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Cra-mediated regulation of *Escherichia coli* adenylate cyclase

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In *Escherichia coli*, expression of certain genes and operons, including the fructose operon, is controlled by Cra, the pleiotropic catabolite repressor/activator protein formerly known as FruR. In this study we have demonstrated that *cra* mutant strains synthesize 10-fold less cAMP than isogenic wild-type strains, specifically when grown in fructose-containing minimal media. The glucose-specific IIA protein (IIA^{glc}) of the phosphotransferase system, which activates adenylate cyclase when phosphorylated, is largely dephosphorylated in *cra* but not wild-type strains growing under these conditions. Dephosphorylation of IIA^{glc} in *cra* strains apparently results from enhanced fructose operon transcription and fructose uptake. These conclusions were supported by showing that fructose-grown *cra* strains possess 2·5-fold higher fructose-1-phosphate kinase activity than fructose-grown wild-type strains. Moreover, artificially increasing fructose operon expression in cells transporting fructose dramatically decreased the activity of adenylate cyclase. The results establish that Cra indirectly regulates the activity of adenylate cyclase by controlling the expression of the fructose operon in cells growing with fructose as the sole carbon source.

Keywords: *Escherichia coli*, adenylate cyclase, phosphotransferase system, fructose operon, catabolite repressor/activator protein

INTRODUCTION

Although the role of cAMP in many physiological processes in *Escherichia coli* is well established (Botsford & Harman, 1992), the regulation of adenylate cyclase (AC) activity *in vivo* is not yet fully understood, especially in response to the carbon source present in the culture medium (Dumay *et al*., 1996). When cells are grown in the presence of glucose, cAMP is present at low levels in agreement with a model involving the phosphotransferase system (PTS) (Saier & Feucht, 1975). Enzyme IIA^{glc} (IIA^{glc}), a glucose-specific component of the PTS, is phosphorylated at the expense of phosphoenolpyruvate (PEP) via a phosphoryl transfer chain, including the general, non-sugar-specific components of the PTS, Enzyme I and HPr (Postma *et al*., 1993; Saier & Reizer, 1994). In the absence of exogenous glucose, IIA^{glc} is phosphorylated and it activates AC. When glucose is transported, leading to the generation of cytoplasmic glucose 6-phosphate, the concentration of phosphorylated IIA^{glc} decreases causing a decrease in AC activity (Feucht & Saier, 1980; Postma *et al*., 1981).

In the work described in this report, we investigated AC regulation by fructose, a sugar which is transported by a fructose-specific PTS that exhibits several features not found in other PTSs (Kornberg & Prior, 1989). For example, *ptsH* mutant strains (lacking HPr) can grow on fructose but not other PTS-sugars (Saier *et al*., 1970). This is due to the presence of a unique carboxy-terminal domain of the FruB protein [the diphosphoryl transfer protein or DTP (Reizer *et al*., 1994)] which is homologous to and can substitute for HPr (Geerse *et al*., 1989a). As regards AC regulation, mutant strains lacking FruB have been reported to exhibit reduced AC activity as compared to wild-type strains during growth in rich media (Gershanovitch *et al*., 1989). This observation led Gershanovitch and colleagues to suggest that the amino-

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Abbreviations: AC, adenylate cyclase; Cra, catabolite repressor/activator protein; IIA^{glc}, Enzyme IIA glucose; PEP, phosphoenolpyruvate; PTS, phosphotransferase system.

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terminal IIA^{fru} domain of FruB, like IIA^{glc}, can activate AC.

Expression of the *fruBKA* operon encoding FruB, FruK (fructose-1-phosphate kinase) and FruA [the fructose-specific Enzyme II B′BC (Reizer *et al*., 1994)] is negatively controlled by a transcription factor originally designated FruR and recently renamed Cra for 'catabolite repressor/activator' (Saier & Ramseier, 1996; Saier, 1996). *cra* mutant strains were first isolated as suppressor mutants that allowed *ptsH* mutant strains to grow on PTS-sugars (Chin *et al*., 1987; Kornberg & Prior, 1989). It was subsequently established that Cra acts as a global regulatory protein which controls carbon metabolism by transcriptional activation or repression of many genes (Saier & Ramseier, 1996). cAMP is also essential for transcriptional regulation of many genes *via* its receptor protein CRP. When complexed with cAMP, CRP binds to specific sites upstream of promoters, causing transcriptional activation or repression (Kolb *et al*., 1993). A CRP binding site is present within the regulatory region of the fructose operon which has been shown to be positively regulated by the CRP–cAMP complex (Feldheim *et al*., 1990).

