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The lactose system in *Klebsiella aerogenes* V9A

3. Specific repression of the *lac* operon by melibiose and raffinose

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SUMMARY

In *Klebsiella aerogenes* strain V9A melibiose and raffinose fail to induce the *lac* operon and strongly repress its induction by isopropyl-β-D-thiogalactopyranoside (IPTG). This repression is specific to the *lac* system and is not released by cAMP, which releases the catabolite repression exerted by glucose and sucrose. It is concluded that melibiose and raffinose bind to the *lac* repressor molecule in *Klebsiella* V9A, competitively displacing IPTG, and that the resulting complex is able to repress the *lac* operon. Other *Klebsiella* strains tested show the same repression phenomenon.

INTRODUCTION

In *Escherichia coli* melibiose is a good inducer of the *lac* operon (Pardee, 1957; Jacob & Monod, 1961; Schmitt, 1968), while raffinose, which may be considered as either sucrosyl-galactose or fructosyl-melibiose, can enter by but cannot induce the *lac* permease. Neither raffinose nor sucrose is metabolised by *E. coli* (Lester & Bonner, 1957; Schaefler, 1967).

Klebsiella species are normally able to grow on melibiose, raffinose and sucrose, as well as on lactose as sole carbon source, and we have shown that in *Klebsiella aerogenes* strain V9A melibiose and raffinose induce and enter by a single permease: if this permease is inactivated neither sugar is taken up unless another permease (either the *lac* permease or a third galactoside permease) is switched on by another inducer (Reeve & Braithwaite, 1973). Thus *Klebsiella* differs from *E. coli* in the interactions of melibiose and raffinose with the *lac* operon. Data on these interactions in *Klebsiella aerogenes* V9A are presented below.

MATERIALS AND METHODS

Abbreviations. c-AMP, Adenosine 3′,5′-cyclic monophosphoric acid. Lac, lactose. Mel, melibiose. IPTG, Isopropyl-β-D-thiogalactopyranoside. ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

Bacteria, media and chemicals. The experiments were all performed with *K. aerogenes* V9A Thy3, a thymine-requiring mutant of the wild-type strain V9A (Reeve & Braithwaite, 1972). Growth tests and enzyme assays were carried out on cells growing in M 9 minimal medium, containing 0·6 % Na_2HPO_4, 0·3 % KH_2PO_4, 0·05% NaCl, 0·1% NH_4Cl, 0·025 % $MgSO_4$ and 0·0022 % $CaCl_2$. Thymine was added at 80 μg/ml and carbon sources at the concentrations indicated. The carbon sources were obtained from Sigma London Chemical Co., except for glycerol and tri-sodium citrate, which were AnalaR grade from BDH Chemicals Ltd, Poole, England. cAMP, IPTG and ONPG were from Sigma.

Assay of β-galactosidase followed the protocol of Reeve & Braithwaite (1973).

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RESULTS

Table 1 shows the specific activities of β-galactosidase in cells of strain V9A growing in minimal medium on citrate, melibiose and raffinose as carbon source, with and without previous induction by IPTG. In uninduced cultures the three carbon sources give about the same enzyme level, so it is clear that melibiose and raffinose fail to induce the *lac* operon. More surprising, both these sugars strongly repress the ability of IPTG to induce the *lac* system, the induced enzyme level being less than 10 % of that in citrate-grown cells.

One possible explanation of these results is that melibiose and raffinose fail to bind to the *lac* repressor molecule of *Klebsiella* and also exert catabolite repression on the *lac* operon. If this is the case, then cAMP will have the same effect on repression by melibiose and raffinose as it does on glucose repression (Pastan & Perlman, 1968). cAMP had no effect on glucose repression in V9A when tested at 2 mM, and treatment of the cells with EDTA by the method of Leive (1965) before cAMP treatment was also found ineffective.

Table 1. *β-galactosidase levels in* Klebsiella *V9A*

Carbon source	Not induced	Induced
Citrate	2·36	202
Melibiose	1·95	18·0
Raffinose	1·98	16·2

Bacteria grown fully in minimal medium on the carbon sources indicated were diluted into the same medium and grown until the cells were in log phase, and then divided to give the induced and uninduced cultures. Induction was for 2 h before assay. Sonicates were assayed and then enzyme activity adjusted to OD 550 1·0 for live cells to obtain the figures quoted. Incubation at 37 °C.

