INDUCTION AND INHIBITION OF β-GALACTOSIDASE BY 1-THIO-β-D-GALACTOPYRANOSIDES

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1-Thio-β-D-galactopyranosides, substituted on sulphur by short unmodified aliphatic chains or short chains modified by hydroxyl groups, are good inducers of β-galactosidase. Cyanoalkyl, carbamoylalkyl, methoxyalkyl and epoxyalkyl thiogalactosides are also inducers. Sodium salts of mercaptoalkyl thiogalactosides are inducers and inhibitors of β-galactosidase. The sodium salt of mercaptoethyl thiogalactoside was found to be a competitive inhibitor with $K_I = 5 . 10^{-4}$M.

A number of modified galactosides as well as the naturally occuring carbohydrates have been tested for their ability to induce the lactose operon (lac operon)[1-6]. As a measure of the induction, the activity of β-galactosidase (EC 3.2.1.23) was often used. In general one may benefit from such studies by obtaining information on the structural requirements of the repressor for an effector molecule. However, it is usually difficult to interpret the data obtained, since permeation of the compounds across the cell membrane is a process not well understood. To eliminate the differences in the affinities of the galactosides to the permease, the enzyme coded by cistron y of the lac operon, strain *Escherichia coli* y^- was used[3,6]. However, the existence of other, more specific permeases was not excluded by these experiments. In fact, presence of such a permease was anticipated[6].

The situation is further complicated by possible polyvalent action of the compounds tested[3,6]. The compounds that in some way affect the induction process or the activity of induced β-galactosidase may be separated into several classes depending on their action: Inducers and substrates (many β-D-galactosides)[1]; "inducteurs gratuits" (many thiogalactosides)[1,2,7]; inhibitors (competitive such as galactose and noncompetitive such as glucose or sucrose)[8]; substrates (*e.g.* *o*-nitrophenyl β-D-galactopyranoside)[9]; inducers and inhibitors (*e.g.* sodium salt of mercaptoethyl 1-thio-β-D-galactopyranoside); inhibitors of induction (*e.g.* 2-nitrophenyl β-D-fucoside)[2,3]. The natural substrate of β-galactosidase, lactose, has a special position among the galactosides. It is not an inducer (although sometimes used as such); rather the product of its conversion by β-galactosidase[5], allolactose[10], has the inducing capacity.

In the present communication, several new 1-thio-β-D-galactopyranosides have been tested for their ability to induce and inhibit β-galactosidase.

EXPERIMENTAL

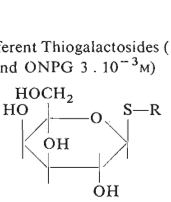
All derivatives of D-galactose were synthesized as described elsewhere[11]. Experiments were performed with bacterial strain *Escherichia coli B* grown in mineral medium[12] on a Dubnoff shaker at 37°C. Induction of the lac operon was performed by adding inducers in a concentration

of 10^{-3}M to growing bacteria, unless otherwise indicated. After appropriate time, aliquots (0·5 ml) of the culture were mixed with one drop of toluene and 10 μg/ml of deoxycholate in an ice bath.

The activity of β-galactosidase was determined spectrophotometrically with *o*-nitrophenyl β-D-galactopyranoside (ONPG) (Lachema, Czechoslovakia) as substrate[13].

TABLE I

Induction of β-Galactosidase by Different Thiogalactosides (10^{-3}M) and Their Effect on β-Galactosidase Activity (thiogalactosides and ONPG 3 . 10^{-3}M)



No	R	Induction %	Inhibition %
I	$-CH(CH_3)_2$	100	
II	$-CH_3$	90	
III	$-CH_2CH_2CH_2CH_2OH$	88	
IV	$-CH_2CH_2OH$	87	
V	$-CH_2CHOHCH_3$	63	
VI	$-CH_2CH_2CH_2OH$	55	
VII	$-CH_2CH_2CN$	45	45
VIII	$-CH_2CH_2CH_2SNa$	45	60
IX	lactose	44	
X	$-CH_2CH_2CHOHCH_3$	35	
XI	$-CH_2CH_2OCH_3$	34	
XII	$-CH_2CH_2SNa$	30	55
XIII	$-CH_2CHOHCH_2Cl$	20	
XIV	$-CH_2CH_2SCN$	15	45
XV	$-CH_2CH_2CONH_2$	12	
XVI	$-CH_2CH-CH_2$ (O)	9	
XVII	$-CH_2CH_2-$	0	
XVIII	$-CH_2CH_2COCH_3$	0	
XIX	$-(CH_2)_7CH_3$	0	55
XX	$-(CH_2)_5CH_3$	0	50
XXI	$-CH_2CHOHCH_2-$	0	
XXII	$-CH_2CH_2NH_2.HCl$	0	
XXIII	$-CH_2COOH$	0	
XXIV	$-CH_2CH_2COONa$	0	
XXV	$-CH_2CH_2COOCH_3$	0	

