STUDIES ON THE INDUCTION OF β-GALACTOSIDASE IN A CRYPTIC STRAIN OF *ESCHERICHIA COLI*

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SUMMARY

1. The absence of a galactoside permease in a cryptic strain of *E. coli* was established.

2. The kinetics and specificity of induction of β-galactosidase in this strain were studied and compared with a normal strain (with galactoside permease).

3. The differential rate of synthesis of β-galactosidase in the cryptic is constant from the moment of inducer addition, at all levels of inducer which give measurable induction. This is in contrast to the situation in the normal strain where at subsaturating concentrations of inducer, there is a lag and a period of increase in the differential rate of synthesis before a constant rate is attained.

4. The increasing differential rate of synthesis in the normal was shown to be due to the action of the inducible galactoside permease.

5. The constant differential rate in the cryptic and in the permease-blocked normal strain was discussed as evidence that the enzyme forming system for β-galactosidase does not increase in amount in response to inducer.

6. Certain aryl-thiogalactosides are competitive inhibitors of induction in permeaseless strains. This result indicates that a sterically specific site, distinct from galactosidase or permease, is involved in induction.

INTRODUCTION

Most of the experimental evidence bearing on the mechanism of enzyme induction concerns the kinetics of induced enzyme synthesis and the specificity requirements for inducing compounds. An implicit or sometimes explicit assumption of all such studies is that the kinetic and specific properties of the system are characteristic of the enzyme-forming system itself. There are however reasons to suspect that other systems may alter or conceal the true properties of the enzyme-forming system itself. Among those systems are those which allow or control the permeation of substrates and inducers into the cell.

It has recently been discovered that in normal (wild type) *Escherichia coli*, the entry and intracellular concentration of galactosides is controlled by a specific inducible permeation factor (galactoside-permease) independent of β-galactosidase,

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References p. 538.

which is a strictly intracellular enzyme[1,2]. Since the induction of galactosidase is dependent upon intracellular galactosides, it is clear that in normal strains, the kinetics and specificity of the phenomenon must depend on the properties of the permease[3].

Certain of the basic problems concerning the mechanism of induction started therefore to be reexamined in the light of this new knowledge. Two of these problems appear particularly critical:

a. Does the mechanism of induction involve an inherently autocatalytic (self accelerating) reaction as it has often been assumed?

b. Does the inducer act by virtue of a stereospecific combination with a pre-existing complementary site or is such a site formed only as a result of inducer action?

It is evident that if induction were studied only in wild type strains, both these questions might be answered in the affirmative since galactoside-permease constitutes a structurally specific site involved in induction and is inherently autocatalytic, since it concentrates intracellularly its own inducer. We have therefore undertaken to study the induction of β-galactosidase in mutant strains (permeaseless or "cryptics") devoid of galactoside-permease. It had previously been observed that these strains could be induced under certain conditions[4,5]. In the first part of the present study, we have endeavoured to determine whether such cryptic strains are really entirely devoid of permease and whether induction can take place when all traces of permease activity are blocked.

In the second part, we have studied the kinetics and the specificity of induction and inhibition of β-galactosidase in permeaseless strains with a view to answering the questions stated above.

METHODS

Strain

Two strains of *E. coli* ML were used in this work. A lactose-negative, cryptic strain (ML 3) and a lactose-positive, normal strain (ML 30) derived by spontaneous mutation from ML 3. The strains were maintained by bi-weekly transfers on liquid medium "63".

Medium

Medium "63" was used throughout this work. Composition: KH_2PO_4 (13.6 g), $(NH_4)_2SO_4$ (2.0 g), $MgSO_4 \cdot 7\,H_2O$ (0.2 g), $FeSO_4 \cdot 7\,H_2O$ (0.005 g), H_2O (1 l) adjusted to pH 7 with KOH. Twice recrystallized maltose (2 g/l) separately sterilized was added before use.

Conditions of culture

For experiments, a 1:200 dilution of the maintenance culture was made in conical flasks and shaken overnight at 30°. The following morning the culture was diluted to a suitable cell density and allowed to grow aerobically at 34° through at least 1½ doublings, by which time it was growing exponentially with a doubling rate of approximately one per hour.

Where preinduced cells were required, inducer was present throughout the growth period.

References p. 538.

Cell density was determined either with the aid of a Meunier colorimeter fitted with a blue or yellow filter, or a Beckman DU spectrophotometer at 600 mμ. Standard curves were used to convert the reading obtained to bacterial dry weight per ml.