In this study, we establish that *cra* mutant strains grown on fructose exhibit cAMP levels that are much lower than those in wild-type strains grown on fructose. We report potential regulatory interactions between AC, IIA^{glc} and the fructose PTS which appear to account for the Cra-mediated regulation of cAMP levels in cells grown on fructose.

METHODS

Bacterial strains. The *E. coli* K-12 strains used in this work are listed in Table 1. The growth medium was either LB or minimal medium 63 (MM63) (Miller, 1992) supplemented with the required amino acids (1 mM), thiamin (5 μg ml^{-1}) and a carbon source (0·4%). When present, Casamino acids were added at 0·1%, and ampicillin, kanamycin or tetracycline were added at 100, 50 or 10 μg ml^{-1}, respectively. P1 transduction was performed as described by Miller (1992). *cra* strains were routinely checked for their sensitivity to 0·2% xylitol (Reiner, 1977) as well as their inability to grow on lactate and pyruvate (Geerse *et al*., 1986).

cAMP assays. Total cAMP in boiled cell extracts was measured employing the Gilman assay with the cAMP binding protein from beef muscle purified through a DEAE-cellulose column chromatography step (Gilman, 1970). Total cAMP per mg dry weight of bacteria was essentially constant during the exponential phase of growth. The amount of cAMP produced is expressed in pmol (mg dry wt bacteria)$^{-1}$ and values are the means of three determinations.

Measurement of cAMP synthesis was performed *in vivo* as described by Harman & Botsford (1979). Bacteria were grown in MM63 to mid-exponential phase, washed and resuspended in fresh medium supplemented with chloramphenicol at 100 μg ml^{-1}. The cell suspensions were aerated at 37 °C, and samples were periodically removed for total cAMP determination. Rates of cAMP synthesis were calculated from the slopes of the plotted data.

PEP measurements. PEP was extracted from exponential phase cultures of wild-type and *cra* strains grown in MM63 supplemented with fructose. Aliquots (0·5 ml) of a growing cell suspension were rapidly pipetted into 1·5 ml boiling water and kept at 100 °C for 15 min. Tubes were then placed on ice and chilled to 0 °C. Particulate material was removed by centrifugation, the supernatants were mixed with 5% acid-washed charcoal for 5 min and the charcoal was removed by centrifugation. PEP was measured using pyruvate kinase from Sigma to generate ATP from PEP (McCoy & Doeg, 1975). The bioluminescence assay for ATP was conducted using the Luciferin–Luciferase system (Sigma) as described by the manufacturers.

***β*-Galactosidase assay and inducer exclusion.** *β*-Galactosidase activity in intact bacteria was measured during exponential growth in MM63 containing 200 μM IPTG. A unit of *β*-galactosidase activity was defined as the amount of enzyme that hydrolyses 1 nmol *o*-nitrophenyl *β*-D-galactoside min^{-1} under standard conditions (Pardee *et al*., 1959).

The extent of inducer exclusion was estimated on MM63 plates supplemented with 1% (w/v) glucose, 0·2% lactose and 40 μg X-Gal ml^{-1}, a medium originally defined by Tyler *et*