To estimate whether certain thiogalactosides were substrates of β-galactosidase, paper chromatography of the reaction products was used[14]. For this purpose, β-galactosidase was induced by isopropyl 1-thio-β-D-galactopyranoside (IPTG) (Lachema, Czechoslovakia) for 3 h. The bacteria were then concentrated by centrifugation, washed with a medium free of any carbon source, suspended in 1/20 of the original volume and sonicated. The suspension was clarified and stored in deep-freeze. The protein concentration of the preparation was 2 mg/ml. 0·1 ml of the preparation was mixed with 10^{-3} mol of the substrates and incubated for 1 h at 37°C. Protein was then removed by barium hydroxide precipitation[15]. The sample was concentrated by vacuum evaporation and chromatographed on paper Whatman No 1 in n-butanol–acetic acid–water (4 : 1 : 5). Carbohydrate derivatives were detected by ammoniacal silver nitrate solution[16].

RESULTS

A variety of inducing capacities for the lac operon, as measured by the β-galactosidase activity, is observed among different thiogalactosides (Table I). Derivatives with short aliphatic chains and those with short aliphatic chains (up to 4 carbon atoms in length) containing hydroxyl groups are the best inducers. Thiogalactosides with a carboxyl group in their alkyl moiety and those with a long aliphatic chain (six carbon atoms and longer) are inactive. Thiogalactosides substituted by other groups have a broad spectrum of activities: An amino group seems to abolish the activity, since 2-aminoethyl 1-thio-β-D-galactopyranoside hydrochloride (*XXII*) does not induce galactosidase. However, 2-carbamoylethyl 1-thio-β-D-galactopyranoside (*XV*) has a weak inducing capacity. Disaccharides such as 1,3-bis-(1-thio-β-D-galactopyranosyl) 2-hydroxypropane (*XXI*) and 1,2-bis-(1-thio-β-D-galactopyranosyl) ethane (*XVII*) are inactive. On the other hand, allolactose, formed by β-galactosidase from lactose[10], is known to be active. The cyano group does not abolish the inducing capacity (compounds *VII* and *XIV*). Also 2-methoxyethyl 1-thio-β-D-galactopyranoside (*XI*) and sodium salts of 2-mercaptoalkyl 1-thio-β-D-galactopyranosides (*VIII* and *XII*) are inducers. Substitutions by a chlorine atom or an epoxy group decrease the activity (*cf.* compounds *XIII* and *XVI* with *V* and *VI* respectively).

Most of the thiogalactosides tested have also inhibitory effects on the reaction of β-galactosidase with ONPG. The most powerful inhibitors are sodium salts of 2-mercaptoalkyl thiogalactosides (*VIII* and *XII*) and thiogalactosides with long aliphatic chains (hexyl and octyl thiogalactosides; *XX*, *XIX*). Mercaptoalkyl thiogalactosides are inducers at the same time. None of the noninducing thiogalactosides (Table I, *XVII*–*XXV*) was found to inhibit the induction.

The kinetics of the induction by several thiogalactosides shows that IPTG (*I*) is the best inducer of all the compounds tested (Fig. 1). No conversion of the inducers by bacteria was detected by paper chromatography during these experiments.

The results indicate that the length of the substituent on sulphur, if shorter than five carbon atoms, is not decisive for the inducing capacity. Thus, the activities of methyl 1-thio-β-D-galactopyranoside (*II*) and 4-hydroxybutyl 1-thio-β-D-galacto-

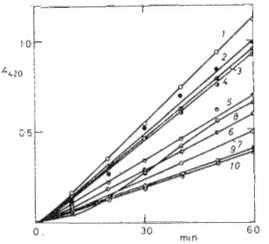


FIG. 1

Induction of β-Galactosidase

Inducers were added in 10^{-3}M concentrations at 0 time. *1* Isopropyl thiogalactoside (*I*); *2* methyl thiogalactoside (*II*); *3* 4-hydroxybutyl thiogalactoside (*III*); *4* 2-hydroxyethyl thiogalactoside (*IV*); *5* 2-hydroxypropyl thiogalactoside (*V*); *6* 3-hydroxypropyl thiogalactoside (*VI*); *7* 2-cyanoethyl thiogalactoside (*VII*); *8* lactose; *9* 3-hydroxybutyl thiogalactoside (*X*); *10* 2--methoxyethyl thiogalactoside (*XI*).

