A NEW ASSAY OF THE PHOSPHOTRANSFERASE SYSTEM IN ESCHERICHIA COLI (°)

Gabriel GACHELIN
Service de Biochimie Cellulaire
INSTITUT PASTEUR - PARIS

Received January 7, 1969

A phosphotransferase system (Kundig et al., 1964) has been shown to mediate transport of some carbohydrates in Salmonella typhimurium (Simoni et al., 1967) and Escherichia coli (Kaback, 1968). This system involves two sequential phosphorylations which transfer a phosphoryl group from PEP to the 6 position of hexoses. In vivo activities of this enzymatic complex may be deduced from initial rates of uptake of αMG (Kaback, 1968). However disruption of the cells by usual means leads to a significant loss of phosphorylative ability of extracts. Moreover, comparative studies on uptake and on phosphorylation cannot be performed simultaneously on the same bacterial suspension, since preparation of a crude extract requires at least one centrifugation followed by disruption of the cells. The purpose of this note is to describe a convenient method of decryptification of the phosphotransferase system in Escherichia coli, permitting direct assay of this activity on small samples of bacterial suspension.

Materials and methods. Wild type 3000 of E. coli K 12, or 3300 (i^-z^+) and a Car^- mutant G10 were used in these experiments. Cells were grown on mineral medium with glycerol (4‰) and casaminoacids (10‰) at 37°C with constant shaking. 15 minutes before each experiment,

(°) - Research in the Department of Cellular Biochemistry, at the Pasteur Institute, has been aided by grants from the U.S. National Institutes of Health, the "Délégation Générale à la Recherche Scientifique et Technique", the "Centre National de la Recherche Scientifique" and the "Commissariat à l'Energie Atomique".

cell growth was stopped by chloramphenicol (50 γ/ml), and kept under shaking. ^{14}C methyl αMG was purchased from the Radiochemical Center. αMG uptake was followed according to Kepes (1960). αMGP was estimated by precipitation of the labelled sugar phosphate as its barium salt in 80% ethanol, followed by vacuum filtration on millipore filters (0.45μ) and washing with 25 ml 80% ethanol (cf. Buttin, 1963). Dried millipore filters were measured for retained radioactivity in a Packard spectrometer.

Addition of small volumes of toluene to E. coli K 12 cells leads to immediate passive equilibration of intracellular content of any small molecule (MW below 2,000) with any given extracellular concentration. Such a property is used in measurements of enzymatic activities, the substrates of which do not enter the cells (ATP for galactokinase, Buttin, 1963) or penetrates at a limiting rate (ONPG for β-galactosidase) ; in the particular case of membrane bound enzymes, toluene treatment seems to leave most of the proteins in sites conserving their interactions with other membrane or cytoplasmic proteins, while breakdown of the permeability barrier allows free access of substrates to specific sites. If toluene does not by itself denature the phosphotransferase system, we may expect to observe, after its addition, a decryptification of the activity, without having to concentrate and disrupt the cells.

Decryptification of phosphotransferase system in E. coli K 12 by toluene

In these experiments, the trisodium salt of PEP was added at a final concentration of 10^{-3}M and labelled αMG at 5.10^{-4}M. Rates of phosphorylation were determined by following αMGP production at 2 minute interval during 10 minutes, at 37°C. Sodium phosphate buffer 0.2M pH 7.2 was used, with $MgCl_2$ 2.10^{-3}M. Uptake activity of the preparation was measured in the same sample by filtering aliquots on millipore filters after 20 minutes exposure to labelled αMG.

Method of decryptification.

A given volume of toluene is added to 10 ml of bacterial suspension in phosphate Mg buffer. The mixture is then gently shaken for one minute and then incubated at 37°C with occasional shaking. The assay is started usually after a 30 minute period of incubation, by adding to a mixture of PEP and αMG an aliquot of the cell suspension. It was found that decryptification increases with time of incubation, reaching a plateau after 20 minutes and then remaining stable for at least two hours.

Uptake or retention is immediately abolished with 0.5 µl/ml of toluene.

Pattern of decryptification.

