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Metabolite Gene Regulation: Imidazole and Imidazole Derivatives Which Circumvent Cyclic Adenosine 3',5'-Monophosphate in Induction of the *Escherichia coli* L-Arabinose Operon

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Imidazole, histidine, histamine, histidinol phosphate, urocanic acid, or imidazolepropionic acid were shown to induce the L-arabinose operon in the absence of cyclic adenosine 3',5'-monophosphate. Induction was quantitated by measuring the increased differential rate of synthesis of L-arabinose isomerase in *Escherichia coli* strains which carried a deletion of the adenyl cyclase gene. The *crp* gene product (cyclic adenosine 3',5'-monophosphate receptor protein) and the *araC* gene product (P2) were essential for induction of the L-arabinose operon by imidazole and its derivatives. These compounds were unable to circumvent the cyclic adenosine 3',5'-monophosphate in the induction of the lactose or the maltose operons. The L-arabinose regulon was catabolite repressed upon the addition of glucose to a strain carrying an adenyl cyclase deletion growing in the presence of L-arabinose with imidazole. These results demonstrated that several imidazole derivatives may be involved in metabolite gene regulation (23).

The L-arabinose operon, *araBAD*, the permease gene, *araE*, and a gene for the binding protein for L-arabinose, *araF*, comprise the regulon for L-arabinose in *Escherichia coli* B/r (Fig. 1) (5, 19, 22, 29). Conversion of L-arabinose to D-xylulose-5-phosphate is a result of the action of enzymes produced from the *araA*, *araB*, and *araD* structural genes. The *araBAD* operon is located between *pyrA* and the leucine operon and can be fused to the leucine operon in such a way as to place the *araBAD* structural genes under control of the leucine operon (6). The remaining genes, *araE* and *araF*, are separated genetically and are unlinked to the arabinose operon (5, 22, 29). Another permease which is not part of the L-arabinose regulon also functions in L-arabinose accumulation within the *E. coli* cell (18). A regulator gene, *araC*, is adjacent to the L-arabinose operon and is one of the primary proteins that is responsible for repression and induction at the control region for the *araBAD-*, *araE-*, *and araF*-inducible regulon (9, 15, 17).

Induction of the L-arabinose operon and other catabolic systems such as the maltose and the lactose operons requires a general positive control mechanism which utilizes cAMP and the cAMP receptor protein (CRP) (2, 10, 14, 31, 32, 39). Strains carrying an adverse mutation in the adenyl cyclase structural gene (*cya*), the enzyme required for the synthesis of cAMP, can no longer utilize various carbohydrates (31). This lack of carbohydrate utilization can be reversed with the addition of 1 mM cAMP (31). In addition, strains with a nonfunctional *crp* gene also lack the ability to utilize a variety of carbohydrates including L-arabinose (10, 14, 19). However, in contrast to the strains carrying mutation in the adenyl cyclase gene (*cya*), the inducible operons in strains carrying mutations in the cAMP receptor protein gene (*crp*) cannot be compensated for the addition of exogenous cAMP (10, 14).

Another mutation which can render *E. coli* negative for L-arabinose utilization, even in the presence of cAMP and CRP, is a mutation in the *araC* gene (15, 19). All three components, cAMP, CRP, and the product of the *araC* gene, are essential for the binding of RNA polymerase and the transcription of the structural genes involved in L-arabinose degradation (19).

Heffernan et al. (19) further demonstrated that certain mutations in the *araC* gene ($araC^i$) eliminated the requirements for the cAMP-CRP complex in RNA polymerase binding to the transcription initiation site of the L-arabinose operon. These observations suggested to us the possible presence of overlaps in the binding sites for the positive effectors cAMP-CRP and *araC* gene product (P2).

Recently we demonstrated that imidazoleacetic acid could circumvent the necessity for

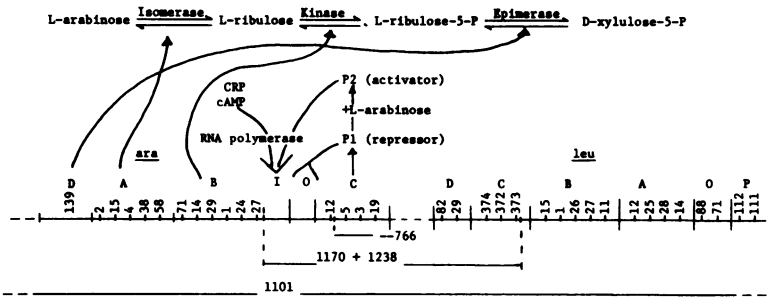

FIG. 1. *The L-arabinose and L-leucine operons in E. coli B/r which were transferred into E. coli K-12, showing the extent of various deletions used, the enzymes involved in the conversion of L-arabinose to D-xylulose-5-P, and various control factors involved in the regulation of the L-arabinose operon. The deletion indicated as 1170 + 1238 in the figure is Δ(ara-leu)1170 ara-1238. We formerly (6) used ara-leu 1170+1238$^{\Delta}$ to designate this deletion.*