Table 1. *E. coli* strains used in this study

Strain	Genotype	Source or reference
TP2503	F$^-$ *xyl-5 argH1 ilvA*	De Reuse & Danchin (1988)
LJ2725	Δ(*argF–lac*)*-169 zai-736*::Tn*10 leu-2725 cra*::*kan*	Ramseier *et al*. (1995)
HK1543	*ptsG*::Tn*9 ptsLMP*::Tn*9 cysA argHBCE thr leu rpsL cra*::Tn*10*	Kornberg & Lambourne (1992)
LJ2801	F$^-$ *xyl-5 argH1 ilvA cra*::*kan leu-2725*	TP2503 × [P1 LJ2725, Kan]
LJ2806	F$^-$ *xyl-5 argH1 ilvA cra*::Tn*10*	TP2503 × [P1 HK1543, Tet]
FB8	F$^-$ prototrophic	Bruni *et al*. (1977)
LJ2808	F$^-$ *cra*::Tn*10*	FB8 × [P1 HK1543, Tet]
TP9500	F$^-$ *xyl-5 argH1* Δ*cya-854*	Crasnier & Danchin (1990)
LJ2809	F$^-$ *xyl-5 argH1* Δ*cya-854 cra*::Tn*10*	TP9500 × [P1 HK1543, Tet]
TP2006	F$^-$ *xyl-5* Δ*cya-854* Δ*lacX74 glp8306*	Roy & Danchin (1982)
LJ2805	F$^-$ *xyl-5* Δ*cya-854* Δ*lacX74 glp8306 cra*::Tn*10*	TP2006 × [P1 HK1543, Tet]
TP2862	F$^-$ *xyl-5 argH1 ilvA aroB* Δ*crr*::*kan*	Lévy *et al*. (1990)
LJ2807	F$^-$ *xyl-5 argH1 ilvA aroB* Δ*crr*::*kan cra*::Tn*10*	TP2862 × [P1 HK1543, Tet]
KL16-21-23	λ^- Δ(*manZ–kdgR*)*-82 fruA3 relA1 spoT1 thi-1*	Ferenci & Kornberg (1971)

al. (1969) for their studies on catabolite repression of *β*-galactosidase. Under these conditions, dephosphorylated IIA^{glc} prevents lactose entry by interacting with the lactose permease (Osumi & Saier, 1982). Consequently, wild-type strains grow as white colonies. Replacement of glucose by fructose in these plates allowed estimation of the capacity of this carbon source to promote inducer exclusion.

Fructose-1-phosphate kinase assay. Fructose-1-phosphate kinase was assayed as previously described (Fraenkel, 1968) in extracts derived from sonically disrupted cells. The incubation mixture (1 ml) contained 50 mM Tris/HCl, pH 7·8, 10 mM $MgCl_2$, 10 mM KCN, 0·2 mM NADH, 1 mM fructose 1-phosphate, 2 mM ATP, 37 µg fructose-1,6-diphosphate aldolase (Sigma), 22 µg α-glycerophosphate dehydrogenase–triosephosphate isomerase (Sigma) and an aliquot of a supernatant obtained by high speed centrifugation of the extracts for 30 min at 27000 ***g***.

Cloning of the *fru* operon. The promoterless *fru* operon (*fruBKA*) was obtained by PCR using chromosomal DNA. The upstream primer (TTCTCATAGAGGCTGGATCCTT TCAATT) was located 18 nt upstream of the ATG codon of the *fruB* gene. The downstream primer (TTACAGGACAG-GGAATTCCTGCCCTGTA) was located 12 nt downstream from the stop codon of the *fruA* gene. The amplified 3·9 kb fragment was digested with *Bam*HI and *Eco*RI (whose restriction sites were introduced into the primers) and cloned into pJF118HE (Fürste *et al*., 1986). The plasmid thus obtained, pFRU118, carries the *fru* operon under the control of the *tac* promoter.

RESULTS AND DISCUSSION

cAMP levels in wild-type versus *cra* strains

The carbon source present in the culture medium indirectly regulates AC activity *in vivo* (Epstein *et al*., 1975; Joseph *et al*., 1982). cAMP levels were measured in isogenic wild-type and *cra* strains grown in MM63 supplemented with various carbon sources (Table 2). With fructose as carbon source, the cAMP level of a *cra* strain (either LJ2801 or LJ2806, Table 1) was 10-fold lower than that of the wild-type strain TP2503. The same difference in cAMP level was observed with another set of isogenic wild-type and *cra* strains (FB8 and LJ2808, Table 1, data not shown). Similar doubling times were obtained for the wild-type and *cra* strains grown on fructose (100 min at 30 °C in MM63 without Casamino acids). With carbon sources other than fructose, cAMP levels of the wild-type and *cra* strains were comparable (Table 2).

