ROLE OF THE *crr*-GENE IN GLUCOSE UPTAKE BY *ESCHERICHIA COLI*

M. C. JONES-MORTIMER, H. L. KORNBERG, R. MALTBY and P. D. WATTS
Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, England

Received 20 December 1976

1. Introduction

The phosphoenolpyruvate-dependent phosphotransferase (PT)-system [1] plays a necessary role in the uptake of a number of sugars ('PT-sugars') by *Escherichia coli* but is not directly involved in the uptake of others ('non PT-sugars') [2]. However, mutants devoid of the enzyme that catalyses the first step in the sequence of reactions required for the phosphorylation of PT-sugars (Enzyme I) not only fail to grow on PT-sugars but are also unable, or reluctant, to grow on many non PT-sugars [3,4].

Since mutants devoid of Enzyme I activity (*ptsI*) appear to be much more susceptible to catabolite repression than their $ptsI^+$-parents, it has been postulated that traces of PT-sugars, such as glucose, suffice to prevent the induction of the proteins needed for the uptake and utilization of non PT-sugars [4]. In support of this view, further mutants have been described, in strains of both *Salmonella typhimurium* [5] and *E. coli* [6], in which the sensitivity to the presence of PT-sugars has been overcome: the gene that specifies this resistance to repression by carbohydrates (*crr*) is co-transducible with *ptsI*. In particular, the uptake of non PT-sugars by such *ptsI crr* double-mutants is no longer inhibited by glucose or methyl-α-glucoside [4]. This implies either that a protein involved in the uptake of PT- and non PT-sugars has been altered to lose its sensitivity to glucose and methyl-α-glucoside, or that the uptake of glucose and its analogue by *crr*-mutants is impaired. Although results of experiments with cell-free extracts suggest that the crr^+-gene specifies a soluble protein involved in glucose phosphorylation [7,8], this cannot be tested in intact cells since glucose and other PT-sugars cannot be utilized by mutants devoid of Enzyme I activity.

It is the main purpose of this paper to report experiments with *E. coli* mutants that contain a temperature-sensitive Enzyme I, active at 30°C but inactive (and rapidly destroyed) at 40°C; these mutants also carry alleles of *crr* and of the *ptsM*-gene that specifies one of the two ports of glucose uptake. The results obtained suggest that the crr^+-gene specifies a component of the uptake system for glucose and methyl-α-glucoside in which the $ptsG^+$-gene product also plays a part. The resistance of *crr*-mutants to inhibition by methyl-α-glucoside is thus due mainly to impairment of uptake and phosphorylation of this analogue.

2. Experimental

The Hfr. C strain *ts19*, which carries a gene ($ptsI^{ts}$) specifying an Enzyme I active at 30°C but inactive (and rapidly destroyed) at 40°C, was isolated by Bourd et al. [9]. This organism, like other *ptsI*-mutants, does not grow on maltose at 40°C. The $ptsI^{ts}$ *crr*-mutant JM1219 was isolated from *ts19* as follows:

Maltose minimal medium was inoculated with a culture of strain *ts19* and incubated at 40°C until the resultant culture was fully grown (4 days). Bacteriophage P1 was then propagated on this culture and was used to transduce strain JM 1169 (a $ptsI\ crr^+$-derivative of strain *ts19-1*△ [6]) mannitol-positive transductants being selected at 30°C. Some such transductants did not grow on mannitol at 40°C and had thus inherited the $ptsI^{ts}$-allele of strain *ts19* that must have still been

present also in the donor. Indeed, many of the *ptsI*^{ts}-transductants also grew on maltose at 40°C as well as at 30°C: they were therefore presumed to be *crr*. One such was purified by isolation of single colonies and, designated JM 1219, was used for subsequent experiments.

The *ptsI*^{ts}*crr*-genes were transferred to *ptsI*-derivates of strain-K2.lt [10] by phage Pl-mediated transduction, colonies being selected that grew on fructose at 30°C but not at 40°C. As controls, similar transductants were obtained with phage Pl that had been propagated on the *ptsI*^{ts}*crr*$^{+}$ parental strain *ts19*. Cultures of these transductants were grown, aerobically with shaking, at 30°C on defined media [11]. The methods used for measurements of growth and of the incorporation of ^{14}C-labelled sugars have been previously described [12].