Table 2. *Effect of cAMP on repression by glucose and melibiose of β-galactosidase activity in the presence of IPTG: strain V9A*

	Increase in enzyme activity (units/ml), cAMP added:	
Other additions	Nil	10^{-2} M
None	22·7	22·7
0·4 % glucose	4·3	22·2
0·4 % melibiose	6·3	3·6

Cells growing in minimal citrate (0·5 %) were induced for two cell generations with 0·1 mM IPTG and aliquots then received the additions shown, at time 0. Samples were chilled, sonicated and assayed for β-galactosidase activity 30 and 60 min later. The table shows the increase in enzyme activity (units/ml) from 30 to 60 min. Duplicate cultures received each treatment. At time 0, the OD 550 was 0·165. Incubation at 37 °C.

However, glucose repression was completely released by 10 mM cAMP (used without EDTA treatment), a concentration which Brenchley & Magasanik (1972) also found effective on another strain of *K. aerogenes*. Addition of cAMP to 10 mM reduced growth rate of cells utilizing all the carbon sources tested, but the cause of this effect is unknown.

Table 2 shows the effect on β-galactosidase activity of growing strain V9A for two cell generations in minimal citrate plus IPTG, then adding glucose or melibiose, either alone or together with cAMP. The increase in enzyme activity during the period 30–60 min

after the additions were made is taken as the index for measuring repression. Glucose reduced the index by about 75 %, and this repression was completely released by adding cAMP at the same time as the glucose. Melibiose also repressed the enzyme level sharply, but cAMP had no effect on this repression. A similar test on the effects of sucrose and raffinose is given in Table 3, and shows that both sugars caused a sharp repression of enzyme activity, but only that by sucrose was affected by cAMP. Thus glucose and sucrose exert catabolite repression on the *lac* operon of V9A, while melibiose and raffinose exert an equally drastic effect which cannot be attributed to catabolite repression.

Table 3. *Effect of cAMP on repression by sucrose and raffinose of β-galactosidase activity in the presence of IPTG: strain V9A*

	Increase in enzyme activity (units/ml), cAMP added:	
Other additions	Nil	10^{-2} M
None	8·9	—
0·4 % sucrose	2·9	15·4
0·4 % raffinose	0·3	2·6

Methods as Table 2. At time 0, OD 550 was 0·102. —, not measured.

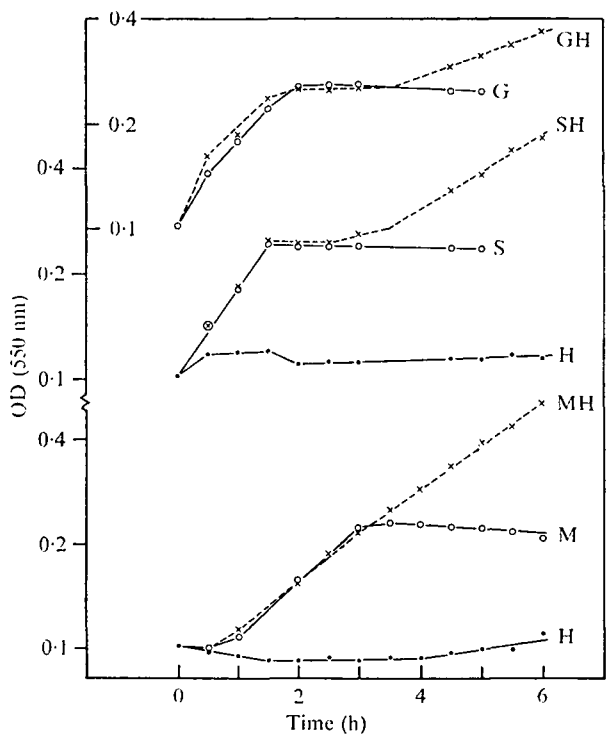

Fig. 1. Repression of histidase biosynthesis in strain V9A. Minimal-grown cells were washed and transferred to M 9 minimal medium containing a limiting supply of a carbon source (0·01 % glucose (G), sucrose (S) or melibiose (M)) with or without 0·4 % histidine (H). A control series had histidine 0·4 % as sole carbon source. All carbon sources were added to warm medium at time 0. Incubation at 37 °C.