Uptake of thiogalactoside

Washed cells were aerated for 20 min at 37° in growth medium containing chloromycetin (50 mg/l), ^{35}S-labeled β-D-thiogalactosyl, β-D-galactoside (TDG) and other indicated additions. Duplicate 1 ml samples were rapidly pipetted onto well-chilled millipore filters*, the medium sucked through and the retained bacteria washed twice with 2 ml of iced medium. This whole procedure takes about 15 sec and speed is essential. After drying, the filters were counted directly in a gas-flow counter.

In vivo hydrolysis of o-nitrophenyl β-D-galactoside (ONPG)

Washed cells were aerated at 37° in growth medium plus chloromycetin (50 mg/l), ONPG and other additions at the concentrations indicated. The reaction was stopped at convenient intervals by diluting aliquots into two parts of *M* Na_2CO_3 at 0°. The cells were removed by centrifugation in the cold and the extent of hydrolysis determined by the difference in O.D. at 420 mμ. The hydrolysis of ONPG at room temperature in Na_2CO_3 is avoided by the low temperature maintained in this procedure. The rate of hydrolysis by the cells is constant over a period of at least 1 hour.

β-galactosidase determinations

Cell suspensions, at a density of not more than 150 μg/ml bacterial dry weight were added to tubes containing two drops of toluene. The tubes were corked and shaken for 30 min at 37°. The β-galactosidase activity was determined at 28° by the increase in O.D. at 420 mμ in a Beckman DU spectrophotometer of a mixture containing ONPG (*M*/375), sodium phosphate buffer, pH 7 (*M*/20) and a suitable dilution of the toluenized suspension. One unit of β-galactosidase is that amount which hydrolyzes 1 mμmole ONPG per min under these conditions.

Induction of β-galactosidase

All the inducers used in this study are β-D-thiogalactosides, in which the oxygen atom of the galactosidic linkage is replaced by a sulfur atom. These compounds allow induction under gratuitous conditions as they are neither hydrolyzed by β-galactosidase nor used as carbon source by the organisms[6].

Inducer was added to exponentially growing cultures containing 35 μg/ml bacterial dry weight. At intervals during the first division and a half after the addition of inducer, samples were taken for galactosidase assays. The galactosidase activity (Z), in units galactosidase per ml is plotted against bacterial dry weight (X), in μg/ml. The rate of induction is defined as the differential rate of synthesis, $p = \Delta Z/\Delta X$[7]. This method of expressing induction rates has the advantage of being independent of minor changes in the growth rate of bacteria.

EXPERIMENTAL

Galactoside permeation in the cryptic strain

Before describing the experimental studies on galactoside permeation in the

* Millipore Filter Corp., Watertown 72, Mass.

References p. 538.

cryptic strain, it may be useful to review the characteristics of the galactoside permease found in the normal strains of *E. coli*.

COHEN AND RICKENBERG[8] found that ^{35}S-thiomethyl β-D-galactoside (TMG) is accumulated by cells of *E. coli* which have been grown in the presence of an inducing galactoside. Subsequent work showed that the amount of TMG taken up (G_{in}) by the cells is a function of the external concentration (G_{ex}) following, fairly closely, the relation:

$$G_{in} = Y \frac{G_{ex}}{G_{ex} + k}$$

where Y is the capacity (maximum amount of galactoside accumulated/unit dry weight of bacteria) and k is the dissociation constant of the bacteria–galactoside "complex"[4]. Other galactosides competitively inhibit the permeation and accumulation of TMG and are taken up by the cells in the same manner as TMG. For each galactoside the capacity and the dissociation constant vary independently.

To account for their observations, these authors proposed a specific permeation factor, galactoside permease, which catalyzes the transfer of galactosides across the cellular osmotic barrier into the cell; and a non-specific exit mechanism, the rate of exit by which is proportional to the intracellular concentration of galactoside.

In the cryptic strain, we find that cells grown in the presence of concentrations of galactoside which induce the same maximum specific activity of galactosidase as in the normal strain, take up at most 0.1 % of the amount of thiogalactoside (TDG) taken up by the normal (Table I). The induced cryptic organisms therefore lack the ability to accumulate galactosides[4].

TABLE I

ACCUMULATION OF RADIOACTIVE TDG BY THE NORMAL AND THE CRYPTIC

TDG radioactive	*TDG non-radioactive*	*Normal*		*Cryptic*	
		counts/min	*μmoles TDG/g dry wt.*	*counts/min*	*μmoles TDG/g dry wt.*
$2 \cdot 10^{-4}$ *M*	—	779*	19	6.7	0.01
		717		6.3	
$2 \cdot 10^{-4}$ *M*	10^{-2} *M*	29	0.04	5.2	0.01
		26		6.3	

Duplicate samples, 0.157 mg dry weight per sample. Samples counted for 10 minutes.
* Background, 4.2 counts/min, not subtracted.