It is well established that cAMP levels correlate with *β*-galactosidase activities (Epstein *et al*., 1975). Therefore, to confirm that cAMP levels in the *cra* strain were substantially different from those in the wild-type strain only when fructose was used as the carbon source, *β*-galactosidase activities in wild-type and *cra* strains were measured following growth in MM63 containing any one of a variety of carbon sources and IPTG. As shown in Table 3, there was little difference in *β*-galactosidase activities for the wild-type and *cra* strains grown with several of these carbon sources (glucose 6-phosphate, mannitol, mannose, glucitol and glycerol). Slight differences were observed with glucose and gluconate, but a large difference was seen only with fructose. In general, *β*-galactosidase activities (Table 3) correlated with cAMP levels (Table 2).

To analyse the role of IIA^{glc} in promoting synthesis of cAMP by the wild-type versus the *cra* mutant strain when fructose was used as the carbon source, we measured cAMP levels in a Δ*crr* strain lacking IIA^{glc} (TP2862) and a Δ*crr cra* strain (LJ2807), both grown in MM63-fructose. Levels were low but comparable [200 and 125 pmol (mg dry wt bacteria)$^{-1}$, respectively], suggesting that the high cAMP production in fructose-grown wild-type cells is due to activation of AC by IIA^{glc}. They further indicate that FruB cannot substitute for IIA^{glc} as an activator of AC, contrary to an earlier report (Gershanovitch *et al*., 1989). These conclusions were substantiated by measurement of AC activity in resting cell suspensions of wild-type, *cra* and *crr* strains (Fig. 1). In the fructose-grown wild-type strain, 30 pmol cAMP were produced min^{-1} (mg dry wt bacteria)$^{-1}$, but fructose-grown *cra* and *crr* strains produced only 1–5 pmol cAMP min^{-1} (mg dry wt bacteria)$^{-1}$.

Levels of AC and IIA^{glc} in fructose-grown wild-type and *cra* mutant strains

To analyse for a possible effect of Cra on transcription of the *cya* gene encoding AC, strains TP2006 (Δ*cya* Δ*lac*) and LJ2805 (Δ*cya* Δ*lac cra*) were transformed with

Table 2. cAMP levels in wild-type and *cra* strains grown in the presence of different carbon sources

Carbon source	Total cAMP [pmol (mg dry wt bacteria)$^{-1}$]*	
	TP2503 (wild-type)	**LJ2801** (*cra*)
Glucose 6-phosphate	200 ± 20	250 ± 30
Glucose	430 ± 40	380 ± 40
Gluconate	1230 ± 160	1160 ± 60
Fructose	3370 ± 230	350 ± 50
Glycerol	4750 ± 490	4000 ± 400

* Values reported represent the means of three determinations ± SEM.

Table 3. *β*-Galactosidase activities in wild-type and *cra* strains grown on different carbon sources

Carbon source	β-Galactosidase activity [units (mg dry wt bacteria)$^{-1}$]*	
	TP2503 (wild-type)	LJ2801 (*cra*)
Glucose 6-phosphate	570 ± 30	600 ± 50
Glucose	1050 ± 70	670 ± 40
Mannitol	1540 ± 170	1290 ± 60
Gluconate	2500 ± 200	1840 ± 240
Mannose	2590 ± 190	2150 ± 40
Glucitol	2950 ± 190	2430 ± 140
Fructose	4200 ± 520	1550 ± 120
Glycerol	6280 ± 80	6140 ± 300

* Values reported represent the means of three determinations ± SEM.

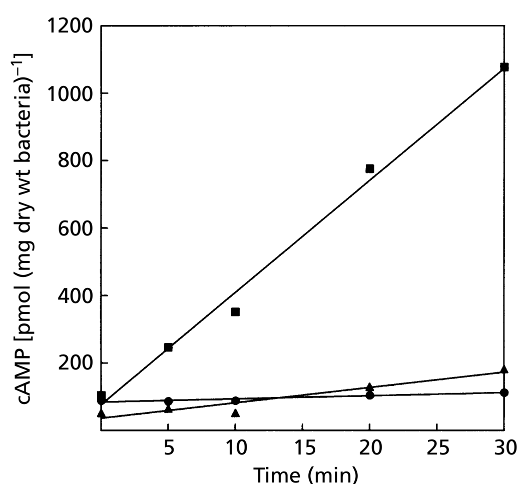

Fig. 1. cAMP synthesis in resting cell suspensions of strains grown in MM63-fructose. cAMP synthesis was measured as described in Methods with strains TP2503 (wild-type) (■), LJ2801 (*cra*) (▲) and TP2862 (*crr*) (●).