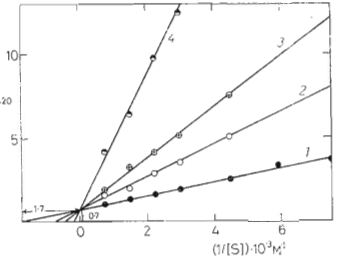


FIG. 2

Effect of the Sodium Salt of Mercaptoethyl Thiogalactoside (*XII*) on the Kinetics of the Degradation of ONPG by β-Galactosidase (Lineweaver—Burk plot)

[s], Concentration of ONPG; *1* control; *2* 3.10^{-4}M *XII*; *3* 1.10^{-3}M *XII*; *4* 4.10^{-3}M *XII*.

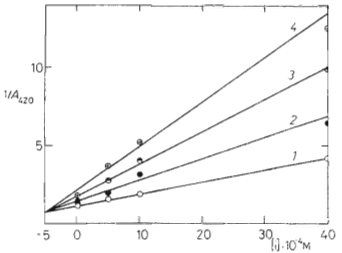


FIG. 3

Estimation of K_I of Sodium Salt of Mercaptoethyl Thiogalactoside (*XII*) (Plot according to cit.[18])

[i], Concentration of *XII*; ONPG concentrations: *1* $1{\cdot}3.10^{-3}$M; *2* $6{\cdot}6.10^{-4}$M; *3* $3{\cdot}3.10^{-4}$M; *4* $1{\cdot}3.10^{-5}$M.

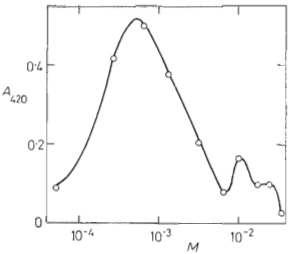


FIG. 4

Optimum Inducing Concentration of the Sodium Salt of Mercaptoethyl Thiogalactoside (*XII*)

β-Galactosidase activity is plotted against the *XII* concentration.

pyranoside (*III*) aproach that of IPTG (*I*). Rather, the character of the side chain substitution is important.

It was interesting to investigate the type of inhibition by 2-mercaptoethyl thiogalactoside (*XII*), one of the most powerful inhibitors of all tested. First, the K_m value of ONPG was calculated and found to be $5{\cdot}7 \cdot 10^{-4}$M (Fig. 2). It is in good agreement with the value obtained with the enzyme isolated from *Escherichia coli K 12* (cit.[8]). As shown in Fig. 2, compound *XII* is a competitive inhibitor with $K_I = 5 \cdot 10^{-4}$M (Fig. 3). It is a better competitive inhibitor of β-galactosidase than *p*-aminophenyl 1-thio-β-D-galactopyranoside[17].

We were not able to reveal any significant degradation of the compound *XII* by a crude β-galactosidase preparation. Lactose and ONPG were completely converted to galactose under the same conditions. The lysate of a noninduced culture did not degrade any of the above mentioned substrates. These results indicate that the compound *XII* is not a substrate of the enzyme.

After the induction of β-galactosidase by *XII*, the inducer may be removed from the medium so that it does not interfere with the subsequent assay of the enzyme. If the inducer was left in the medium during the enzyme assay, it had an inhibitory effect, especially at high concentrations (Fig. 4). The optimum concentration of *XII* for the induction of galactosidase is in the range of $5 \cdot 10^{-4}$ to $1 \cdot 10^{-3}$M.

DISCUSSION

It is difficult to correlate the results obtained so far by us and other authors concerning the structural requirements of the repressor for an effector molecule, since the effects of galactosides were followed *in vivo*. The affinity of the purified repressor to these compounds is unknown.

Another serious limitation of our experiments is the uncertainty about the permeation of these compounds into the cell. Thus, it might be possible that some galactosides have no inducing activity because they do not penetrate the cell membrane. To show convincingly the lack of the functional affinity to the repressor, radioactive thiogalactosides would have to be available.

In spite of these limitations one can see that thiogalactosides substituted by short aliphatic hydrocarbon chains, both unsubstituted and hydroxyl substituted, are the best inducers. The cyano group, carbamoyl group, methoxy group, mercapto group and epoxy group in short aliphatic chains are also active. The substitutions of thiogalactosides by the mercaptoalkyl group, cyanoalkyl group or long aliphatic chain are necessary for inhibiting β-galactosidase. Among the compounds tested, several had inductory as well as inhibitory properties. One of them, sodium salt of mercaptoethyl thiogalactoside, was shown to be a competitive inhibitor that binds to the enzyme with an affinity similar to that of ONPG.

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