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IMIDAZOLE ACETIC ACID AS A SUBSTITUTE FOR cAMP

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SUMMARY

The ability of imidazole acetic acid (IA) to substitute for cAMP was demonstrated by use of a series of strains carrying a lesion in the cya structural gene. The substitution of IA for cAMP was specific for the L-arabinose operon in that this compound was ineffective in substituting for cAMP in the lactose or maltose catabolic systems. The cAMP receptor protein (CRP) and the araC gene product were necessary for the IA mediated induction of the L-arabinose system.

Catabolite repressed systems in Escherichia coli require cyclic adenosine-3',5' monophosphate (cAMP) and the cAMP receptor protein (CRP) for induction (1-8). In addition, the product of the araC gene (P2) is needed for transcription of the structural genes involved in L-arabinose degradation (7-8). Hence, adverse mutations in cya (the gene coding for adenyl cyclase), crp (the gene coding for CRP) or araC can result in the inability of a strain to utilize L-arabinose as a carbon source.

Certain mutations in the araC gene were observed to circumvent the requirements for the cAMP-CRP complex in the induction process (8). The cAMP-CRP independent transcription initiation of the L-arabinose operon in a strain carrying a mutation in the araC gene ($araC^1$) led to the idea that small molecules other than cAMP might facilitate transcription of the L-arabinose operon. In this paper we present evidence that the non-mutagenic [as determined by the Ames test (9)] compound imidazole acetic acid can substitute for cAMP in the induction of the L-arabinose operon.

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TABLE I. Bacterial Strains

Strains	Genotype	Source
F⁻ Strains		
B/r UP1007	Wild type	E. Englesberg
K-12 LS853	his-85 λ⁻ trpA9605 trpR55 cya-2	B. J. Backmann
B/r DC1	ara-leu-1119 pro-1 T6^r try-10 dau-5 str^r	D. P. Kessler
B/r DC7	araC766 dau-5 arg-2	P1bt SB1085 x SB5004
B/r DC71	leuB1 mal$^+$ λ^s	D. P. Kessler
B/r DC74	ara-leu-1170+1238 dau-5 str^r mal$^+$ λ^s	P1bt DC11 x SB2074
K-12 CU356	gal⁻ ilvDAC115 leu-455	EMS-induced Leu⁻ mutant of CU344 (18)
K-12 CU359	gal⁻ ilvDAC115 pdxA204	P1bt WG1473 x CU356
K-12 KC2	gal⁻ ilvDAC115 araC766	P1bt 7 X CU359
K-12 KC4	gal⁻ ilvDAC115 ara-leu-1170+1238	P1bt 74 X CU359
K-12 KC7	gal⁻ ilvDAC115	P1bt DC1 X CU359
K-12 KC8	gal⁻ araC766 cya-2	P1kc KC13 X KC2
K-12 KC10	gal⁻ ara-leu-1170+1238 cya-2	P1kc LS853 X KC4
K-12 KC13	gal⁻ cya-2	P1kc LS853 X KC7
K-12 KC14	gal⁻	P1kc LS553 X KC7
K-12 KC15	gal⁻ araC766	P1kc KC13 X KC2
B/r SB5616	ara-leu-1119 dau-5 cya-4 crp str^r	G. Wilcox
B/r EB1078	dau-5 cya-4 crp str^r	P1bt UP1007 X SB5616
B/r DC455	ara-leu-1101 araD139 str^r	D. P. Kessler

a. Auxotrophic requirements: ara-leu-1170-1238, leucine; ara-leu-1119, leucine; ara-leu-1101, leucine; leuB1, leucine; his⁻, histidine; trp⁻, tryptophan; pro⁻, proline; ilv, isoleucine and valine, pdxA204, arginine and uracil.

b. Abbreviations: dau-5, D-arabinose negative; str^r, streptomycin resistant; λ^s, E. coli B/r sensitive to lambda infection; EMS, ethylmethyl sulfonate; cya⁻, adenyl cyclase deficient; crp⁻, a negative cAMP receptor protein; gal⁻, D-galactose negative; ara⁻, L-arabinose negative.

MATERIALS AND METHODS

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

Media. The minimal base media and the complex media used have been described by Sheppard and Englesberg (10). 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 acetic acid (IA).

Chemicals. L-arabinose, D-xylose, cAMP, imidazole acetic acid (sodium or HCl salt), glycylglycine and the amino acids were obtained from Sigma Chemical Company. Ethyl methyl sulfonate was purchased from Calbiochem. Carbazole, sulfuric acid, manganous chloride and other reagent salts were of highest purity and were obtained from Fisher Chemical Company.

Transductions. Constructions of derivative strains were performed by P1 transduction experiments as described by Gross and Englesberg (11).