Phosphotransferase activity measured following such a treatment depends closely upon the amount of toluene added. It shows a striking peak of activity between 0.75 µl/ml and 1.5 µl/ml with a clear optimum at 1 µl/ml. Addition of an excess of toluene (3 µl/ml) decreases phosphotransferase activity down to a level of 10% of maximal. Such a pattern is very reproducible. The optimum concentration of toluene is not a function of bacterial cell concentration. It remains at 1 µl/ml in concentrations ranging from 0.1 mg of bacterium/ml (wet weight) to 1 mg/ml, which are the usual concentrations of growing cultures of E. coli, and appears to be related to solubilisation of toluene in buffer. Is the decryptified phosphorylation of αMG due to the phosphotransferase system ?

The following table shows characteristics of phosphotransferase activity in either sonicated cells (Kundig, 1964) or treated by the described treatment.

In both cases, PEP is the only donor of phosphoryl group. Neither ATP nor other triphosphonucleotides, acetyl P carbamyl P are able

Characterisation	Toluenised cells	Sonicated cells
Buffer : Nature	Na-K phosphate	Na-K phosphate
: pH	7.2	7.2, 7.4
: molarity	0.01 -- 0.2M	0.025M
Ionic requirement and half activation	Mg^{++} (5.10^{-4}M)	Mg^{++} (-)
K_M for PEP	2.10^{-4}M	2.10^{-4}M
K_M for αMG	2.10^{-4}M	-
K_M for glucose	3.10^{-5}M	3.10^{-4}M
Specific activity		
-αMG	13.9 µM/g/min	-
-glucose	57 "	10 µM/g/min
Product of reaction	αMG 6P	αMG 6P (Kaback,1968)
	G-6P	G-6P
Activity in Car^- mutant	Undetectable (<0.05 µM/g/min)	Undetectable

to replace PEP for αMGP synthesis. In both cases, PEP does not act through production of ATP (Kundig, 1964).

It was verified that treating the cells as above in mineral medium 63 gives exactly the same results as in pure sodium and magnesium phosphate buffer. This simplification may therefore be used when cells are grown in presence of sugars or aminoacids which do not interfere with the phosphotransferase assay.

Under these conditions, at 37°C, with optimal concentration in PEP and substrate, phosphorylation is linear with concentration of cells in a range from 0.1 mg/ml of bacteria (W/W) to 1 mg/ml.

Toluenised phosphotransferase is more specific of α glucosides than the activity extracted from sonicated cells. Toluenised preparations are able to phosphorylate glucose, αMG, 2-deoxyglucose and

mannose, but not galactose, fructose or maltose. TMG is not phosphorylated although the strains used had high β-galactoside permease activity. Melibiose, sorbitol, galactose, TMG, fructose, mannitol, maltose, 3.0-Me glucose and glucosamine do not exhibit significant affinity for the transferase system, as estimated by competition for αMG phosphorylation. Decryptified phosphotransferase thus appears specific for glucose and some of its derivatives and also for mannose.

It should be pointed out, in relation with this observation, that under the conditions of growth used, the only carbohydrates permeases expressed in the cells were αMG and β-galactoside permeases.

In spite of these differences in specificity, it seems very likely that the two activities are expressions of the same enzymatic complex. Differences might be due to growth conditions and are under investigations.

In conclusion, decryptification of the phosphotransferase system by toluene constitutes an easy method for in vivo studies of this system, avoiding alterations found to result from centrifugation, exposure to cold or delays in performing the assay. This method appears to be of particular interest for studies of the correlation between phosphotransferase and uptake activities.

REFERENCES

Buttin, G. J. Mol. Biol., 7, 164 (1963).

Kaback, H.R. J. Biol. Chem., 243, 3711 (1968).

Kepes, A. Biochim. Biophys. Acta, 40, 70 (1960).

Kundig, W., Ghosh, S., and Roseman, S. Proc. Nat. Acad. Sci. (USA), 52, 1067 (1964).

Simoni, R.D., Levinthal, M., Kundig, F.D., Kundig, W., Anderson, B., Hartman, P.E., and Roseman, S. Proc. Nat. Acad. Sci. (USA), 58, 1963 (1967).

Abbreviations used :

αMG : α methyl glucose

αMGP : α methyl glucose 6 phosphate

PEP : Phosphoenol pyruvate

Car^- : G10 is an EMS mutant lacking ability to grow on : fructose, melibiose, lactose, glycerol, mannose, mannitol, sorbose, glucose and maltose, growing on pyruvate, arabinose and glucose 6 phosphate. This mutant is altered in accumulation of carbohydrates. Simoni et al., 1967, found that this category of mutants is lacking enzyme I of phosphotransferase system.

TMG : Thio-methyl-β-D-galactopyranoside.