cAMP in the induction of the *ara* operon but was ineffective in the induction of the *lac* or *mal* operon, a phenomenon which we defined as metabolite gene regulation (MGR) (23). Indoleacetic acid, was also observed to function in this capacity (E. L. Kline et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, K104, p. 162; Proc. Natl. Acad. Sci., in press). These studies suggested that cellular metabolites other than cAMP have the potential of facilitating gene expression in vivo.

In this manuscript we have expanded the concept of MGR by defining other imidazole derivatives which can control the expression of the L-arabinose operon and are thus potential MGR molecules. Furthermore, we have shown that this induction by imidazole compounds can be catabolite repressed by glucose.

MATERIALS AND METHODS

Bacterial strains. The genotypes and the derivations of bacterial strains used are given in Table 1.

Media. The minimal base media and the complex media used have been described by Sheppard and Englesberg (34). When required, the minimal base medium was supplemented with the following to yield a final concentration of: 0.5% D-glucose, 0.4% L-arabinose, 0.4% D-maltose, 0.4% D-lactose, 0.4% D-rhamnose, 0.4% D-xylose, 0.4% mM L-leucine, 0.4 mM L-histidine, 0.4 mM L-tryptophan, 1 mM cAMP, and 10 mM imidazole.

Chemicals. L-Arabinose, D-xylose, cAMP, imidazole, histamine (free base), histidinol dihydrochloride, histidinol phosphate, imidazolelactic acid, imidazoleacetic acid, urocanic acid, glycylglycine, and the amino acids were purchased from Sigma Chemical Co. Thiazole was purchased through Pfaltz and Bauer, and ethyl methyl sulfonate was obtained from Calbiochem. Imidazolepropionic acid was a gift from Irving Klotz. Carbazole, sulfuric acid, manganous chloride, and other reagent salts were of highest purity and were obtained from Fisher Chemical Co.

Transductions. Constructions of derivative strains were performed by P1 transduction experiments as described by Gross and Englesberg (18).

Mutagenesis screen. Imidazole and a variety of imidazole derivatives were screened for their mutagenic potential by the method of B. N. Ames et al. (1) by using strains TA98, TA100, and TA1537 and the suggested control mutagens to check for reversion.

Reversion analysis. Reversion analysis was carried out as previously described (23).

Plate induction test. A 36-h single colony of strain KC13 (Δ*cya-2*) which had been phenotypically characterized was diluted by removing the colony from a glucose-leucine minimal plate and streaking radially onto separate leucine-supplemented plates containing lactose, maltose, arabinose, or glucose as the sole carbon source. Crystals of the compound to be tested were then placed in the center of the radiating streaks as indicated in Fig. 2. A control colony was radially streaked on another part of the same plate and was not supplied with crystals. A single colony of strain KC14 grown on minimal glucose medium was streaked in an identical manner onto a minimal medium plate with no carbon supplement. Crystals of imidazole or one of its derivatives were placed in the center of the streaks to determine whether the compound tested could be used as a carbon source.

Growth kinetics and extract preparation. Cell growth was monitored and cell extracts were prepared by the method of Kline et al. (23).

In the catabolite repression studies, the cells were grown in L-arabinose and 10 mM imidazole to an absorbance at 660 nm (A_{660}) of 0.35. At this density the culture was split. One-half of the culture was allowed to grow without treatment as it had before the split. To the other half of the culture was added glucose to a final concentration of 0.5%. Growth was subsequently followed with time in both of these cultures. Samples were also taken at each of these times for determination of L-arabinose isomerase activity.

L-Arabinose isomerase assay. L-Arabinose isomerase (EC 5.3.1.4) activity was assayed as described elsewhere (23).

Protein determination. Protein concentrations of the cell extracts were determined by the method of Lowry et al. (24).

