal analysis was carried out on the well-washed precipitates.

Determination of enzymic activities

The lactase and amylomaltase activities were carried out by determining the glucose liberated with notatin as described previously[2,9]. The niphegalase activity was determined by measuring spectrometrically at λ-420 mμ *ortho*-nitrophenol liberated from *ortho*-nitrophenol-β-D-galactoside. All activities are expressed as μM/h at 28° C, measured at pH 7.0 in the presence of potassium ions for lactase and of sodium ions for niphegalase (1). All specific reactants were maintained at saturating concentrations for the enzyme. It should be recalled that the "lactase" and "niphegalase" activities are associated with the single enzyme β-galactosidase. Under the above conditions of determinations, the activity ratio $\frac{\text{niphegalase}}{\text{lactase}}$ was equal to 3.6 ± 0.18, with all the strains and mutants, and whichever type of preparation was used.

Immunological assays for Pz and Gz

These technics have been described in detail previously[4], and will be briefly reviewed here. When increasing amounts of a mixture of Pz and Gz are precipitated by a given fixed volume of anti-enzyme sera, a point is reached where all of the Pz, Gz, and antibody to Gz are no longer detectable in the supernatant. This condition defines the equivalence point T, and determines the total antigen Tz which is the sum of Pz and Gz.

The immunological titration of Gz depends upon the higher reactivity of Gz than Pz with antibody to Gz. In mixtures, Pz precipitates only after the preferential precipitation of Gz is complete. At the point where only a trace of excess β-galactosidase is detectable in a supernatant, the separation of Gz and Pz is effected, and the antibody to Gz is precipitated by Gz alone. This condition is referred to as equivalence point G and determines the quantity of Gz present. The difference between the concentrations of Tz and Gz is Pz. The data are expressed in *combining units* per μg N as defined arbitrarily by assigning to a fixed volume of antiserum the value of 100 units.

For inactive extracts the titration for Pz is carried out by determining equivalence point P where all of the Pz and all of the anti-enzyme precipitable by Pz, is precipitated. For 100 units antiserum this points determines 100 units Pz.

References p. 289.

All of the preparations of Gz from *Enterobacteriaceae* (see Table III) titrated to the same end-point G, a fact which shows these various β-galactosidases to be immunologically identical.

EXPERIMENTAL

THE BEHAVIOR OF Pz DURING THE SYNTHESIS OF Gz

a. *Effects of nitrogen starvation on the synthesis of Pz and Gz.* The simplest hypothesis concerning the relationship between the proteins Pz and Gz in biosynthesis is that Pz is the precursor of Gz. If the synthesis of Gz could be induced under conditions preventing the concomitant synthesis of Pz, one should, according to this hypothesis, find that Pz decreases in proportion to the amount of Gz formed. Such conditions appear to be difficult or impossible to achieve. We have not been able to discover any agent or condition that would *electively* prevent the synthesis of Pz in otherwise normally metabolizing cells. The only available method, therefore, appeared to be to prevent indiscriminately the synthesis of new protein by depriving the cells of a source of nitrogen.

Suspensions of L^+ cells (ML 32400) when thoroughly washed by centrifugation, and resuspended in synthetic medium containing an energy source and an inducer, but lacking the nitrogen (NH_4^+) source, did synthesize some β-galactosidase, but only to a very low level (about 1/50th of a control suspension in the presence of N). Furthermore, if the nitrogen source was eliminated, not by the usual method of washing by centrifugation, but rather by letting the cells themselves consume all the available nitrogen, in the presence of an excess of other essential nutrients, then the cells were found unable to synthesize any detectable amount of enzyme.