Although this inability to accumulate galactosides suggests that the cryptic has no galactoside permease, the possibility remains that the specific permeation factor, for which the term permease should be reserved, is still present and is responsible for letting galactosides into the cell, but that another part of the system, such as the energy coupling which permits *accumulation*, is not present in this strain[3]. In order to examine this possibility, the permeation of galactosides into the cells had to be studied independently of intracellular accumulation.

This can be done by measuring the rate of hydrolysis of ONPG by intact cells. It has been found by RICKENBERG *et al.*[4] that, in the normal strain, the overall rate

References p. 538.

of ONPG hydrolysis is limited by the rate of *entry* of the galactoside into the cell rather than by the activity of the hydrolytic enzyme (β-galactosidase). In the cryptic, where the amount of galactosidase is the same as in the normal, but where the rate of hydrolysis of ONPG by intact cells is much lower, it follows that the rate of entry of ONPG again is limiting.

Fig. 1 shows that the rate of ONPG hydrolysis in the normal increases with concentration of substrate according to the Henri-Michaelis relationship, and is essentially maximum at $5 \cdot 10^{-3}$ *M*, while the rate of hydrolysis in the cryptic increases *in proportion* to substrate concentration. Therefore, the faster rate in the normal is limited by a saturable factor (the galactoside permease) while the rate in the cryptic (at least up to *M*/25 ONPG) is limited only by a non-saturable diffusion-like process.

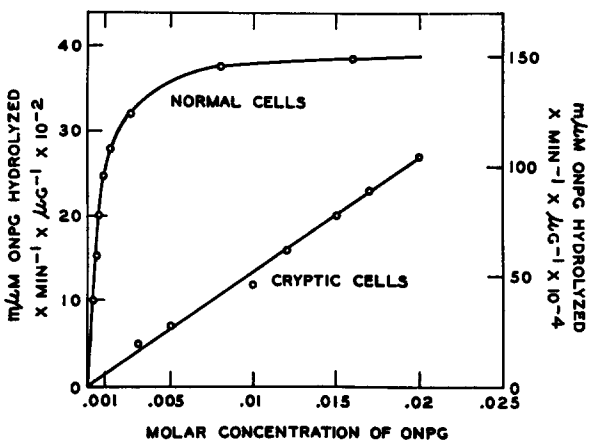

Fig. 1. Relationship of rate of *in vivo* ONPG hydrolysis to substrate concentration in the normal and the cryptic.

Growth of the cryptic in presence of thioisopropyl β-D-galactoside (IPTG), a good inducer of galactoside permease in the normal strain and of galactosidase in both the normal and cryptic strains, does not change the rate of galactoside (ONPG) permeation into the cells. As is shown by Fig. 2, the specific rate of ONPG hydrolysis by intact cryptic cells does not change during induction or de-induction except during the first few minutes of induction when galactosidase itself is limiting. Therefore, the rate of entrance of galactoside into the cells of the cryptic does not depend on a factor which is induced by galactosides as would be expected if the rate depended on the formation of the inducible permease.

Table II shows that, in contrast with the strong competitive inhibition of the permease by TDG, there is no effect of this compound on the rate of hydrolysis of ONPG in the cryptic. IPTG, which has a lower affinity than TDG for the permease, but a much higher affinity for β-galactosidase, inhibits the hydrolysis of ONPG in both strains.

These observations on galactoside accumulation and permeation allow the conclusion that the cryptic strain is devoid of detectable galactoside permease, and that the entry of galactoside into the cell is accomplished by a process similar to diffusion.

This conclusion is further strengthened by the observation that the diffusion-like

References p. 538.

permeation of galactosides exists in the normal strain as well as in the cryptic. This "passive" permeation can be demonstrated in the normal provided the permease-activated entry is blocked. This can be accomplished by using TDG as an inhibitor: $2 \cdot 10^{-2}$ *M* TDG completely blocks ONPG entry by the permease in the normal strain and causes the rate of ONPG hydrolysis to be decreased to the rate found with the cryptic at the same ONPG concentration (Table II). We shall refer to this observation in the studies on induction presented later.

