THE ENZYMATIC LESION OF STRAIN MM-6, A PLEIOTROPIC CARBOHYDRATE - NEGATIVE MUTANT OF ESCHERICHIA COLI

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For many microorganisms the phosphorylation by ATP of several utilizable carbohydrates has never been adequately demonstrated in extracts. Wood has recently discussed the problem under the title, "The mystery of missing kinases", and suggested that several newly described ATP-independent phosphotransferases might be used (1966).

On the basis of mutant analysis Fraenkel, Falcoz-Kelly, and Horecker (1964) suggested that glucose*, fructose, and mannose were phosphorylated in E. coli by an unknown "nonspecific hexokinase" distinct from the well known glucokinase. Their scheme is summarized in Table 1. The mutant MM-6 grew poorly on glucose, and not at all on fructose or mannose. A glucose-negative strain, GN-2, was isolated from MM-6 after mutagenesis; this strain was missing glucokinase. A fructose-revertant (FR-1) of strain GN-2 grew also on glucose and mannose, but remained glucokinase negative. Thus it appeared that the unknown "hexokinase" was absent in both strains MM-6 and GN-2, but reverted in strain FR-1.

Tanaka, Lerner, and Lin (1967) showed that a mutant of Aerobacter aerogenes unable to grow on mannitol but growing normally on other carbohydrates was lacking an enzyme II (acting on mannitol and inducible by mannitol) of a PEP-phosphotransferase system similar to that discovered by Kundig, Ghosh,

* All carbohydrates mentioned are D-isomers.

TABLE I

Growth and Kinase Patterns of E. coli Strains*

Strains	Growth**			Enzymes	
	Galactose	Glucose	Fructose	Glucokinase (measured)	"Hexokinase" (predicted)
Hfr 3300	++	++	++	+	(+)
MM-6	++	+/-	-	+	(-)
GN-2	++	-	-	-	(-)
FR-1	++	++	++	-	(+)

* Modified from Fraenkel et al. (1964).

** The list of compounds on which MM-6 and GN-2 fail to grow but which can be used by Hfr 3300 and FR-1 include mannitol and sorbitol in addition to the previously reported fructose, mannose, and succinate (Fraenkel and Horecker, unpublished).

and Roseman in E. coli (1964). This system consists of three protein compounds acting according to the following scheme:

$$\text{PEP} + \text{HPr} \xrightleftharpoons{\text{enzyme I}} \text{P-HPr} + \text{pyruvate}$$

$$\text{P-HPr} + \text{sugar} \xrightarrow{\text{enzyme II}} \text{sugar-P} + \text{HPr}$$

where PEP denotes phosphoenolpyruvate; HPr, a small heat-stable protein; and P-HPr, the phosphorylated protein.

Tanaka and Lin (1967) recently described two more classes of mannitol-negative mutants of A. aerogenes. They lacked, respectively, enzyme I or HPr, and, in contrast to the mutant affected in enzyme II, were unable to grow on a variety of carbohydrates. Enzyme I mutants, for example, did not grow on mannitol, sorbitol, or fructose, and grew poorly on glucose and mannose. This growth pattern is strikingly reminiscent of the E. coli mutant MM-6. We have therefore assayed enzyme I, HPr, and enzyme II (for mannitol) in this mutant and its related strains. Table II shows that enzyme I was missing in MM-6 and

GN-2 but present in the wild-type and revertant strains. Since the mutant did not grow on mannitol despite the presence of its enzyme II, it is also likely that other enzymes II are not affected. Kundig et al. (1966) have suggested that there may be a family of such inducible enzymes. Hence the particular lesion of strains MM-6 and GN-2 is in enzyme I and the PEP-phosphotransferase system is the missing "hexokinase".

TABLE II

Specific Activities of Enzyme I, HPr, Enzyme II and Glucokinase in Parental and Mutants Strains*

Strain	Enzyme I	HPr	Enzyme II	Glucokinase
	mμmoles per min per mg protein			
Hfr 3300	4.9**	15.3	6.3	52
MM-6	0.0	7.1	4.3	46
GN-2	0.0	7.6	7.6	0
FR-1	5.3	13.7	2.3	0

The cells were grown in 250 ml of medium 63 (see Fraenkel et al., 1964) containing 1% Bacto-tryptone (Difco) and 2% mannitol. They were harvested in early stationary phase and washed once with 0.9% NaCl. After suspension in 0.1 M Tris-HCl buffer, pH 7.6, the cells were disrupted by ultrasonic treatment, and unbroken cells and debris removed by centrifugation at 4,000 x g for 15 min at 2°. Samples of the resulting extract were assayed for enzyme I, HPr (Tanaka and Lin, 1967) and enzyme II (Tanaka et al., 1967) by measuring C^{14}-mannitol incorporation into a negatively charged product in a system including an excess of the remaining two components. These components were prepared from A. aerogenes as previously described. Glucokinase in the supernatant fractions recovered from 30 min of centrifugation at 17,000 x g was assayed spectrophotometrically (Fraenkel et al., 1964). Protein concentration was measured with the phenol reagent (Lowry et al., 1951).