These nitrogen-starved cells, however, contained normal levels of the Pz protein. For example in one experiment ML 32400 ($M^+L^+G^-$) in the presence of excess maltose, before nitrogen starvation, showed 41 units Pz/mg N, after 1 h starvation at 37° C 38 units Pz/mg N and after 24 h starvation 52 units Pz/mg N. The figures would even suggest that cells, starved for a long time, contained a slightly increased Pz fraction. Therefore, the inability of nitrogen-deprived cells to synthesize β-galactosidase cannot be attributed to the absence, in these cells, of the presumed precursor. These negative results appear to contradict the simplest hypothesis according to which the synthesis of Gz consists of a reorganization of material pre-existing as Pz. They seem to indicate that Gz is formed partially or entirely *de novo*. However, it is well known that induced enzyme synthesis is linked to cellular metabolism, in particular to energy-transferring reactions. It is quite conceivable that certain critical steps in this metabolism may be affected by such a drastic condition as complete nitrogen starvation, which can hardly be considered as preventing only *de novo* protein synthesis. Therefore no final conclusions could be drawn from such observations alone. Clearly the synthesis of Gz, and its effects on Pz levels, had to be studied with normally metabolizing, *i.e. growing cells.*

β-GALACTOSIDASE AND Pz LEVELS IN GROWING CELLS

In a cellular population growing at a constant rate, in a constant environment, the composition of the cells may be expected to reach a stable state, in which the level of any one constituent depends on its relative net rates of synthesis and disappearance.

References p. 289.

In a population of *E. coli* growing at a constant rate in the absence of inducer, the Pz protein should be maintained at a given level. If an inducer is added, β-galactosidase will begin to be synthesized, and should eventually also reach a constant level. If Pz and Gz are interrelated in their synthesis, (*i.e.* if the synthesis of Gz results in either increasing the rate of disappearance, or decreasing the rate of synthesis of Pz) the stable-state level of Pz should be lower when Gz is also synthesized.

These expectations have been tested in a variety of experiments in which we used the continuous-culture principle developed by MONOD[8], and NOVICK AND SZILARD[11], which insures reproducible stable-state conditions for a growing culture, and permits control of the growth-rate.

For a general discussion of this method, we refer to the above-mentioned publications. It may be recalled very briefly that the method consists in constantly diluting a growing culture with new medium, while maintaining a constant volume by drawing out an equivalent amount of liquid from the culture. It is easily shown that, provided the dilution rate does not exceed a critical value (corresponding to the maximal growth rate of the organism in the presence of excess nutrient), a stable state is reached in which the composition of the medium is constant, and the growth rate is equivalent to the dilution rate, controlled by the experimenter.

The experiment described in Fig. 1 illustrates the general result obtained concerning the relationship of Pz and Gz levels, as well as some of the significant features of the method. In this experiment, a culture of ML 30 was maintained at a constant growth rate and constant density in a medium containing maltose, the sole carbon source, as limiting factor of the growth. Melibiose, which is not metabolized by the bacteria, nor split by the enzyme, was used as an inducer. The levels of Pz and Gz (immunological titration), β-galactosidase and amylomaltase (activity measurements) were determined on extracts of the bacteria. (The β-galactosidase activity was also determined directly on toluene-treated suspensions[2]).

It is seen that the β-galactosidase activity appears a few minutes after the addition of melibiose, and increases for about two and a half hours. The results of the activity measurements are exactly parallel with results of the immunological titration of Gz. The level of Pz, which was stable before the addition of melibiose, drops very significantly as Gz increases and reaches a new equilibrium value about 44% lower at about the same time that Gz reaches its stable maximum.

In terms of antigenic equivalents, the decrease in Pz is much less than the increase in Gz. This is expressed by the "T" curve, which shows the change in *total* anti-galactosidase antigen (Pz + Gz = Tz). When the rate of dilution of the continuous culture is taken into account it can be calculated that at no time does the decrease in Pz level exceed the dilution rate. In other words, there is never an absolute decrease in the total amount of Pz in the system.