Ames test and reversion analysis. The inability of high concentrations of IA (0.1 mM - 1.0 M) to cause reversion of TA98 (hisD3052), TA100 (hisG46) and TA1537 (hisC1537) demonstrated that the Ara^+ phenotype induced by the presence of IA was not due to a secondary mutation. After each growth and enzyme kinetic experiment the cells used in these experiments were analyzed for Ara^+ revertants. The presence of Ara^+ revertants was determined as follows: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions of each culture used were plated on L-arabinose supplemented minimal medium and on D-glucose supplemented minimal medium. The lack of colony formation on the L-arabinose minimal medium and growth on the D-glucose minimal medium indicated no significant Ara^+ reversions.

Plate induction test. A 36 hr phenotypically characterized single colony of KC13 (cya-2) 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. This allowed observation of induction for a wide range of compound concentrations and cell densities. On another area of the same plate, a control colony was radially streaked without any crystal supplement. A single colony of DC455 (ara-leu-1101) grown on glucose minimal medium containing leucine was streaked in an identical manner onto the L-arabinose leucine plate to demonstrate whether the compound tested could be used as a carbon source.

Extract preparation. A colony of phenotypically characterized KC13 (cya-2) or KC14 (cya^+) were grown at 37°C in a gyrotory New Brunswick shaking water bath (model G76). Growth was followed by measuring the absorbance at 660 nm with a Spectronic 20 and samples for assay of L-arabinose isomerase were removed at various stages of growth before and after a shift which was performed when the cells reached an A_{660} of 0.35. Cell samples were immediately chilled, harvested at 4°C, and washed three times with 15 ml of pre-chilled minimal salts solution. The harvested and washed cells were resuspended in a total of 1.5 ml cold 1 mM ethylenediamine tetraacetic acid (EDTA), pH 7.4, and disrupted with a Heat Systems sonicator W220F. Cell debris was removed by centrifugation at 15,000 rpm for 30 minutes at 4°C.

L-arabinose isomerase assay. L-arabinose isomerase activity was determined by the method of Cribbs and Englesberg (12) except that glutathione was omitted from the disruption medium. L-ribulose production was determined by the cysteine-carbazole test (13). One unit of L-arabinose isomerase activity was defined as the production of one micromole of L-ribulose per hour.

Protein determination. Protein concentrations of the cell extracts were determined by the method of Lowry et al. (14).

RESULTS AND DISCUSSION

Plate analysis of the effect of IA on the induction of selected operons. The ability of IA to substitute for cAMP in the induction of the L-arabinose operon is demonstrated in Table 2. The Ara^+ response to IA was shown in an adenyl cyclase negative strain, KC13 (cya-2), which had been plated on media containing L-arabinose as the sole carbon source. These results indicated that IA helped to initiate transcription of the L-arabinose operon even in the absence of cAMP.

As in the case of cAMP mediated initiation of transcription of the L-arabinose operon, the dependence of the IA induction on the cAMP receptor protein (CRP) and the araC gene product (P2) was manifested by the lack of induction in cya^- strains carrying either a crp lesion (EB1078) or an araC lesion (KC15).

Failure of IA to elicit similar responses for the utilization of lactose and maltose in the cya^- background demonstrated the specificity of IA for the L-arabinose operon (Table 2). IA was also unable to overcome the repression of the leucine biosynthetic operon in strain KC10 (cya-2 ara-leu-1170+1238) plated on leucine minimal medium with L-arabinose as the sole carbon source (Table 2). This strain requires leucine and carries an ara-leu fusion which places the L-arabinose genes under leucine control (15).

Failure of IA to serve as a carbon source. The inability of IA to serve as a carbon source was demonstrated by the fact that a strain carrying a total deletion of the ara operon (DC455) was unable to grow on L-arabinose minimal medium when supplemented with IA (Table 2).

Determination of the optimal IA concentration needed for induction of the L-arabinose operon. The ability of IA to replace cAMP in initiating transcription of the L-arabinose operon was strictly dependent on the concentration of IA used. If the concentration of IA added to L-arabinose minimal medium liquid cultures was $\geq$ 100 mM, or $\leq$ 0.1 mM, KC13 (cya^-)

TABLE 2: Phenotypic Characterization of Imidazole Acetic Acid (IA) Substitution for cAMP

Strains	Pertinent Genotype	Carbon[a] Source PCM[b]	D-Lactose cAMP	D-Lactose IA	D-Lactose H_2O	D-Arabinose cAMP	D-Arabinose IA[c]	D-Arabinose H_2O	D-Maltose cAMP	D-Maltose IA	D-Maltose H_2O
KC14	isogenic gal⁻ wild type		+	+	+	+	+	+	+	+	+
KC13	cya-2 gal⁻		+	-	-	+	+	-	+	-	-
KC10	cya-2 ara-leu-1170+1238 gal⁻		+	-	-	-	-	-	+	-	-
KC8	cya-2 araC766 gal⁻		+	-	-	-	-	-	+	-	-
EB1078	cya-4 crp⁻		-	-	-	-	-	-	-	-	-
DC455	ara-leu-1101[d]		+	+	+	-	-	-	+	+	+

a. Methodology described in "Materials and Methods".

b. "PCM" (positive control molecule) designates the molecule that substitutes for cyclic adenosine 3'-5' monophosphate in the text.

c. Abbreviations: IA, imidazole acetic acid; cAMP, cyclic adenosine 3'-5' monophosphate; other abbrevations and symbols are defined in Table 1.

d. Auxotrophic requirements: Refer to Table 1.