pDIA483 which carries a *cya–lacZ* protein fusion (Crasnier *et al*., 1994). The *β*-galactosidase activities of the two strains grown in MM63-fructose were the same [60 units (mg dry wt bacteria)$^{-1}$], showing that Cra does not regulate *cya* gene transcription. We also showed that *crr* gene expression was not affected by Cra as revealed by IIA^{glc} assays of extracts derived from wild-type and *cra* strains (performed as reported by Saier *et al*., 1976). IIA^{glc} levels were the same (±15%) in the two strains, regardless of whether glucose or fructose was present in the growth medium (data not shown). When a *crr* mutant strain was assayed for IIA^{glc}, less than 10% of the amount found in the isogenic wild-type strain was measured. In conclusion, cellular levels of AC and IIA^{glc} were not altered by the presence of the *cra* mutation.

Inducer exclusion in *cra* strains

To explain the difference in cAMP levels observed for fructose-grown wild-type versus *cra* strains, it was considered that IIA^{glc} in *cra* mutants might be less phosphorylated than in wild-type cells. If this were the case, inducer exclusion should occur in *cra* strains because dephosphorylated IIA^{glc} would prevent lactose entry by interacting with the lactose permease (Osumi & Saier, 1982). This scenario was analysed on MM63 plates supplemented with fructose and lactose (see Methods). Strain TP9500 lacking AC (Δ*cya*) and LJ2809 (Δ*cya cra*) were transformed with different plasmids encoding active truncated ACs which are not subject to regulation by IIA^{glc} (Crasnier *et al*., 1994). These truncated ACs constitutively synthesize cAMP at low (plasmid pDIA1940) or high (plasmid pDIA1920) levels, respectively. Differences in *β*-galactosidase activities in wild-type versus *cra* mutant cells bearing any one plasmid can only be explained by different sensitivities to inducer exclusion (and not by differences in cAMP levels). The transformed strains were analysed on MM63 plates for their phenotypes: white colonies indicating the occurrence of inducer exclusion, blue colonies indicating the absence of inducer exclusion. As expected, TP9500 (*cra*$^+$) containing plasmid pDIA1900, which carries a wild-type *cya* gene, exhibited a blue phenotype. With LJ2809 (*cra*$^-$) containing the same plasmid, a white phenotype was observed which can be related to the low cAMP level characteristic of fructose-grown *cra* strains. With plasmid pDIA1920, which allows the production of high cAMP levels, the strains did not exhibit inducer exclusion. This was not surprising as high levels of cAMP, by causing enhanced transcription of the lactose operon, counteract the inhibitory effect of IIA^{glc} on the lactose permease. However, with plasmid pDIA1940, inducer exclusion occurred with the *cra*$^-$ strain but not with the *cra*$^+$ strain. cAMP levels were measured directly in the *cra*$^+$ and *cra*$^-$ strains containing pDIA1940. The results

Table 4. Fructose-1-phosphate kinase activities in wild-type and *cra* strains grown at 37 °C in MM63 supplemented with Casamino acids, fructose and cAMP

[cAMP] (mM)*	Fructose-1-phosphate kinase activity [nmol min^{-1} (mg total protein)$^{-1}$]†	
	TP2503 (wild-type)	**LJ2801** (*cra*)
0	260 (40)	620 (40)
1	250 (40)	1240 (50)
5	170 (50)	1300 (70)

* Exogenous cAMP concentrations are indicated.

† Values in parentheses are the generation times in minutes of the strains grown under the indicated conditions.

established that the level was the same for the two strains [400 pmol (mg dry wt bacteria)$^{-1}$], in agreement with published data (Crasnier *et al.*, 1994). These results imply that IIA^{glc} is in the dephosphorylated state in LJ2809(pDIA1940) but not in TP9500(pDIA1940).