These results show that the net rate of synthesis of Pz is affected by the synthesis of β-galactosidase while, by contrast, that of amylomaltase is not. A rather large number of experiments under a variety of conditions was performed to verify this conclusion. These experiments carried out as described in Fig. 1, consisted in determining Pz, Gz, β-galactosidase, and sometimes amylomaltase, in extracts from continuous-culture cells, after attainment of the equilibrium.

Table I shows the results obtained when *E. coli* is grown in the presence of various galactosidic or non-galactosidic sugars as carbon and energy source. It will be seen that the synthesis of Gz is invariably accompanied by a decrease of the level of Pz, and

References p. 289.

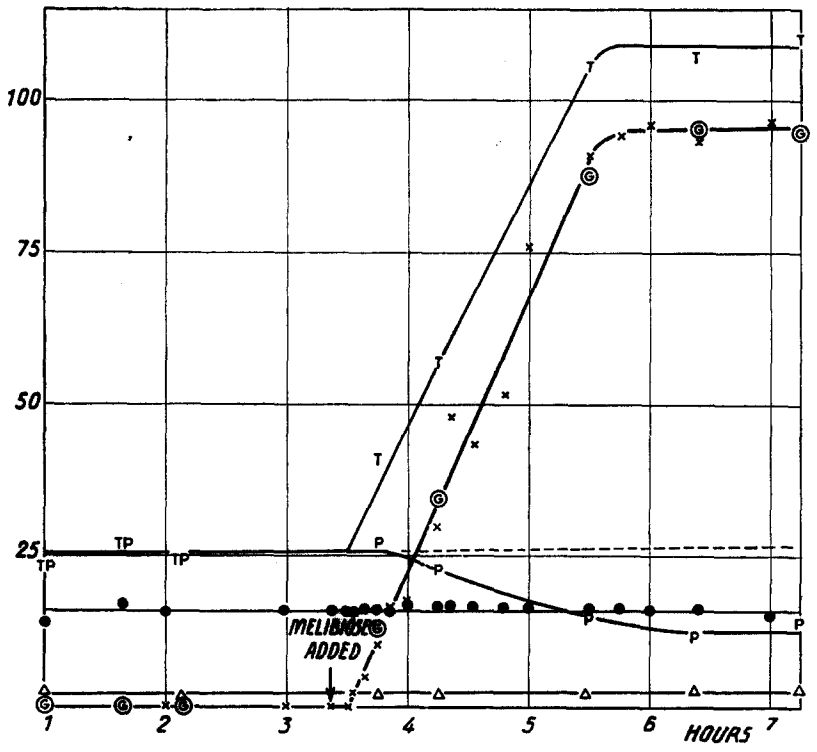

Fig. 1. ML 30 ($M^+L^+G^+$) was stabilized at time 0 in continuous culture with maltose as sole carbon and energy source. At the times indicated 50 ml samples were removed and the extracts analyzed for Tz, Pz, Gz, niphegalase, amylomaltase, and total nitrogen.

		Scale factor
T	= combining units Tz/mg N	× 2
P	= combining units Pz/mg N	× 2
G	= combining units Gz/mg N	× 2
O	= niphegalase units/mg N	× 20
△	= amylomaltase units/mg N	× 10
●	= optical density (D.O.)*	× 100
×	= niphegalase activity in toluenized bacteria**	× 0.1

* D.O. 1000 = 0.7 mg dry weight/ml = 10^9 bacteria/ml.

** Culture toluenized following procedure described in Ref. 1. Values expressed in niphegalase units/D.O. 1000.

It should be noted that the curves representing synthesis of β-galactosidase in toluenized organisms in sand extracts whether measured by enzymic activity or immunological titration are the same.

this is true for the two rather widely different strains ML and K 12. The level of Pz is lowest when β-galactosidase is synthesized, *e.g.* in this case, in the lactose-grown cultures; conversely, the formation of amylomaltase (maltose-grown culture) does not correlate with a decrease of Pz. Actually, the levels of Pz on glucose-grown and maltose-grown cultures are always closely comparable, although there is invariably a slight difference in favour of the maltose-grown, as compared with the glucose-grown culture. (This is also shown in the results of the experiment summarized in Table II).