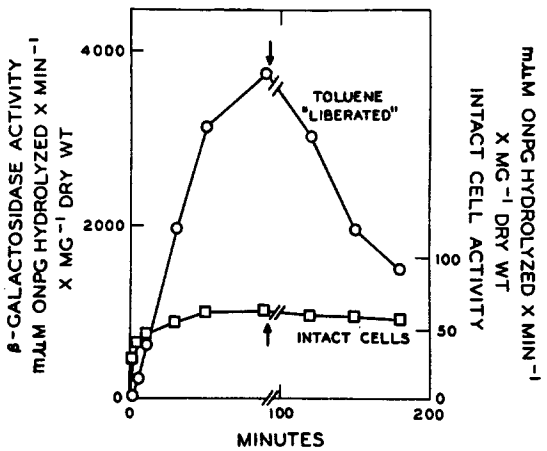

Fig. 2. *In vivo* hydrolysis of ONPG compared with β-galactosidase activity during induction and de-induction of the cryptic. Cells were grown in the presence of $5 \cdot 10^{-4}$ *M* IPTG for 90 min then centrifuged, washed, and grown in the absence of inducer for the remainder of the experiment. Points represent specific activity of sample at indicated time. Concentration of ONPG for *in vivo* activity determination was $5 \cdot 10^{-3}$ *M*. β-galactosidase activity was determined with "toluenized" cells.

TABLE II

EFFECT OF THIOGALACTOSIDES ON THE *in vivo* HYDROLYSIS OF ONPG IN THE NORMAL AND THE CRYPTIC

ONPG (M)	*Inhibitor (M)*	*ONPG hydrolysis*	
		Cryptic	*Normal*
10^{-3}	—	11.6	306
10^{-3}	TDG $2 \cdot 10^{-2}$	11.6	12.1
10^{-3}	IPTG $5 \cdot 10^{-3}$	10.7	59.5
$5 \cdot 10^{-3}$	—	52.9	815
$5 \cdot 10^{-3}$	TDG $2 \cdot 10^{-2}$	51.7	60.5
$5 \cdot 10^{-3}$	IPTG $5 \cdot 10^{-3}$	36.3	440

Kinetics of induction of β-galactosidase

In Fig. 3 we present families of curves which represent galactosidase induction in the normal and cryptic strains of *E. coli*. It is apparent that the absence of permease results not only in decreased sensitivity of the cells to inducer, but actually in a qualitative change of the kinetics of induction.

With the normal strain, at low inducer concentrations there is a period of increase in the (differential) rate of induction before a constant rate is attained. As the concen-

References p. 538.

tration of inducer is increased, the acceleration period is shortened, until, at high concentrations of inducer, the rate is maximal from the start of induction.

With the cryptic strain, in contrast, at low inducer concentrations as well as at high, the rate of induction is *constant from the point of addition of inducer*. At the lowest concentrations giving measurable induction, the rate of synthesis is only 1 % of maximum but there is no evidence of any lag in the attainment of this rate.

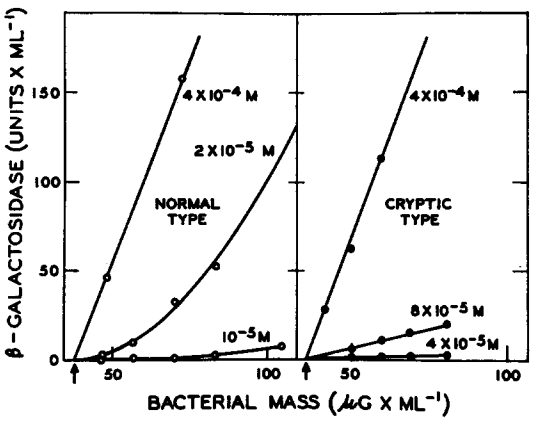

Fig. 3. Kinetics of induction by IPTG of β-galactosidase in the normal and the cryptic. Arrow indicates inducer addition.

The linear induction curves obtained with the cryptic allow a quantitative examination of the relationship between the concentration of inducer and the rate of induction. Fig. 4 a and b are curves showing this relationship for two inducers, TMG and IPTG. The shape of the curve for each inducer, and the concentration which gives the half-maximal rate of enzyme production are the same in different experiments, but the absolute rates of induction can vary as much as 50 % from week to week. Obviously, the relationship found here is a complex one, not the simple hyperbola described for some induction systems[9,10]. An S-shaped curve, such as this, could be due to several entirely different causes and a discussion of them is not likely to be useful at this time.