3. Results and discussion

In contrast to *crr*$^{+}$-derivatives of strain ts 19, that are strongly inhibited in their growth on fructose by many non-catabolizable analogues of glucose [12,13], otherwise isogenic *crr*-strains grew on fructose in the presence of methyl-α-D-glucoside and of 5-thio-D-glucose; however, such strains were still inhibited by 2-deoxy-D-glucose (fig.1). It is known that methyl-α-glucoside is taken up and phosphorylated via the sugar-specific component of the PT-system specified by *ptsG*$^{+}$ [14], whereas 2-deoxy-D-glucose is taken up predominantly via a component specified by *ptsM*$^{+}$ [15]. The selective inhibitory effects of glucose analogues therefore suggest that *crr*-mutants are impaired in the former system of glucose transport. This conclusion was confirmed by measurements of the relative proportions of [^{14}C] fructose and glucose utilized by a variety of mutants growing on equimolar mixtures of these hexoses, and by studies of the growth of mutants also affected in the *ptsM*-gene.

It has been established that when glucose is added to cultures of strain K2.lt growing on PT-sugars such as fructose, the continued uptake and utilization of fructose is inhibited and glucose is used preferentially [12,13]. This applies also to the utilisation of non PT-sugars, such as lactose, when glucose is added [16]. However, *crr*-mutants do not exhibit this behaviour: although glucose is used when added to cultures of these mutants growing on fructose (fig.2), on sorbitol, or on lactose, it is not used preferentially.

Although *ptsI*^{ts}*crr*-mutants grew on glucose as sole carbon source at virtually the same rate as did their *ptsI*^{ts}*crr*$^{+}$-counterparts, removal of *ptsM*-function caused a drastic reduction in growth rate in the former strains but not the latter. Thus, both the mutants PW 7 (*ptsI*^{ts} *crr*$^{+}$ *ptsM*$^{+}$) and PW 8 (*ptsI*^{ts}*crr ptsM*$^{+}$) grew on glucose at 30°C with mean doubling time of about 2 h, whereas

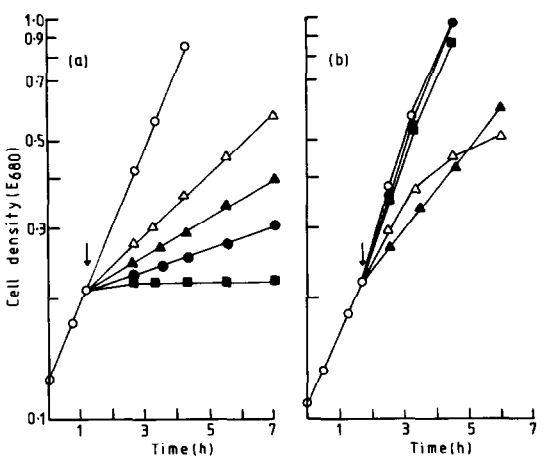

Fig.1. Effect of glucose analogues on the growth of (a) strain *ts19* (*crr*$^{+}$) and (b) strain JM 1219 (*crr*) at 30°C with fructose as carbon source. (○) No addition, (●) + methyl-α-D-glucoside (2 mM), (△) + 2-deoxy-D-glucose (2 mM), (■) + 5-thio-D-glucose (1 mM), (▲) + 3-deoxy-3-fluoro-D-glucose (0.2 mM).

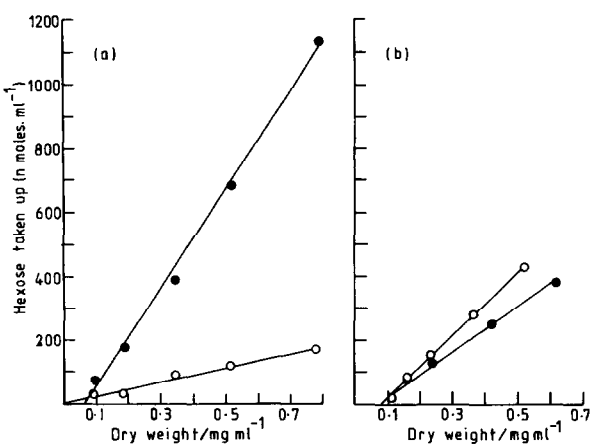

Fig.2. Incorporation of carbon from glucose (●) and fructose. (○) by (a) strain PW 7 (*crr*$^{+}$) and (b) strain PW 8 (*crr*) during growth at 30°C on equimolar mixtures of the two sugars.