References p. 289.

TABLE I

LEVELS OF β-GALACTOSIDASE (Gz) AND PROTEIN Pz IN *E. coli* GROWN ON VARIOUS SUGARS

Streins	*Carbon source*	*Amylomaltase*	*β-galactosidase*	*Pz*	*Gz*	*Tz*	*Pz relative units*
				Combining units			
ML 30	glucose	0	0	97	0	97	88 (—12%)
M+L+G+	maltose	92	0	110	0	110	100 —
	lactose	0	2010	75	195	270	68 (—32%)
K 12	glucose	0	0	50	0	50	92 (— 8%)
M+L+G+	maltose	88	0	54	0	54	100 —
	lactose	0	2180	35	208	243	65 (—35%)
ML 32400	glucose	0	0	50	0	50	91 (— 9%)
M+L+G−	maltose	203	0	55	0	55	100 —
	mannose	0	0	50	0	50	91 (— 9%)
	fructose	0	0	53	0	53	100 —
	lactose	0	562	41	59	100	74 (—26%)

All determinations were carried out on sand extracts of cells grown in continuous culture at 37° C with specified sugar (1 gram/litre) as sole carbon and energy source. The cultures were stabilized three hours at a growth rate of 0.85 divisions/h. Enzymic activity expressed in μM/hr/mg N.

TABLE II

EFFECTS OF GALACTOSE ON Pz LEVELS IN L⁻ AND L⁺ STRAINS

Strain	*Carbon source*	*Amylomaltase*	*β-galactosidase*	*Pz*	*Gz*	*Tz*	*Pz relative units*
				Combinant units			
ML 30	glucose	0	0	39	0	39	87 (—13%)
M+L+G+	maltose	130	0	45	0	45	100 —
	galactose	0	345	28	38	66	62 (—38%)
ML 3	glucose	0	0	45	0	45	90 (—10%)
M+L−G+	maltose	62	0	50	0	50	100 —
	galactose	0	0	53	0	53	106 —

Conditions identical with those of Table I.

Strictly speaking, these observations demonstrate that a significant, although not extremely large drop in level of Pz, and a considerable accumulation of the (otherwise virtually absent), Gz protein, are associated with the presence of galactosidic sugar during the growth of a culture. The conclusion that the drop of Pz is associated with the rise of Gz is only an inference, since these two events might conceivably represent independent consequences of the presence of galactosidic sugars. This inference is a very good one, however, since these effects are highly specific, and independent of whether or not the inducing sugar is metabolized as carbon and energy source (*cf.* melibiose). From the experiment described in Table II, it will be seen that with the normal (L⁺) strain, β-galactosidase is synthesized in the presence of galactose, and Pz drops 38% by comparison with the maltose-grown strain. With the L⁻ strain, no β-galactosidase is formed, and the level of Pz is, if anything, slightly increased. This shows that a strain that has lost the capacity to synthesize β-galactosidase in response to galactose, does not present the characteristic drop of Pz, when cultivated on galactose.

References p. 289.

THE GENETIC ASSOCIATION OF PROTEINS Pz AND Gz

If the proteins Pz and Gz are closely related in biosynthesis, one should also expect a close genetic correlation in the capacity to synthesize both proteins. Strains or species that do synthesize one, should also in general be capable of synthesizing the other, and conversely.

A survey was conducted in which the presence of Pz and the capacity to synthesize Gz were tested in a number of mutants of the ML strain, and in a number of different species and genera of *Enterobacteriaceae* (Table III).