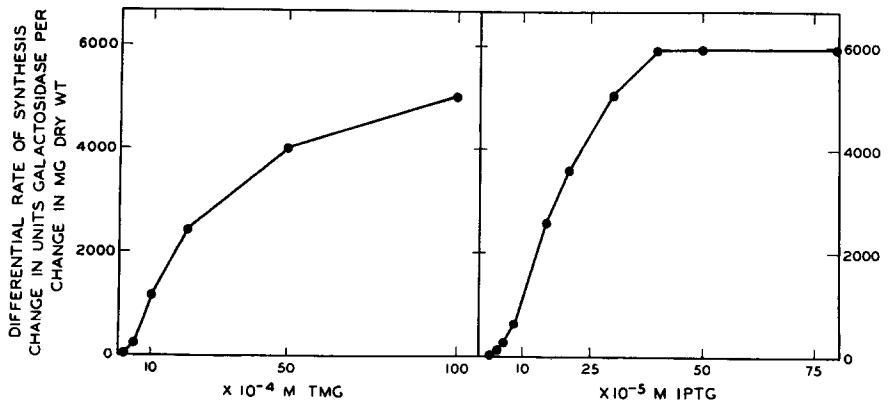

Fig. 4. Relationship between differential rate of synthesis of β-galactosidase and inducer concentration. (a) TMG, (b) IPTG.

References p. 538.

The basic induction kinetics illustrated in Fig. 3 are relevant to the discussion of the mechanism of enzyme induction[11–15]. Examples of a lag in induction after exposure of the cells to inducer (as seen at all but very high inducer concentrations in the normal strain) have been used as evidence for the formation by the inducer of an autocatalytic complex which both increases in amount per cell and produces the induced enzyme[16]. The complete absence of a lag in the rate of β-galactosidase production in the cryptic strain, however, leaves no experimental justification for assuming an autocatalytic mechanism of enzyme induction. Rather, the linear course of induction suggests that the function of inducer is to activate a system, already present in the cell, which does not increase in response to inducer.

Autocatalytic kinetics are not an inherent property of the galactosidase induction system in the normal strain, either. Linear induction at rates of enzyme production much below maximal can be demonstrated for the normal, provided only the permease is not functioning. As seen earlier (Table II), TDG, due to its high affinity for the permease, is an excellent inhibitor of this system. In addition, TDG is not an inducer of β-galactosidase in *E. coli*. It should therefore be an inhibitor of induction when induction depends on concentration of inducer into the cell by the permease. This expectation is borne out by experiment.

The addition of TDG renders the normal cells much less sensitive to inducer: 10^{-5} *M* IPTG no longer gives measurable induction (Fig. 5, curve a), although, when there is no TDG present, that concentration of IPTG induces (see Fig. 3).

If the IPTG concentration is raised above the minimal inducing concentration for the cryptic (*i.e.*, $2–3 \cdot 10^{-5}$ *M*), the normal cells respond to inducer, even in the presence of TDG at concentrations which completely block the permease. Moreover, under these conditions, the differential rate of synthesis of galactosidase is constant from the point of addition of inducer, and that rate is the same as the rate obtained in the cryptic in response to the same inducer concentration (Fig. 5, curves b, c; d, e).

This experiment is relevant to another aspect of the relationship of the permease

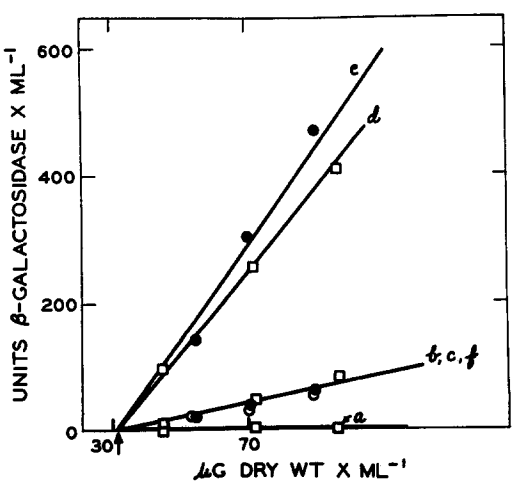

Fig. 5. Conversion, by TDG, of kinetics of induction of β-galactosidase in the normal to kinetics of induction in the cryptic. (□, a) Normal, 10^{-5} *M* IPTG, $4 \cdot 10^{-3}$ *M* TDG; (□, b) Normal, $8 \cdot 10^{-5}$ *M* IPTG, $4 \cdot 10^{-3}$ *M* TDG; (□, d) Normal, $4 \cdot 10^{-4}$ *M* IPTG, $4 \cdot 10^{-3}$ *M* TDG; (●, c) cryptic, $8 \cdot 10^{-5}$ *M* IPTG; (●, e) cryptic, $4 \cdot 10^{-4}$ *M* IPTG; (○, f) cryptic, $8 \cdot 10^{-5}$ *M* IPTG, $4 \cdot 10^{-3}$ *M* TDG.