TABLE 1. *Bacterial strains*[a]

Strains	Genotype	Source
F⁻ strains		
B/r UP1007	Wild type	E. Englesberg
K-12 LS853	*his-85* λ⁻ *trpA9605 trpR55 Δcya-2*	B. J. Bachmann
B/r DC1	*Δ(ara-leu)1119 pro-1* T6ʳ *trp-10 dau-5* Strʳ	D. P. Kessler
B/r SB2074	*Δ(ara-leu)1170 ara-1238 dau-5* Strʳ	D. P. Kessler
B/r SB5000	*leuB1 dau-5*	Nancy Lee
B/r SB5004	*leuB1 dau-5 arg-2*	DES-induced Arg⁻ mutant of SB5000
B/r DC7	*ΔaraC766 dau-5 arg-2*	P1 *bt* SB1085 × SB5004
B/r DC71	*leuB1 mal*⁺ λˢ	D. P. Kessler
B/r DC74	*Δ(ara-leu)1170 ara-1238 dau-5 mal*⁺ λˢ Strʳ	P1 *bt* DC71 × SB2074
K-12 CU356	*galT-12 ΔilvDAC115 leu-455*	EMS-induced Leu⁻ mutant of CU344 (8)
K-12 CU359	*galT-12 ΔilvDAC115 pdxA204*	P1 *bt* WG1473 × CU356
K-12 KC2	*galT-12 ΔilvDAC115 ara C766*	P1 *bt* 7 × CU359
K-12 KC4	*galT-12 ΔilvDAC115(ara-leu)1170 ara-1238*	P1 *bt* 74 × CU359
K-12 KC7	*galT-12 ΔilvDAC115*	P1 *bt* DC1 × CU359
K-12 KC8	*galT-12 ΔaraC766 Δcya-2*	P1 *kc* KC13 × KC2
K-12 KC10	*galT-12 Δ(ara-leu)1170 ara-1238 Δcya-2*	P1 *kc* LS853 × KC4
K-12 KC13	*galT-12 Δcya-2*	P1 *kc* LS853 × KC7
K-12 KC14	*galT-12*	P1 *kc* LS853 × KC7
K-12 KC15	*galT-12 ΔaraC766*	P1 *kc* KC13 × KC2
K-12 KC22	*galT-12 Δcya-2 crp* Strʳ	P1 *kc* EB1078 × KC13
B/r SB5616	*Δ(ara-leu)1119 dau-5 cya-4 crp* Strʳ	G. Wilcox
B/r EB1078	*dau-5 cya-4 crp* Strʳ	P1 *bt* UP1007 × SB5616
B/r DC455	*Δ(ara-leu)1101 araD139* Strʳ	D. P. Kessler
B/r EB1079	*dau-5* Strʳ	P1 *bt* EB1015 × DC455

[a] Auxotrophic requirements: *Δ(ara-leu)1170 ara-1238*, leucine; *Δ(ara-leu)1119*, leucine; *Δ(ara-leu)1101*, leucine; *leuB1*, leucine; *his*, histidine; *trp*, tryptophan; *pro*, proline; *ilv*, isoleucine and valine; *pdxA204*, arginine and uracil. Abbreviations: *dau-5*, D-arabinose negative; Strʳ, streptomycin resistant; λˢ, *E. coli* B/r sensitive to lambda infection; EMS, ethyl methyl sulfonate; *cya*, adenyl cyclase deficient; *crp*, a negative cAMP receptor protein; *gal*, D-galactose negative; *ara*, L-arabinose negative.

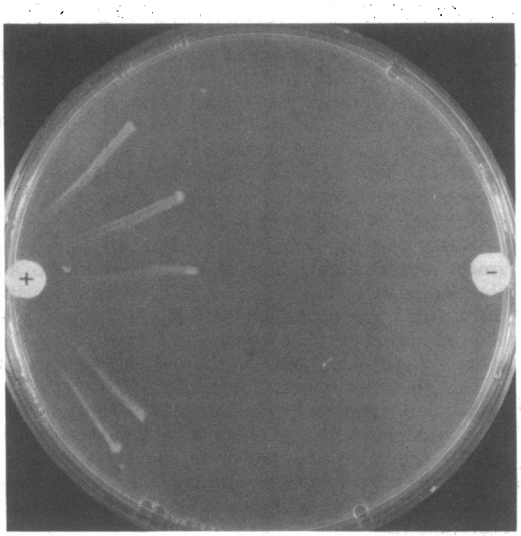

FIG. 2. *A 48-h plate induction response for* L*-arabinose utilization by KC13 (Δcya-2) using imidazole or its derivatives. +, Presence of compound crystals; −, absence of crystals.*

RESULTS

Ability of imidazole and imidazole derivatives to regulate selected operons. As indicated by plate analyses (Tables 2 and 3), imidazole, histidine, histamine, histidinol phosphate, urocanic acid, and imidazole propionic acid were able to substitute for cAMP in eliciting an Ara⁺ but not a Lac⁺ or a Mal⁺ response in a *cya* deletion strain (KC13). The induction response was dependent on the cAMP receptor protein (CRP) and the *araC* gene product (P2) as demonstrated by the lack of induction in *Δcya-2* strains carrying either a *crp* lesion (KC22) or an *araC* partial deletion (KC15) (Tables 2 and 3). Other imidazole derivatives (imidazole-lactic acid, histidinol, and thiazole) could not replace cAMP in the utilization of L-arabinose in the *Δcya* background.