TABLE III

DISTRIBUTION OF Pz IN VARIOUS ML MUTANTS AND OTHER *Enterobacteriaceae*

Strains ML mutans	*Energy source*	*Units Pz/mg N*	*Ability to synthesize Gz in presence β-methyl-galactoside*
	Group I		
M+L-G+f (ML 3s)	maltose	63	+
M+L-G+f (ML 3f)	maltose	50	+
M+L-G- (ML 3241)	maltose	40	+
M-L-G- (ML 324)	glucose	72	+
M-L-G- glucose- (ML 307)	peptone	present	+
M-L+G+ (ML 320)	glucose	48	+
M+L-G+ (2) (ML 3080a)	maltose	34	+
	Group II		
M+L-G+ (ML 3080b)	maltose	present	—
	Group III (Other Enterobacteriaceae)		
Aerobacter aerogenes	maltose	present	+
Paracoli coliform	maltose	present	+
Shigella sonnei	maltose	present	+
Shigella flexneri	casein hydrolysate	absent	—
Salmonella enteritidis	maltose	absent	—
Proteus vulgaris OX 19	maltose	absent	—
	Group IV (Species possessing unrelated β-galactosidase)		
Lactobacillus bulgaricus	lactose	absent	—
Saccharomyces fragilis	lactose	absent	—

The 7 different ML mutants which were tested had been selected as unable to metabolize lactose and/or maltose, galactose and glucose (group 1, Table III). However, all were capable of synthesizing β-galactosidase (in reduced amounts) in the presence of methyl-β-D-galactoside as inducer. Only one mutant (ML 3080, b, group II) did not synthesize galactosidase under any known condition. All the ML mutants, including this last one, were found to contain significant amounts of Pz. Moreover, as shown by a series of comparative titrations (Fig. 2), the Pz proteins in each of these mutant strains were immunologically indistinguishable. Therefore, the complete or partial loss of the capacity to synthesize Gz does not necessarily entail a concurrent loss or modification of the protein Pz. A much more significant situation is revealed when the distribution of Pz in a number of species of *Enterobacteriaceae* is considered. As can be

References p. 289.

seen from Table III, a complete correlation between presence of Pz and capacity to synthesize Gz is observed (group III). The three species which were found to possess the Pz protein were also capable of synthesizing Gz. The other three species examined did not contain Pz, and could not be induced to synthesize Gz nor any other β-galactosidase, under any conditions. Attempts at isolating galactosidase-producing mutants from these species failed. The comparison of *Shigella sonnei* and *Shigella fexneri* is particularly instructive. *S. sonnei* possesses Pz, can be induced to synthesize β-galactosidase, and although on fresh isolation from a dysentery case, it does not utilize lactose,

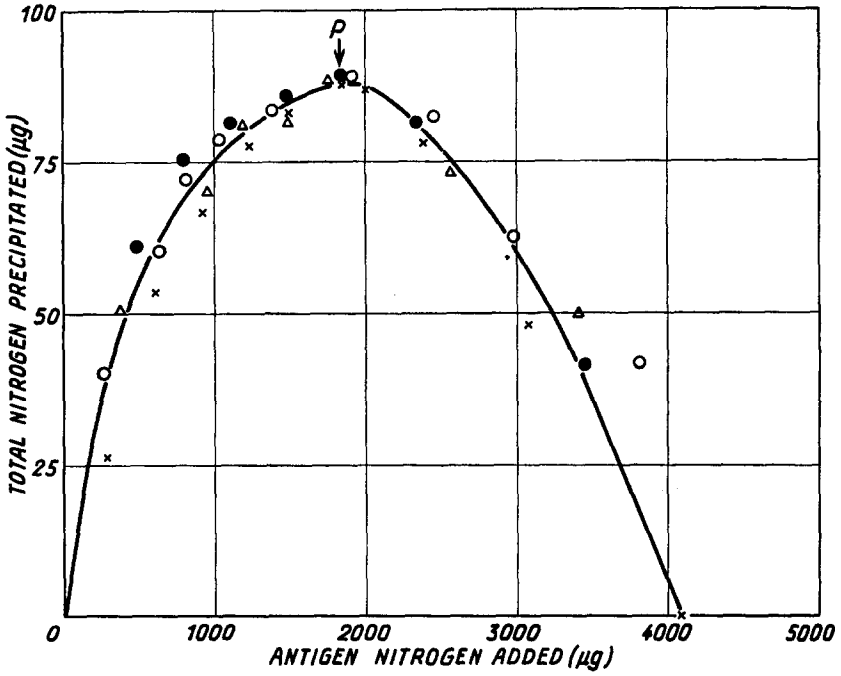

Fig. 2. Quantitative precipitin curve representing reaction between Pz in inactive extracts from various strains of ML and anti-β-galactosidase sera. For technique see (4)