Kornberg & Elvin (1987) showed that transport of fructose by washed suspensions of fructose-grown wild-type cells is inhibited by glucose which is taken up preferentially. However, they did not observe inhibition when glucose was added to fructose-grown *cra* strains in agreement with the proposal that IIA^{glc} is poorly phosphorylated in *cra* strains. Furthermore, when 2 mM methyl α-glucoside (a non-metabolizable glucose analogue) was added to wild-type cells growing in MM63 supplemented with 0·2 % fructose and 0·01 % glucose, there was rapid cessation of growth. With *cra* cells growing under the same conditions, growth was not arrested by addition of 2 mM methyl α-glucoside (our unpublished observations). The lack of effect of methyl α-glucoside in *cra* strains is presumably due to lack of uptake since IIA^{glc} is poorly phosphorylated.

PEP levels in *cra* strains

A possible explanation for the fact that IIA^{glc} is apparently dephosphorylated in *cra* strains grown on fructose may be that there is a lower concentration of PEP in *cra* strains as compared to wild-type strains. Therefore PEP levels were measured in isogenic wild-type (TP2503) and *cra* (LJ2806) strains grown in MM63 containing fructose as the sole carbon source. The PEP levels were similar, i.e. $0·95 \pm 0·18$ and $1·29 \pm 0·24$ μmol (g dry wt bacteria)$^{-1}$ for the wild-type and the *cra* mutant strain respectively. These values are in the same range as those reported by Lowry *et al.* (1971) and Dietzler *et al.* (1979). Thus, a difference in PEP level could not account for the differences in IIA^{glc} phosphorylation in these strains.

Fructose-1-phosphate kinase measurements

Both HPr and the carboxy-terminal domain of FruB interact with phosphorylated Enzyme I. Thus, it was considered that increased concentrations of FruB in *cra* strains may prevent the phosphorylation of IIA^{glc} by HPr. To evaluate levels of expression of the *fruBKA* operon, fructose-1-phosphate kinase activities were measured. As can be seen from the results presented in Table 4, fructose-1-phosphate kinase activity was increased about 2·5-fold in a *cra* strain as compared to a wild-type strain when both strains were grown in the presence of fructose. This observation is in agreement with the proposal that differential transcription of the *fru* operon is indirectly responsible for the differential phosphorylation of IIA^{glc}. It also implies that Cra-mediated repression of *fru* operon expression is not fully reversed in wild-type strains growing on fructose.

Addition of 1 or 5 mM cAMP to the culture medium resulted in a twofold increase in fructose-1-phosphate kinase activity in the *cra* strain but not in the wild-type strain (Table 4). This observation can be interpreted by assuming that, in the wild-type strain but not in the *cra* strain, CRP is saturated with cAMP. If both CRP and Cra act independently, the fivefold difference in fructose-1-phosphate kinase activity [260 versus 1300 nmol min^{-1} (mg total protein)$^{-1}$, Table 4] is likely to represent the repression of the fructose operon by Cra. Alternatively, Cra may prevent the activation of *fru* operon transcription by CRP in fructose-grown wild-type strains. This interpretation is in agreement with previous data indicating that Cra can displace the CRP–cAMP complex from its binding site in the presence of RNA polymerase. In fact, Cra can repress transcription when it is activated by CRP–cAMP and restore transcription when it is repressed by CRP–cAMP (Ryu *et al.*, 1995).

Controlled expression of the fructose operon

The above results suggested that cAMP levels in fructose-grown wild-type cells correlate inversely with the levels of the fructose operon gene products. To further establish a role of fructose operon expression in the regulation of AC, a plasmid, pFRU118, was constructed containing the fructose operon under the control of the *tac* promoter (see Methods). Due to the presence of the *lacI*^{q} repressor gene on the plasmid, expression of the fructose operon was dependent on the concentration of IPTG added to the culture medium.