The data in Table 2 show that imidazole was unable to overcome the repression of the leucine biosynthetic operon in strain KC10 [*Δcya-2 Δ(ara-leu)1170 ara-1238*] plated on leucine minimal medium with L-arabinose as the sole carbon

TABLE 2. *Phenotypic characterization of imidazole (Im) circumvention of cAMP*[a]

Strain	Pertinent genotype	D-Lactose[b]			L-Arabinose			D-Maltose		
		cAMP[b]	Im[c]	H_2O[c]	cAMP	Im	H_2O	cAMP	Im	H_2O
KC14	Isogenic wild-type *galT-12*	+	+	+	+	+	+	+	+	+
KC13	Δ*cya-2 galT-12*	+	−	−	+	+	−	+	−	−
KC10	Δ*cya-2* Δ*(ara-leu)1170 ara-1238 galT-12*	+	−	−	−	−	−	+	−	−
KC8	Δ*cya-2* Δ*araC766 galT-12*	+	−	−	−	−	−	+	−	−
KC22	Δ*cya-2 crp* Str^r *galT-12*	−	−	−	−	−	−	−	−	−
DC455	Δ*(ara-leu)1101*[d]	+	+	+	−	−	−	+	+	+

[a] See Table 1, footnote *a* for abbreviations and auxotrophic requirements.
[b] Carbon source; methodology is described in the text.
[c] Molecule that substitutes for cAMP in the test.

TABLE 3. *Ability of imidazole (Im) and imidazole derivatives to circumvent the necessity for a cAMP in* L-*arabinose operon induction*[a]

Strain	Pertinent genotype	Compound tested with dilution plate method[b]									
		cAMP	Im	IL	His	Hisol	HisolP	His-am	Thiz	Uroc	IP
KC14	Wild-type *galT-12*	+[c]	+	+	+	+	+	+	+	+	+
KC13	Δ*cya-2 galT-12*	+	+	−	+	−	+	+	−	−/+	+
KC8	Δ*cya-2* Δ*araC766 galT-12*	−	−	−	−	−	−	−	−	−	−
DC455	Δ*(ara-leu)1101*	−	−	−	−	−	−	−	−	−	−
KC22	Δ*cya-2 crp* Str^r *galT-12*	−	−	−	−	−	−	−	−	−	−
KC15	Δ*araC766 galT-12*	−	−	−	−	−	−	−	−	−	−

[a] Abbreviations: His, histidine; Hisol, histidinol; HisolP, histidinolphosphate; His-am, histamine (free base); Thiz, thiazole, Uroc, urocanic acid; IL, L-β-imidazolelactic acid; IP, imidazolepropionic acid.
[b] Method for screening compounds is described in Materials and Methods.
[c] +, Strong growth after 36 h; −, no growth after 48 h; −/+, a definite weak growth response after 48 h.

source. This strain requires leucine for growth and carries an *ara-leu* fusion which places the L-arabinose genes under leucine control (6).

Growth kinetics. The rate of growth of a strain carrying the Δ*cya-2* deletion (KC13) on L-arabinose in the presence of 1 mM cAMP approached that of KC14, the isogenic wild-type strain, grown under the same conditions (Fig. 3 and 4A). When these strains were shifted to L-arabinose minimal medium without cAMP, KC14 (*cya*$^+$) continued to grow at the same rate as it did before the shift, whereas KC13 (Δ*cya-2*) began to limit in its growth at approximately 120 min after the removal of cAMP (Fig. 3).

Strain KC13 grown in the presence of L-arabinose and 10 mM imidazole had a generation time of about twice that of strain KC13 cells grown in 1 mM cAMP. Shifting the culture from the presence of 10 mM imidazole also resulted in limitation. The isogenic wild type, KC14, grew at about the same rate in the presence of 10 mM imidazole as it did in its absence (Fig. 4B). Earlier we reported similar growth kinetics for strain KC13 when 10 mM imidazoleacetic acid was used (23).