* It was mentioned in a footnote to a previous paper (Fraenkel et al., 1964) that strain GN-2 contains the PEP-phosphotransferase. That incorrect conclusion was based upon the measurement of a low level of glucose-dependent pyruvate formation from PEP.

** The specific activity of the C^{14}-mannitol was 1,350 cpm/mμmole. In a typical assay 20 μg protein of the wild-type extract gave about 2000 cpm over a blank of 200 cpm.

It may be noted that the measured specific activities of the components of the PEP-phosphotransferase system are far lower than would be calculated

from the in vivo rate of utilization of the substrate (ca. 200 mμmoles per min per mg protein). At present there is no explanation for this discrepancy. It is unlikely to be caused by the fact that the assay system was heterologous - the two components present in excess in each assay being from A. aerogenes - because similar low specific activities were found in the system of A. aerogenes (where the components in the assay system were homologous [Tanaka and Lin, 1967]).

Kundig and coworkers (1966) suggested that the PEP-phosphotransferase system plays a role in carbohydrate transport, enzyme II being a carrier in the membrane and active transport resulting from phosphorylation by P-HPr. Since certain sugars are accumulated in the non-phosphorylated state (e.g. galactose and β-methyl-thiogalactoside) it was assumed that specific dephosphorylation was also a part of the transport process. However, this mechanism does not explain how rephosphorylation of the free sugar by enzyme II can be avoided, or how the escape of the sugar through enzyme II by facilitated diffusion can be avoided if rephosphorylation should fail. A simpler model has therefore been proposed (Tanaka and Lin, 1967) according to which the retention of the substrate is associated with the first step of dissimilation. This would hold true for compounds such as mannitol, sorbitol, glucose, fructose and mannose.

The dependence of the metabolism of these compounds on P-HPr would explain several types of observations: a) the leakage of glucose from cells of strain MM-6 growing on lactose (Asensio et al., 1963); b) the appearance of α-methyl-glucoside, an analogue used for the study of glucose transport, in the cells as a phosphorylated compound (Rogers and Yu, 1962; Hagihira, Wilson, and Lin, 1963; Hoffee, 1963; and Winkler, 1966); and c) the relative insensitivity of the uptake of α-methyl-glucoside to agents inhibiting ATP generation, e.g. dinitrophenol and azide (Englesberg, Watson, and Hoffee, 1961; Hoffee, Englesberg, and Lamy, 1964; Halpern and Lupo, 1966).

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REFERENCES

Asensio, C., Avigad, G., and Horecker, B. L., Arch. Biochem. Biophys., 103, 299 (1963).
Englesberg, E., Watson, J. A., and Hoffee, P. A., Cold Spring Harbor Symp. Quant. Biol., 26, 261 (1961).
Fraenkel, D. G., Falcoz-Kelly, F., and Horecker, B. L., Proc. Natl. Acad. Sci. U.S., 52, 1207 (1964).
Hagihira, H., Wilson, T. H., and Lin, E. C. C., Biochim. Biophys. Acta, 78 505 (1963).
Halpern, Y. S., and Lupo, M., Biochim. Biophys. Acta, 126, 163 (1966).
Hoffee, P.A., Ph.D. Thesis, University of Pittsburgh, 1963.
Hoffee, P., Englesberg, E., and Lamy, F., Biochim. Biophys. Acta, 79, 337 (1964).
Kundig, W., Ghosh, S., and Roseman, S., Proc. Natl. Acad. Sci. U.S., 52, 1067 (1964).
Kundig, W., Kundig, F. D., Anderson, B., and Roseman, S., J. Biol. Chem., 241, 3243 (1966).
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem., 193, 265 (1951).
Rogers, D., and Yu, S. H., J. Bacteriol. 84, 877 (1962).
Tanaka, S., Lerner, S. A., and Lin, E. C. C., J. Bacteriol., in press.
Tanaka, S., and Lin, E. C. C., Proc. Natl. Acad. Sci. U.S., in press.
Winkler, H. H., Biochim. Biophys. Acta, 117, 231 (1966).
Wood, W. A., Ann. Rev. Biochem., 35, 521 (1966).