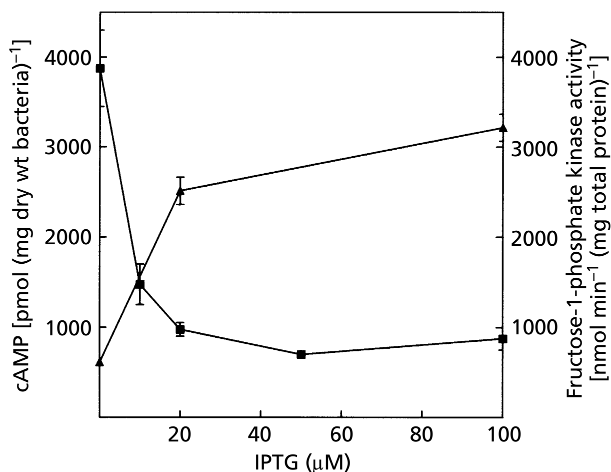

Fig. 2. Variations of cAMP levels and fructose-1-phosphate kinase activities as a function of exogenous IPTG concentration. Strain KL16-21-23 (*fruA ptsM*) containing pFRU118 was grown in MM63 supplemented with 0·4% fructose. Total cAMP (■) and fructose-1-phosphate kinase activity (▲) were measured as a function of exogenous IPTG concentration.

Strain KL16-21-23 (Table 1), containing a mutation in the *fruA* gene and a deletion in the mannose (*ptsM*) operon, did not grow in MM63-fructose. When this strain was transformed with plasmid pFRU118, growth in MM63-fructose was restored. The cAMP levels and the fructose-1-phosphate kinase activities as a function of the IPTG concentration present in MM63-fructose are shown in Fig. 2. Increasing the IPTG concentration resulted in a decrease in cAMP production, correlating with an increase in fructose-1-phosphate kinase activity. Significant variation in cAMP level was not observed with a control plasmid. With glycerol as the carbon source, there was a less than twofold change in cAMP level upon addition of IPTG. These results demonstrate that cAMP levels in cells transporting fructose are dependent on the amounts of fructose operon gene products. Finally there was only slight variation in the doubling time over the range of IPTG concentrations used (data not shown), in agreement with the fact that wild-type and *cra* strains showed essentially the same growth rate. These observations suggest that fructose operon gene products are not limiting for fructose utilization by wild-type cells.

Concluding remarks

This study presents insight into the mechanism by which fructose indirectly controls AC activity and reaffirms a role for phosphorylated IIA^{glc} as an activator of AC. The difference in cAMP levels between a wild-type and a *cra* strain grown on fructose was primarily due to the lack of activation of AC by phosphorylated IIA^{glc}. The proposal that dephosphorylated IIA^{glc} may be able to inhibit AC under some conditions (Feucht & Saier, 1980; Liberman *et al.*, 1986) is not applicable to the studies reported here since the cAMP level observed in a *crr cra* strain is reproducibly lower than that in a *cra* strain.

In strains grown on fructose, Cra mediates AC regulation indirectly by controlling expression of the fructose operon. Growth on fructose diminishes but does not totally abolish the repression of fructose operon transcription by Cra. Only in *cra* strains is the level of *fru* operon transcription sufficient to impair AC activation by preventing phosphorylation of IIA^{glc}. This is most likely due to rapid uptake of fructose leading to competition between FruB and HPr for phosphorylated Enzyme I.

Cra regulates expression of genes encoding key enzymes of the Embden–Meyerhof and Entner–Doudoroff glycolytic pathways as well as of the gluconeogenic, glyoxylate shunt and Krebs cycle pathways (Chin *et al.*, 1989; Geerse *et al.*, 1989b; Ramseier *et al.*, 1995). It will be of interest to know if the observations reported here regarding transcriptional regulation of the fructose operon by Cra can be generalized to other Cra-regulated operons. In addition, it is important to note that many of the genes regulated by Cra are also regulated by the CRP–cAMP complex, and many other genes regulated by CRP–cAMP are not directly regulated by Cra. Thus by regulating the cAMP level, Cra indirectly influences the transcription of hundreds of genes under CRP control. However, this effect depends on the rapid metabolism of fructose and is therefore observed only in fructose-grown cells. Finally, it is worth noting that, in *cra* strains, fructose transport can substitute for glucose transport both in causing inducer exclusion and in regulating the cAMP level.

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