Effect of imidazole on L-arabinose isomerase expression. To quantitate the induction of the L-arabinose operon in the presence of imidazole and cAMP, we determined the enzyme activity of L-arabinose isomerase (the *araA* gene product) at various times during the growth of strain KC13 or KC14. The differential rate of L-arabinose isomerase synthesis in KC13 (Δ*cya-2*) grown in L-arabinose minimal medium supplemented with 10 mM imidazole was ca. 2.6-fold less than when this strain was grown in the presence of L-arabinose and 1 mM cAMP (Fig. 5). After the Δ*cya* strain was shifted to an L-arabinose minimal medium without cAMP or imidazole, the transcription of the L-arabinose operon (as indicated by L-arabinose isomerase activity) continued for a period of time and subsequently decreased. When growth limitation occurred after shift from the cAMP, the levels of L-arabinose isomerase were ca. 2.2-fold greater than the levels observed when limitation occurred after the shift from imidazole. A differential rate of synthesis of L-arabinose isomerase similar to that seen with imidazole has also been observed in strain KC13 during growth and limitation on imidazoleacetic acid (23). This difference in expression of the operon in the presence of cAMP versus imidazole or imidazoleacetic acid (23) perhaps reflects a difference in the metabolic state of cells grown on the positive effector, cAMP, compared to those grown on

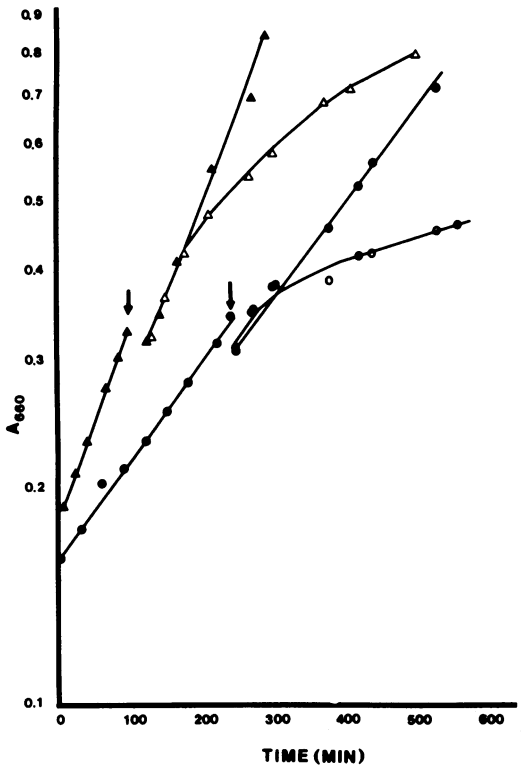

FIG. 3. *Growth of KC13 (Δcya-2) with L-arabinose as a carbon source in the presence (▲) or absence (△) of 1 mM cAMP and the presence (●) or absence (○) of 10 mM imidazole. Time of shift is indicated by the arrows. For details see text.*

imidazole or the metabolic intermediate, imidazoleacetic acid (23).

The effect of cAMP and imidazole on the expression of the L-arabinose operon in the isogenic wild-type strain KC14 is shown in Fig. 6A and B. Compared to expression of the operon in the absence of exogenous inducer, the differential rate of L-arabinose isomerase synthesis was not altered when the strain was grown in the presence of 1 mM cAMP (Fig. 6A). Comparisons of strain KC14 grown in the presence or absence of imidazole revealed a decreased rate of L-arabinose isomerase synthesis when the strain was grown in the presence of 10 mM imidazole (Fig. 6B). Shifting the imidazole-grown cells to medium with no imidazole resulted in an increase in L-arabinose isomerase activity to about the same level as that seen in cAMP-grown KC14 shifted to medium without cAMP.

Catabolite repression of the L-arabinose operon by glucose in imidazole-induced cells. When glucose was added to strain KC13 (*Δcya-2*) growing in the presence of L-arabinose and 10 mM imidazole, an increase in growth rate occurred (Fig. 7). An apparent catabolite repression in strain KC13 also occurred upon the addition of glucose as evidenced by the transient decreased differential rate of expression of L-arabinose isomerase (Fig. 8).

DISCUSSION

The ubiquitous involvement of cAMP in cellular metabolism of both eucaryotic and procaryotic systems has been clearly defined within the past 20 years (14, 26, 31, 35). In multicellular organisms, Sutherland et al. (35) first demonstrated that the action of certain hormones (the primary messenger) stimulated the activity of membrane-bound adenyl cyclase with a resultant increase in intracellular cAMP (the second messenger). This increased intracellular concentration of cAMP produced dramatic changes in the metabolic activities of the cell. In addition, Makman and Sutherland (26) revealed that cAMP was also involved in the regulation of procaryotic organisms. Pastan, Perlman, Zubay, and collaborators (10, 14, 31, 39) subsequently defined the requirement for cAMP and the CRP in the induction of the lactose, maltose, and arabinose operons. Unlike the other catabolic systems, the L-arabinose operon was found to require a combination of L-arabinose and the *araC* gene product (P2) as well as the cAMP-CRP complex for initiation of transcription (10, 14, 19, 39).