● = ML 32400 M+L+G−
○ = ML 30 M+L+G+
× = ML 3 M+L−G+
△ = ML 3241 M+L−G−

it is characteristic that this organism mutates to a lactose utilizing form. *Shigella fexneri*, on the contrary, does not possess Pz, can not be induced to produce Gz, and is never found to mutate to a lactose utilizing form.

As shown elsewhere, the β-galactosidase produced by the various members of the *Enterobacteriaceae* is, in every case, immunochemically and enzymatically identical with the *E. coli* ML β-galactosidase (see [5]). It is important to note this point here, since two organisms *Lactobacillus bulgaricus* and *Saccharomyces fragilis*, which produce a galactosidase, do not possess the Pz protein. However the β-galactosidases synthesized by these organisms are both enzymatically and immunochemically unrelated to the *E. coli* β-galactosidase. One would therefore not expect to find that these organisms contain Pz.

References p. 289.

The complete correlation between the ability to synthesize Gz and the presence of Pz in a variety of related species is further evidence in favor of the hypothesis that the two proteins are related in biosynthesis.

THE BEHAVIOUR OF Pz IN MUTANT ML 3241

As an appendix to the experiments on the relationship of proteins Pz and Gz, some observations on the effects of galactose on the synthesis of Pz in one particular mutant (ML 3241) may be mentioned. This mutant does not metabolize lactose or galactose as carbon sources, nor does it synthesize β-galactosidase in the presence of lactose, except at extremely high concentrations of the sugar. It does, however, synthesize low levels of β-galactosidase in the presence of galactose, provided of course another energy source is present. It should be added that galactose, even at low concentrations, inhibits to a significant extent the growth rate of this mutant. Table IV compares the effects of galactose on this mutant. L^-G^- (ML 3241) and on L^+G^- mutant (ML 32400) in which the Pz–Gz relationship is "normal" *i.e.* such that Pz decreases on appearance of Gz. It may be seen that the latter synthesizes large amounts of β-galactosidase in the presence of galactose, and that, accordingly, Pz drops by about 28% as compared with a control culture on maltose alone. Mutant ML 3241 synthesizes only 12% as much β-galactosidase in the presence of galactose, but the Pz protein, far from decreasing slightly or staying constant, as it might have been expected, increases a great deal, over the level found in the galactoseless control.

TABLE IV

EFFECT OF GALACTOSE ON L^+G^- AND L^-G^- STRAINS

Strain	*Presence of galactose* 3×10^{-3} *M*	*Amylomaltase*	*β-galactosidase*	*Pz*	*Gz*	*Tz* *Combining units*	*Pz relative units*
ML 32400	—	82	0	57	0	57	100 —
$M^+L^+G^-$	+	105	1310	41	133	174	72 (—28%)
ML 3241	—	42	0	48	0	48	100 —
$M^+L^-G^-$	+	40	121	88	16	104	183 (+83%)

Conditions of experiment same as Table I. Maltose sole carbon and energy source. The growth rate was 0.3 divisions/h just below the maximum growth rate which obtains in the presence of maltose + galactose. Cultures stabilized 5 hours before analysis.