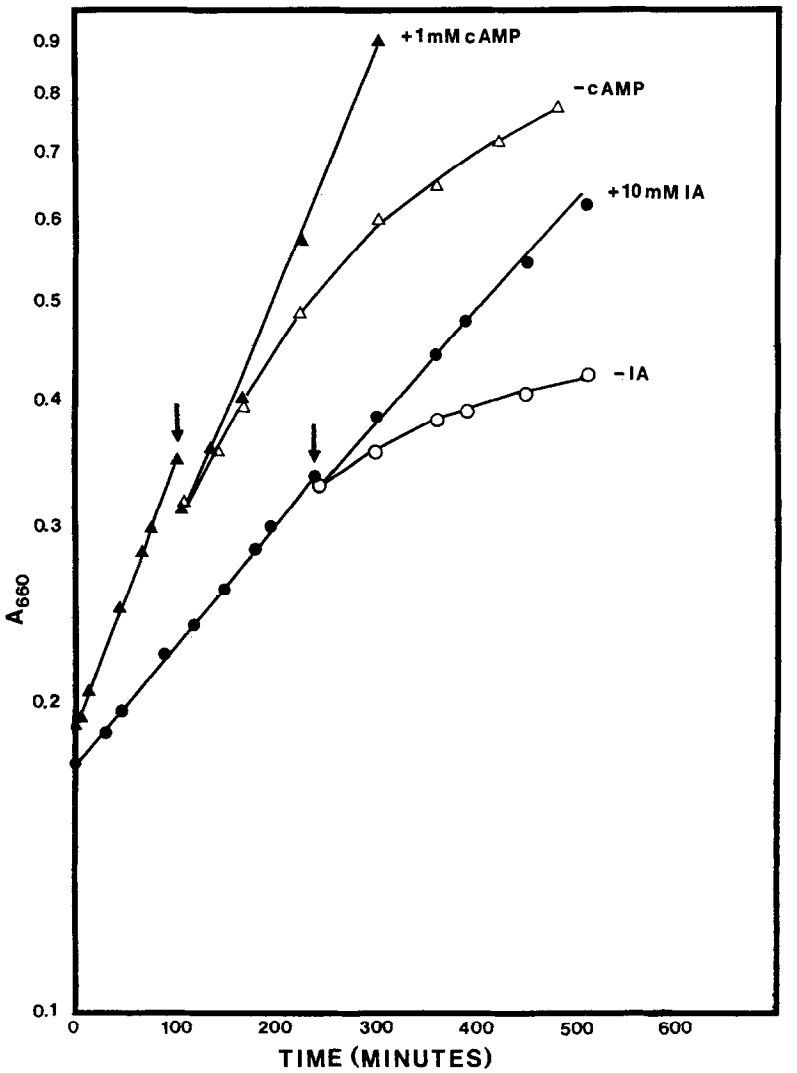

Figure 1. Growth of KC13 (cya-2) with L-arabinose as a carbon source in the presence and absence of positive effectors. Time of shift is indicated by the arrows. (For details see text.)

was unable to utilize L-arabinose as a carbon source. However, at a concentration of 10 mM IA the L-arabinose operon was maximally induced in KC13.

Growth kinetics. As indicated in Table 2, strain KC13 (cya-2) did not grow on L-arabinose unless supplied with a positive effector (cAMP or IA). The isogenic wild type KC14 (cya^+), on the other hand, grew on L-

arabinose in the presence and in the absence of cAMP and IA. The growth kinetics of KC13 in the presence of 1 mM cAMP is shown in Figure 1. When this strain was shifted to L-arabinose minimal medium without cAMP, it began to limit severely in growth at approximately 120 minutes after removal of cAMP.

If KC13 was grown in the presence of 10 mM IA instead of 1 mM cAMP, the generation time was approximately twice that of the cAMP grown cells (Figure 1). After a shift of the culture from the presence of 10 mM IA, limitation occurred as it did when cAMP was removed in the previous experiment.