We recently reported that imidazoleacetic acid could facilitate induction of the L-arabinose operon in the absence of cAMP and proposed the concept of MGR (23). In this paper we have demonstrated that imidazole and several derivatives of this compound (histidine, histamine, histidinol phosphate, urocanic acid, and imidazolepropionic acid) which do not serve as a carbon source for the *E. coli* strains (Table 4) can also circumvent the necessity for cAMP in the induction of the L-arabinose operon (Table 2). Functional *crp* and *araC* gene products are necessary for initiation of transcription of the *ara* DNA in the presence of these imidazole derivatives (Table 3), as is the case with cAMP.

The ability of some of the imidazole derivatives to induce the L-arabinose operon but not the lactose operon agrees with the reports that there are regions of dissimilarity at the promoter locus in these systems (12, 17). The additional requirement for the L-arabinose *araC* gene product (P2) complex for initiation of transcription in the L-arabinose system also supports these observations (19).

The five-membered ring structure of imidazole is a component of the structure of cAMP. Thus, it was not entirely unexpected that such

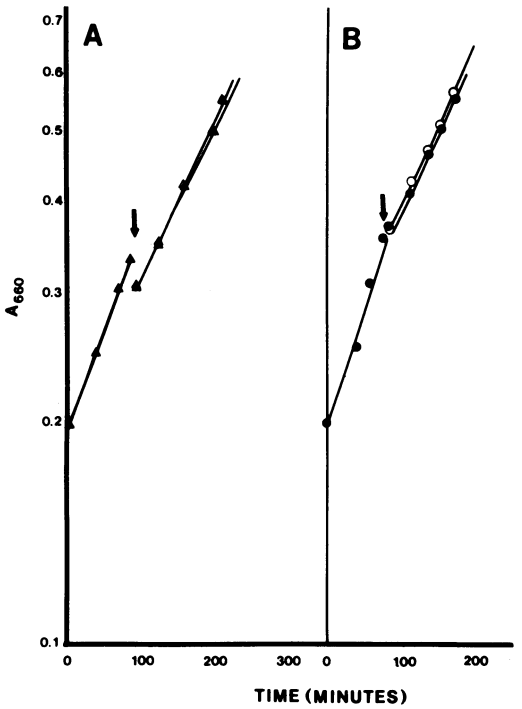

FIG. 4. *(A) Growth of KC14 (isogenic wild type) with* L*-arabinose as a carbon source in the presence* (▲) *or absence* (△) *of 1 mM cAMP. (B) Growth of KC14 with* L*-arabinose as a carbon source in the presence* (●) *or absence* (○) *of 10 mM imidazole. Shift times indicated by arrows.*

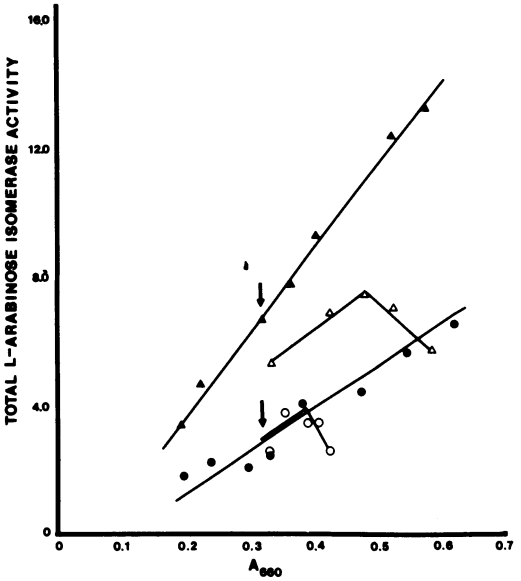

FIG. 5. *Differential rate of synthesis of* L*-arabinose isomerase in KC13 (Δcya-2) grown on* L*-arabinose in the presence* (▲) *or absence* (△) *of 1 mM cAMP or the presence* (●) *or absence* (○) *of 10 mM imidazole. Time of shift indicated by arrows. Total activity = specific activity (μmoles of* L*-ribulose formed per hour per milligram of protein) × A_{660}.*

a compound could exhibit some of the functional properties of cAMP in certain cellular reactions. The fact that the parent compound imidazole could function in the initiation of transcription of the L-arabinose system suggested that initiation of transcription did not require additional structural components attached to the ring structure.

One of these cAMP-circumventing imidazole derivatives, urocanic acid, has been proposed to induce other catabolic systems, histidase and *N*-formino-L-glutamate in *Aerobacter aerogenes* (25). It should be noted, however, that although histidine, histamine, urocanic acid, and histidinol phosphate in themselves may circumvent the cAMP necessity in the L-arabinose system, they can be metabolized by the cell and perhaps some metabolite of these compounds is the molecule that allows for the cAMP circumvention.