References p. 538.

to induction. Although no permease is detectable in the cryptic, the possibility remained that there was a trace of non-inducible permease in the cells which performs some function essential for galactosidase induction[17]. If this were true, then a TDG/IPTG ratio of 50/1 should result in a 99.8 % inhibition of the rate of induction. As curve f in Fig. 5 shows, TDG has no effect on induction in the cryptic. Thus we must conclude that the permease is not necessary for induction.

Specificity of β-galactosidase induction

MONOD and his collaborators[18], in a series of studies on the structural requirements for inducers of β-galactosidase in normal strains of *E. coli*, found that only compounds with a galactosidic configuration were effective inducers. BUTTIN AND MONOD[19], in using a series of thio-galactosides as inducers, found that the aglycone played a large role in determining the inducing activity of the galactoside. As seen in Table III, the response of the normal and cryptic strains to these compounds at about saturating concentrations is generally similar. The short chain alkyl derivatives are the best inducers while the aryl derivatives induce very slightly or not at all.

TABLE III

INDUCTION OF β-GALACTOSIDASE BY THIOGALACTOSIDES IN THE NORMAL AND THE CRYPTIC

Thiogalactoside	*Specific activity**	
	Normal$_{30}$	*Cryptic*
Isopropyl (IPTG)**	5,000	5,000
Propyl	5,000	5,000
Methyl (TMG)	5,000	3,900
Butyl	4,700	3,100
Hexyl	476	1,200
Phenylethyl	212	< 10
p-Aminophenyl	—	< 10
Phenyl (TPG)	7	< 10
Galactosidyl (TDG)	9	< 10
Cyclohexyl	3,600	< 10
Basal activity	7	< 10

* Units β-galactosidase/mg dry wt. bacteria after growth overnight in presence of inducer.
** Inducer concentration $5 \cdot 10^{-3}$ *M*, except cyclohexyl: $2 \cdot 10^{-3}$ *M*.

Structural discrimination such as this suggests that the inducer must combine with specific sites for induction to occur, and that the aglycone may form a part of the attachment of the galactoside molecule to these sites.

If such sites do intervene in induction, then some of the galactosides which are not inducers might competitively inhibit induction by inhibiting the interaction of the inducing galactosides with these sites. As seen earlier, TDG is a competitive inhibitor of induction in the normal strain. Nevertheless, it is clear that this inhibition is due solely to competition with inducer for *entrance* into the cell at the permease. TDG neither inhibits induction in the cryptic strain (Fig. 5, curve f) nor directly inhibits induction in the normal strain. In the experiment represented by curve b in Fig. 5, the ratio of TDG to IPTG in the cell was approximately 200/1, yet there was an inhibition of induction of not more than 15 %. On this basis it can be calculated

References p. 538.

that TDG has an affinity for the induction mechanism of not more than 0.1 % of the affinity of IPTG.

Another non-inducing galactoside, thiophenyl β-D-galactoside (TPG), which had previously been cited as an example of a competitive inhibitor of induction in the normal strain[20] was next tested to determine whether it inhibits induction in the cryptic. As shown in Table IV, TPG does inhibit induction in the cryptic, and the

TABLE IV

INHIBITION OF DIFFERENTIAL RATE OF SYNTHESIS OF β-GALACTOSIDASE BY TPG IN THE CRYPTIC

IPTG (M)	*TPG* $4 \cdot 10^{-3}$ *M*	*Experiment I*		*Experiment II*	
		*ΔZ/ΔX**	*Inhibition (%)*	*ΔZ/ΔX**	*Inhibition (%)*
$8 \cdot 10^{-5}$	—	0.75			
	+	0.3	64		
$1 \cdot 10^{-4}$	—			1.2	
	+			0.64	47
$2 \cdot 10^{-4}$	—	2.9		3.7	
	+	2.0	31	2.2	41
$2.5 \cdot 10^{-4}$	—			4.4	
	+			2.8	27
$4 \cdot 10^{-4}$	—			5.5	
	+			4.4	20
$8 \cdot 10^{-4}$	—	4.5			
	+	3.6	20		
$2 \cdot 10^{-3}$	—	4.5**			
	+	3.9	13		
$4 \cdot 10^{-3}$	—	4.5**			
	+	4.8	0		
$8 \cdot 10^{-5}$	TPGlucoside ($4 \cdot 10^{-3}$ *M*)	0.75	0***		

Experiments I and II conducted on different days.