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Neidhart & Magasanik (1957) observed strong catabolite repression of histidase by glucose in a strain of *Klebsiella aerogenes* when the cells were growing in minimal medium containing glucose, histidine and a source of nitrogen other than histidine, and this repression was also released by cAMP (Prival & Magasanik, 1971). A similar test was therefore applied to V9A, cells grown to stationary phase in minimal glycerol medium being diluted directly about tenfold into warm minimal medium containing 0·4 % histidine and a limited supply (0·01 %) of glucose, sucrose of melibiose. As controls, either histidine or the sugar was omitted, and growth was followed by optical density readings at 500 nm. In these conditions catabolite repression will be revealed as a diauxic lag when the bacteria have exhausted the sugar and start to utilize histidine as carbon source.

This experiment (Fig. 1) shows that, in V9A, both glucose and sucrose repressed histidase biosynthesis (curves GH and SH), but no such repression occurred during growth on melibiose (curve MH showed no lag after exhaustion of the melibiose). In similar experiments raffinose also failed to repress histidase biosynthesis. It is interesting that there was no growth during the 6 h of the test when the stationary phase cells were diluted into medium containing histidine without another carbon source (curves H). This is probably explained by the fact that histidase is induced by urocanic acid, its product, and not by histidine itself (Prival & Magasanik, 1971). Presumably the small amount of urocanate made by the basal level of histidase in the newly transferred cells was metabolized too rapidly to allow induction of histidase.

DISCUSSION

The experiments described above indicate that both melibiose and raffinose exert strong repression on the *lac* operon of *Klebsiella* V9A, which is specific to this operon and is not released by cAMP. Repression occurs both when IPTG is added to cells already growing on melibiose of raffinose, and when either sugar is added to cells already induced to a high level of β-galactosidase activity by IPTG when growing on citrate. In interpreting these results is should be borne in mind that melibiose (disaccharide) is 6-(α-D-galactopyranosyl)-D-glucopyranose, while raffinose (trisaccharide) is α-D-galactopyranosyl-(1 $\rightarrow$ 6)-α-D-glucopyranosyl-(1 $\rightarrow$ 2)-β-D-fructofuranoside, so that raffinose contains melibiose as a subunit and similar induction effects might be expected with both substances. Reeve & Braithwaite (1973) have in fact shown that melibiose and raffinose are both substrates of the melibiose permease and also induce it in *Klebsiella* V9A.

In *Escherichia coli* melibiose induces the *lac* operon, whether or not it is metabolized by an active α-galactosidase (Schmitt, 1968, table 2), and the most probable explanation for the difference between the two species is that melibiose binds to the *lac* repressor in both to form a complex which can bind (or remain bound) to the operator in *Klebsiella* but not in *E. coli*. This hypothesis also requires that melibiose and raffinose can displace IPTG competitively from the previously formed IPTG-repressor complex. A very similar interaction in *E. coli* is the competitive inhibition of induction by TMG of the *lac* operon brought about by certain galactoside analogues such as 2-nitrophenyl-β-D-fucoside and 2-nitrophenyl-6-*O*-methyl-β-D-galactoside (Müller-Hill, Rickenberg & Wallenfels, 1964). Several examples are also known of inducers or compounds structurally related to them which repress enzyme synthesis or induction in certain strains carrying a constitutive mutant, both in the *lac* operon of *E. coli* (Williams & Paigen, 1968; Jobe & Bourgeois, 1972) and in *Pseudomonas aeruginosa* (Brown & Clarke, 1970); but the situation in *Klebsiella* described in this paper seems to be the only case so far of substrates for one inducible enzyme repressing induction of another catabolic enzyme system by interacting with its repressor. This situation seems to be more useful for the species than that in

E. coli, where growth on melibiose maintains in the cells a high concentration of unneeded β-galactosidase.

Tests on a number of other *Klebsiella* strains, unrelated to V9A, show that in all of them melibiose and raffinose repress β-galactosidase synthesis and this repression is not released by cAMP. Thus specific repression of this kind appears to be a general characteristic of *Klebsiellas*. The data from these tests will be published separately.

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