Another point of interest was the inability of thiazole and other imidazole derivatives (histidinol and imidazolelactic acid) to circumvent the cAMP requirement. These observations suggested to us that there are specific structures which the imidazole and imidazole derivatives must have to be capable of MGR (23).

From the data presented here it is evident that imidazole was apparently not as efficient as cAMP in the induction of the L-arabinose operon. For instance, the growth rate of strain KC13 (*Δcya-2*) was slower in the presence of 10 mM imidazole than in the presence of 1 mM cAMP (Fig. 3). There was also a slower differential rate of synthesis of L-arabinose isomerase in strain KC13 grown in the presence of imidazole versus cAMP (Fig. 4). Although the in vivo concentration of cAMP in *E. coli* under noncatabolite-repressed conditions has been estimated to be 43 μM (7), the exogenous concentration of this compound needed for maximum induction of the L-arabinose operon in a *Δcya* strain is 1 mM (26).

The growth rate of isogenic *cya*$^+$ strain KC14 with imidazole was similar to the growth rate without imidazole. However, the extent of induction of the L-arabinose operon was decreased in comparison with the strain grown in the absence of the compound. Shifting of strain KC14 from an imidazole-supplemented medium to medium without the compound resulted in an increase in L-arabinose isomerase activity to the level seen in the strain grown without imidazole (Fig. 6B). Because CRP was required for the imidazole-mediated induction of the L-arabinose

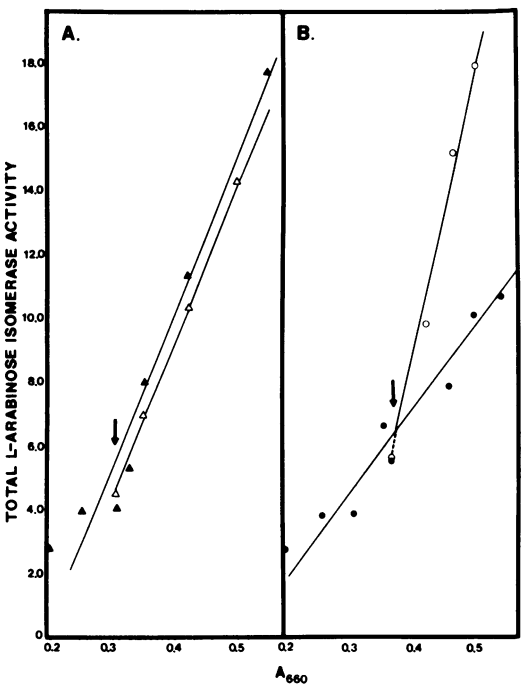

FIG. 6. *(A) Differential rate of synthesis of* L*-arabinose isomerase in* L*-arabinose-grown KC14 (cya$^+$) with* (▲) *or without* (△) *1 mM cAMP. Arrow indicates shift time. (B) Differential rate of synthesis of* L*-arabinose isomerase in* L*-arabinose-grown KC14 (cya$^+$) with* (●) *or without* (○) *the presence of 10 mM imidazole. Arrow indicates shift time.*

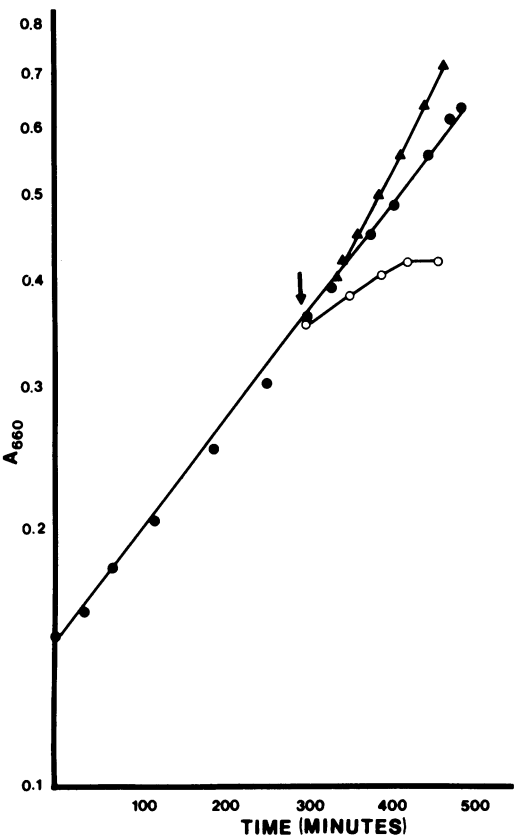

FIG. 7. *Growth of strain KC13 on* L*-arabinose and 10 mM imidazole with* (▲) *or without* (●) *the addition of glucose. The arrow indicates the point at which glucose was added. At the same time, part of the culture was shifted from* L*-arabinose and 10 mM imidazole medium to* L*-arabinose without imidazole* (○) *to check the ability of the culture to limit.*

operon in the *cya*-negative background (Table 2), it was reasoned that the lower expression of L-arabinose isomerase seen in the isogenic *cya*$^+$ background (KC14) in the presence of imidazole was due in part to a competition between imidazole and cAMP for CRP or for the DNA initiation sites. This competition, as well as the increased activation of phosphodiesterase by imidazole (4), would result in a decrease in the concentration of the more effective inducer complex, cAMP-CRP, subsequently leading to a decrease in the degree of expression of the L-arabinose operon.