* Differential rate of synthesis of galactosidase = change in units galactosidase/change mg dry wt. of bacteria.

** Value at $8 \cdot 10^{-4}$ *M* IPTG (saturation).

*** Calculated by comparison with Line 1.

der cent of inhibition decreases as the concentration of inducer is raised. This signifies that TPG and inducer compete at some sites in the cells and that TPG inhibits induction by occupying a portion of these sites, effectively decreasing the number available for inducer. It should be clear that this interpretation is independent of any assumptions regarding the nature of the reaction occurring at these sites.

Since the aglycone of TPG is in part responsible for the properties of this compound as an inhibitor of induction, another thiophenyl glycoside, thiophenyl β-D-glucoside (TPGluc), was tested as an inhibitor. We found that this compound has

References p. 538.

absolutely no effect on galactosidase induction (bottom line, Table IV). As TPGluc differs from TPG only by the steric configuration around carbon 4 of the sugar moiety, the sites at which inducer and TPG compete must have a high degree of stereospecificity, discriminating between the glucose and the galactose configuration.

There are other saccharides which do not have the galactosidic configuration but which, nevertheless, inhibit the induction of β-galactosidase[21, 22]. These are metabolizable sugars or sugar derivatives, of which glucose is the best known example.

TABLE V

INHIBITION OF DIFFERENTIAL RATE OF SYNTHESIS OF β-GALACTOSIDASE BY GLUCOSE IN THE CRYPTIC

IPTG (M)	*Glucose* $4 \cdot 10^{-3}$ *M*	*Experiment I*		*Experiment II*	
		*ΔZ/ΔX**	*Inhibition (%)*	*ΔZ/ΔX**	*Inhibition (%)*
$8 \cdot 10^{-5}$	—	1.1			
	+	0.85	23		
$1 \cdot 10^{-4}$	—			1.2	
	+			0.55	54
$2 \cdot 10^{-4}$	—	4.6		3.7	
	+	3.4	26	1.9	49
$2.5 \cdot 10^{-4}$	—			4.4	
	+			2.2	50
$4 \cdot 10^{-4}$	—	6.1		5.5	
	+	4.6	25	2.8	49
$8 \cdot 10^{-4}$	—	7.7			
	+	6.1	21		
$2 \cdot 10^{-3}$	—	7.7**			
	+	6.1	21		
$4 \cdot 10^{-3}$	—	7.7**			
	+	5.9	23		

Experiments I and II conducted on different days.

* Differential rate of synthesis of galactosidase = change in units galactosidase/change mg dry wt. of bacteria.

** Value at $8 \cdot 10^{-4}$ *M* IPTG (saturation).

To determine whether this inhibition by non-galactosides is similar to the TPG inhibition described above, the effect of glucose on induction of galactosidase in the cryptic was examined. The data in Table V show that glucose, in contrast with TPG, is a non-competitive inhibitor of induction. This indicates that whatever the mechanism of glucose inhibition, glucose itself does not compete with inducer at the stereospecific sites responsible in the galactosidase induction mechanism for the TPG inhibition. It remains possible that a more or less distant product of glucose metabolism may be responsible for the effects interfering perhaps at the same site as TPG.

References p. 538.

DISCUSSION

It is almost trite to say that before any valid conclusions can be drawn as to the *in vivo* function or metabolism of a substance, it is essential to be aware of the factors controlling its permeation into the cell. Nevertheless, ignorance of these factors has led to important misconceptions regarding the mechanism of induction of β-galactosidase, and the hydrolysis of galactosides by intact-cell suspensions.

Crypticity

Recent studies in this and other laboratories have shown that the bacterial cell is only slightly permeable to sugars, amino acids, and organic acids, and that the rapid entry of these compounds into the cell is mediated by specific, catalytic permeation factors (permeases). For example, the diffusion-like entry of galactosides into *E. coli* is 100 to 1,000 times slower than the permease catalyzed uptake (Fig. 1 and Table II). The absence of galactoside permease in the cryptic strains of *E. coli* adequately accounts for the long-troubling, paradoxical fact that this strain cannot grow on lactose or metabolize β-galactosides although it can and does form normal amounts of β-galactosidase[23].