The experiments described in the preceding paragraphs left the question of the functional significance of the Pz protein entirely open, except in so far as they bore on the problem of its possible role as a precursor. The observation just mentioned suggests that Pz may be somehow involved in the metabolism of galactose, and it is of course very tempting to entertain the possibility that Pz represents, in whole or in part, an active enzyme or enzymes. So far however we have not found an enzymic activity associated with Pz nor other enzymes which cross-react with anti-galactosidase serum.

DISCUSSION

The immunological evidence[3,4] and the physiological evidence (presented above) concur in showing that the induced enzyme β-galactosidase, and the Pz protein of *E. coli*

References p. 289.

are related in biosynthesis as well as in structure. The fact that only those species of *Enterobacteriaceae* which possess Pz are capable of synthesizing β-galactosidase adds very strong support to this conclusion. However, the Pz–Gz relationship may belong to one of several different types, which could be summarized as follows:

a. Pz is the precursor of Gz;
b. Pz and Gz have a common precursor or share a common specific building block;
c. Pz and Gz are independently synthesized by the same or similar mechanisms within the cell, perhaps at the same site and under the control of the same genetic determinants.

SUMMARY

E. coli cells possess a protein, Pz, which appears to be synthesized under all conditions of growth. In the presence of a galactoside, the cells also synthesize another protein, Gz, endowed with β-galactosidase properties, and very closely similar to Pz in antigenic structure as well as in solubility properties. The synthesis of Gz interferes very significantly with the net rate of Pz synthesis. Only those species of *Enterobacteriaceae* which possess the Pz protein are competent to synthesize β-galactosidase.

RÉSUMÉ

E. coli possède une protéine, Pz, qui semble être synthétisée dans toutes les conditions permettant la croissance. En présence d'un galactoside, les cellules synthétisent en outre une autre protéine, Gz, qui a les propriétés d'une β-galactosidase, et qui par ses caractères de solubilité ainsi que par sa structure antigénique, est extrêmement proche de la protéine Pz. La synthèse de la protéine Gz retentit d'une manière très marquée sur la vitesse de synthèse de la protéine Pz. Seules les espèces d'*Enterobacteriaceae* qui possèdent la protéine Pz paraissent capables d'effectuer la synthèse de la β-galactosidase.

ZUSAMMENFASSUNG

E. coli besitzt ein Protein, Pz, welches unter allen Wachstumsbedingungen gebildet zu werden scheint. In Gegenwart eines Galactosides synthetisieren die Zellen ausserdem noch ein anderes Eiweiss, Gz, welches die Eigenschaften einer β-Galactosidase hat und welches, was Löslichkeit und Antigenstruktur betrifft, dem Protein Pz ausserordentlich nahe steht. Die Gz-Synthese wirkt sich deutlich aif die Geschwindigkeit der Pz-Synthese aus. Nur die Arten von *Enterobacteriaceae*, welche das Protein Pz besitzen, scheinen zur Synthese der β-Galactosidase befähigt zu sein.

REFERENCES

1 J. MONOD, G. COHEN-BAZIRE AND M. COHN, *Biochim. Biophys. Acta*, 7 (1951) 585–599.
2 M. COHN AND J. MONOD, *Biochim. Biophys. Acta*, 7 (1951) 153–174.
3 M. COHN AND A. M. TORRIANI, *Compt. rend.*, 232 (1951) 115–117.
4 M. COHN AND A. M. TORRIANI, *J. Immunol.* (in press).
5 J. MONOD AND M. COHN, *Adv. Enzymol.*, 13 (1952) 67.
6 M. COHN AND A. M. TORRIANI, Unpublished experiments.
7 J. MONOD AND A. AUDUREAU, *Ann. Inst. Pasteur*, 72 (1946) 868.
8 J. MONOD, *Ann. Inst. Pasteur*, 79 (1950) 390.
9 J. MONOD AND A. M. TORRIANI, *Ann. Inst. Pasteur*, 78 (1950) 65.
10 J. LEDERBERG, *J. Bact.*, 60 (1951) 381.
11 A. NOVICK AND L. SZILARD, *Science*, 112 (1950) 715.

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