Effect of IA on L-arabinose isomerase expression. In an attempt to quantitate the expression of the L-arabinose operon in the presence of IA versus cAMP, the activity of the enzyme L-arabinose isomerase (the araA gene product) was monitored at various times during growth of the organism. As indicated in Figure 2, the differential rate of L-arabinose isomerase synthesis in KC13 (cya-2) grown in L-arabinose minimal medium supplemented with 10 mM IA was approximately 2.6 fold less than when the organism was grown under similar conditions in the presence of 1 mM cAMP. Upon shift of the cya^- strain to an L-arabinose minimal medium free of the positive effector, cAMP or IA, the expression of the L-arabinose operon (as indicated by L-arabinose isomerase activity) decreased with time. Levels of L-arabinose isomerase present when severe growth limitation occurred after shift from the cAMP were approximately 2.2 fold greater than the levels observed after limitation following the shift from IA. Although these data indicated that cAMP was a more efficient inducer of the L-arabinose operon, the fact that KC13 (cya-2) grew on L-arabinose in the presence but not the absence of IA and increased the production of L-arabinose isomerase demonstrated that IA could indeed serve as a positive effector in initiating transcription of the L-arabinose operon.

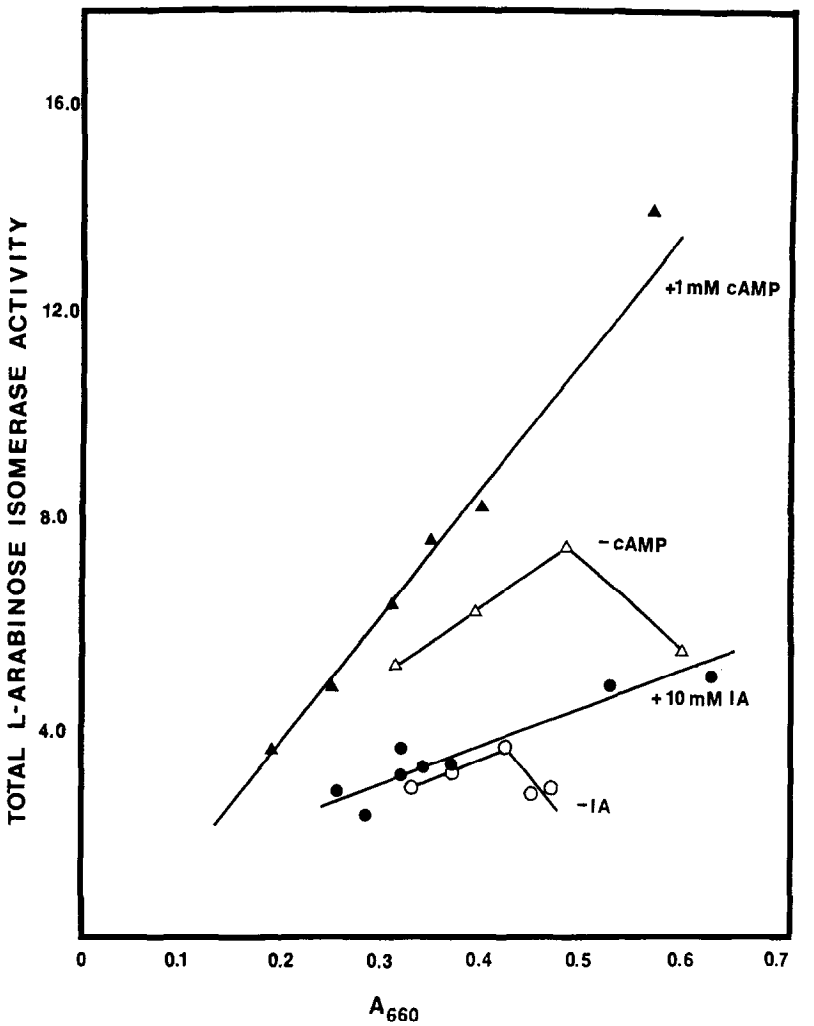

Figure 2. Differential rate of synthesis of L-arabinose isomerase in KC13 (cya-2) in the presence and absence of positive effectors. (For details see text.) Total activity = specific activity (μmoles L-ribulose formed/hr mg protein) X A_{660}.

Since IA is a natural metabolite in both bacterial and mammalian systems (16-17), we have extended our investigations with this compound to a mammalian tissue culture system and have found it to increase the expression of certain enzymes in the eucaryotic cells as well (manuscript in preparation). Preliminary investigations have also suggested that other naturally occurring metabolites such as indole acetic acid (Kline et al., Abstracts of the Annual Meetings of Microbiology, 1979)

can elevate enzyme activity in the bacterial and the tissue culture systems. On the basis of our observations in both the bacterial and the mammalian systems, we propose that critical levels of certain small molecular weight metabolic intermediates other than cAMP may play a role in directing the regulation of gene expression. We have designated this type of control as "metabolite gene regulation" (MGR).

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