the mutant PW 10 (*ptsI*^{ts} *crr ptsM*), required over 6 h to double in mass. Since removal of *ptsM*-function makes little difference to growth on glucose of *crr*$^{+}$-strains, it follows that most of the glucose utilized must enter via the *ptsG*-system: this confirms results obtained by other means [15,18]. The differences in the behaviour of *crr*$^{+}$ and *crr*-mutants, illustrated in figs.1 and 2, therefore point to a difference in functioning of the *ptsG*-system: indeed, washed suspensions of *crr*-mutants take up methyl-α-D-[^{14}C]glucoside less well than do *crr*$^{+}$-strains. Why this difference materially affects growth on glucose only if the (quantitatively minor) *ptsM*-system is also impaired remains to be explained: it is not easily reconciled with various models [8,17] that have been proposed to account for the mechanism and control of the PT-system.

Acknowledgements

We thank Dr G. I. Bourd, (Gamaleya Institute for Epidemiology, USSR Academy of Medical Sciences, Moscow-D98) for the gift of *E. coli* strain *ts19*, the Science Research Council for supporting this work through Grant B/RG 97562 and through the award of a Research Studentship to P. D. Watts and Mrs Vidya Virkar for technical assistance.

References

[1] Kundig, W., Ghosh, S. and Roseman, S. (1964) Proc. Natl. Acad. Sci. USA 52, 1067–1074.
[2] Kundig, W., Kundig, F. D., Anderson, B. and Roseman, S. (1966) J. Biol. Chem. 241, 3243–3246.
[3] Wang, R. J. and Morse, M. L. (1968) J. Mol. Biol. 32, 59–66.
[4] Saier, M. H. and Roseman, S. (1972) J. Biol. Chem. 247, 972–975.
[5] Saier, M. H., Young III, W. S. and Roseman, S. (1971) J. Biol. Chem. 246, 5838–5840.
[6] Jones-Mortimer, M. C. and Kornberg, H. L. (1974) FEBS Lett. 48, 93–95.
[7] Kundig, W. and Roseman, S. (1971) J. Biol. Chem. 246, 1407–1418.
[8] Kundig, W. (1974) J. Supramol. Struct. 2, 695–714.
[9] Bourd, G. I., Bolshakova, T. N., Saprikina, T. P., Klyutcheva, V. V. and Gershanovitch, V. N. (1971) Molekul. Biol. 3, 384–389.
[10] Brice, C. B. and Kornberg, H. L. (1967) Proc. R. Soc. London Ser. B 168, 281–292.
[11] Ashworth, J. M. and Kornberg, H. L. (1966) Proc. R. Soc. London Ser. B. 165, 179–188.
[12] Amaral, D. and Kornberg, H. L. (1975) J. Gen. Microbiol. 90, 157–168.
[13] Kornberg, H. L. (1972) in: The Molecular Basis of Biological Transport (Woessner J. F. and Huijing, F. eds) pp. 157–180. Miami Winter Symp., Academic Press, N.Y.
[14] Kornberg, H. L. and Smith, J. (1972) FEBS Lett. 20, 270–272.
[15] Curtis, S. J. and Epstein, W. (1975) J. Bacteriol. 122, 1189–1199.
[16] Kornberg, H. L. (1973) in: Rate Control of Biological Processes, Symp. Soc. Exp. Biol. 27, 175–193.
[17] Saier, M. H. and Stiles, C. D. (1975) in: Molecular Dynamics in Biological Membranes, pp. 95–105. Heidelberg Science Library, Springer-Verlag, N.Y.
[18] Kornberg, H. L. and Jones-Mortimer, M. C. (1975) FEBS Lett. 51, 1–4.