Kinetics of induction

The discovery of a galactoside permease in *E. coli* also leads to an understanding of the much discussed and often misinterpreted phenomenon: the "autocatalytic" kinetics of induction. One of the earliest and most repeatedly made observations on induced enzymes was that after exposure of the cells to inducer, the rate, (or differential rate), of enzyme production is low at first and increased to a maximum following an exponential relationship of the type

$$\frac{dz}{dx} = kz$$

where z is enzyme, x is time or bacterial mass, and k is a constant[24,25]. Because this relationship can describe an autocatalytic process, the postulation of a self-reproducing enzyme-forming system became an integral part of most theories on the mechanism of enzyme induction.

The essential part of these theories is the assumption that a rate-limiting, autocatalytic step is intimately involved in the production of the induced enzyme. A frequently seen version assumes that the enzyme is formed on templates; that in the uninduced cell there is only one (or a very small number) of the appropriate templates; and that inducers cause these templates to replicate, at a rate which is a function of inducer concentration, until the maximum number per cell is reached[16].

The linear kinetics of induction of β-galactosidase observed at saturating concentrations of inducer appeared to be in disagreement with this type of theory of induction[26]. However, within the framework of the theory, it could be assumed that in those cases where the rate of enzyme production is *maximal* from the time of addition of inducer, inducer-activated replication of the templates was not the limiting step in the synthesis. If inducer does indeed cause template replication, then the decreased rate of enzyme synthesis resulting from lowering the concentration of inducer must express a decreased rate of template replication at such subsaturating

References p. 538.

concentrations. According to the theory, therefore, the replication process should constitute the limiting step in enzyme production and autocatalytic kinetics should reappear.

But, in the cryptic strain, linear kinetics of induction are observed at subsaturating concentrations of inducer. That is, the production of enzyme (z) is a constant fraction of the increase in bacterial mass (x) at all concentrations of inducer which give measurable induction. The differential rate of synthesis, $p = dz/dx$, is a function only of inducer concentration under a given set of experimental conditions (Fig. 3). Even at a concentration of inducer which causes enzyme production at a rate 100 times less than the maximum rate, the kinetics of induction show no trace of an "autocatalytic" tendency.

It has recently been suggested that the properties of galactoside permease could account for the "autocatalytic" induction kinetics of β-galactosidase in the normal strain of *E. coli*[3,27]. The results presented here, which show that in the cryptic strain, as well as in the normal when the permease is not functioning, linear kinetics of induction are always observed, confirm and extend this conclusion. The results presented here show that it is *only* the galactoside permease which causes the "autocatalytic" kinetics of induction.

It must be concluded that any hypothesis which includes an autocatalytic step as being an integral part of the mechanism of galactosidase induction is experimentally unjustified. More generally the linear kinetics of induction contradicts any model for enzyme induction which involves an increase in the amount of enzyme forming system as a result of inducer addition. Rather, we must consider the induction mechanism (enzyme-forming-system[16], apo-organizer[14]) as always present in the cell and only "activated" by inducer.

Whether a specific, galactoside-combining site involved in β-galactosidase induction exists in the cell before inducer is present is a question of fundamental importance in designing a model for enzyme induction. MONOD AND COHN[20] found that aromatic thiogalactoside can competitively inhibit induction of β-galactosidase by other galactosides, whereas the corresponding thioglucoside had no effect on the system. The discovery of the galactoside permease raised the problem of whether the site of competitive interaction was not exclusively at the permease level. The results reported here (*cf.* Table IV) show that a specific and competitive interaction of galactosides occurs in the permease-less strain, indicating that there are stereospecific sites involved in induction which are not the permease. Moreover, since the degree of inhibition is independent of the state of induction, it may be concluded that the sites of interaction do not increase or decrease as a result of induction. It should be noted that the stereospecificity of these sites has not been completely determined. All that is known of their specificity is that they interact with β-galactosides but not with at least one β-glucoside.

Recently, a third β-galactoside-handling system, in addition to β-galactosidase and galactoside permease has been discovered in *E. coli*[28]. This system catalyzes the formation of acetyl-thiogalactosides from thiogalactosides. A tempting possibility is that these compounds are formed as a result of interaction of the thiogalactosides with the specific sites discussed above. Actually, the acetyl-thiogalactosides have been shown to be completely inactive in galactosidase induction and thus their synthesis is probably not involved in this process.

References p. 538.

A knowledge of the nature of the reaction occurring at these specific sites would doubtless aid us in discovering the mechanism of enzyme induction.

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