Catabolite repression has formerly been attributed to changes in intracellular concentrations of cAMP when glucose was added to bacterial cells growing on an alternate carbon source such as lactose, maltose, xylose, or galactose (33). However, as shown in this study, glucose can apparently repress the catabolites of strain KC13 (Δ*cya-2*, adenyl cyclase deletion) growing on L-arabinose and 10 mM imidazole. These results demonstrate that in *E. coli* the phenomenon of catabolite repression by glucose can occur independently of changes in concentrations of cAMP. They may, in fact, suggest that factors such as the catabolite modulator factor described by Ullman and co-workers (11, 36) are involved in this repression.

It is becoming increasingly evident from studies in our laboratory, on both procaryotic and eucaryotic cells, that metabolites other than cAMP can participate in the control of cellular activity at the genetic level. This concept we have defined previously as MGR (23). The understanding of the mechanism through which these metabolites circumvent the cAMP necessity at the molecular level is not clear. An interesting point, however, is that the work by Hendry and co-workers with CPK molecular models has shown that indoleacetic acid and histidine (20, 21, 37), as well as cAMP, (L. B. Hendry, personal communication) can stress hydrogen bonding of double-stranded DNA or RNA. This disruption may very well be one of the ways of

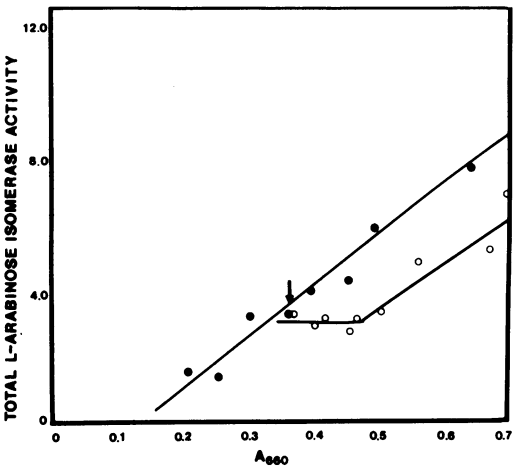

FIG. 8. *Differential rate of synthesis of L-arabinose isomerase in strain KC13 growing on L-arabinose and 10 mM imidazole with (○) or without (●) glucose. Arrow represents the point at which glucose was added to the culture.*

TABLE 4. *Carbon utilization analysis of imidazole (Im) derivative compounds*[a]

Compound tested	Growth of KC14 (isogenic wild type) at h tested:[b]				
	12	24	36	48	96
D-Glucose (control)	+	+	+	+	+
L-Arabinose (control)	+	+	+	+	+
cAMP	−	−	−	−	−
Im	−	−	−	−	−
His	−	−	−	−	−
HisolP	−	−	−	−	−
His-am	−	−	−	−	−
Thiz	−	−	−	−	−

[a] See Table 3, footnote *a* for abbreviations.

[b] See text for explanation of carbon utilization analysis.

enhancing RNA polymerization at selective regions on the DNA.

In this paper we have provided additional support for the concept of MGR by identifying other imidazole metabolites (histidine, histamine, histidinol phosphate imidazolepropionic acid, and urocanic acid) which can circumvent the necessity for cAMP in the induction of the L-arabinose operon. We have also recently shown that indoleacetic acid (Kline et al., in press) can control gene expression in various bacterial systems and possibly in an animal tissue culture system. Hence, there is a growing body of evidence which suggests that naturally occurring metabolites can control genetic expression in a manner analogous to gene control by cAMP.

On the basis of our findings and those of Hendry et al. (20, 21, 37; personal communication), we suggest that MGR may occur in the following manner. (i) Metabolite or metabolites selectively direct a pre-initiation complex to associate with specific DNA regions producing local disruption of hydrogen bonding at promoter sites. (ii) Binding of these metabolite pre-initiation complexes produces a high affinity site for RNA polymerase. (iii) RNA polymerase binds to the promoter regions affected and initiates transcription.

Further elucidation of the actual mechanism involved in MGR is presently under investigation in our laboratory.

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