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EVIDENCE THAT GLUCOSE 6-PHOSPHATE IS TRANSPORTED INTACT IN *ESCHERICHIA COLI*

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Summary

Conservation of the $^{3}H/^{32}P$ ratio in [2-^{3}H]glucose 6-[^{32}P]phosphate transported by *Escherichia coli* strain DF2000 indicates that the Glc-6-*P* molecule is transported intact, without a sequential mechanism involving hydrolysis and rephosphorylation.

Escherichia coli, and a number of other bacteria [1], have an active transport system for the uptake of hexose phosphates [2—5]. Studies on the induction properties [3,7—9], the specificity [3], genetic mapping and mutant strains [3,10—13] and energy coupling [14—16], all indicate that the uptake of glucose 6-phosphate by this system is distinct from that of glucose, and represents an active transport system, rather than a group translocation [17]. No direct evidence has been presented showing that the glucose and phosphate moieties remain intact during transport, although the $^{3}H/^{32}P$ ratio was shown to be conserved after metabolism of the glucose 6-phosphate to nucleic acid [2]. In this report we demonstrate in a brief, simple and direct way what has been assumed from the published indirect studies: the ratio of $^{3}H/^{32}P$ in the intracellular pool of glucose 6-phosphate is the same as the extracellular pool of doubly labeled [2-^{3}H]glucose 6-[^{32}P]phosphate, showing that hexose phosphates are transported across the cell membrane intact and not by a sequential mechanism, involving hydrolysis, transport of the neutral sugar, and rephosphorylation.

Strain DF2000 (18, 19) of *E. coli* was used in these studies, as in previous studies, because it lacks phosphoglucose-isomerase (blocking formation of fructose 6-phosphate from Glc-6-*P* and glucose-6-phosphate dehydrogenase

(blocking the phosphogluconate-oxidative pathway formation of 6-phosphogluconate from Glc-6-*P*, thus limiting the metabolism of Glc-6-*P* to conversion to glucose 1-phosphate and polysaccharides. The *E. coli* cells were induced with Glc-6-*P*, washed, and then allowed to transport [^{3}H]Glc-6-^{32}*P* (see footnote to table) in a medium with excess cold phosphate. Dilution of the ^{32}P label, with respect to ^{3}H would be expected if transport involved hydrolysis of the Glc-6-*P* moiety with subsequent Glc-6-*P* reformation. High voltage electrophoresis was used to purify the transported sugar phosphate in the initial extraction of Glc-6-*P*, as well as in the subsequent purification steps, as described in the footnote to the table. The ratio of ^{3}H/^{32}P in the extracellular [^{3}H]Glc-6-^{32}*P* whether determined in the extract, the purified Glc-6-*P*, or enzymatically derived 6-phosphogluconate, was essentially the same, 4.8 ± 0.2 (Table I). Likewise, the ^{3}H/^{32}P ratio of the intracellular Glc-6-*P* was,

TABLE I

CONSERVATION OF ^{3}H/^{32}P RATIO DURING [^{3}H]Glc-6-^{32}*P* UPTAKE

The doubly labeled [^{3}H]Glc-6-^{32}*P* was prepared with hexokinase plus [2-^{3}H]glucose and [γ-^{32}P]ATP, and non-radioactive carriers with a slight excess of ATP. The reaction was followed by both the fall in pH and enzymatic determination of the resultant Glc-6-*P* photometrically with glucose-6-phosphate dehydrogenase. Nucleotides were adsorbed with charcoal, and the final product purified by high voltage electrophoresis [buffer system: pH 6.0 pyridine—glacial acetic acid—H_2O (1:25:475, by vol.)], to yield [^{3}H]Glc-6-^{32}*P* (specific activity of 0.13 mCi of ^{3}H and 0.027 mCi of ^{32}P per mmole).
The cells were grown in Davis medium A (20), supplemented with thiamine (1 μg/ml) $FeSO_4 \cdot 7H_2O$ (0.0005%), and Difco Casamino Acids (1%) as carbon source. Induction was accomplished by the addition of Glc-6-*P* (1 mM). The cells were harvested and washed twice in minimal medium (without Glc-6-*P* before use. Uptake assays were performed, following a 2 min room-temperature incubation, with [^{3}H]Glc-6-^{32}*P* (1 mM), at a cell turbidity of 200 Klett units (number 42 filter) by filtering an aliquot on a Millipore filter, as previously described [16]. These filters were dried and dissolved with ethanol—dioxane (1:3, by vol) prior to being counted in toluene-based scintillation fluid containing 33% Triton X-100. To determine the intracellular ratio of ^{3}H/^{32}P, the rest of the incubation mixture was filtered after a 2 min incubation, washed, and extracted in boiling water for five minutes. A control run to determine the ^{3}H/^{32}P ratio of the extracellular [^{3}H]Glc-6-^{32}*P* used 0.2 μmoles [^{3}H]Glc-6-^{32}*P*, a clean filter, and an appropriate amount of heat-killed cells mixed without filtering. This mixture was boiled 5 min and carried through the separation procedures, exactly as with the intracellular pool determinations. The boiled extracts were clarified, evaporated to dryness, and resuspended in distilled water. A portion was counted, and the remainder placed on paper for high voltage electrophoresis to separate Glc-6-*P*. The area of the electropherogram containing Glc-6-*P* was eluted, evaporated to dryness, and redissolved in Tris—HCl (10 mM, pH 7.0) with 0.1 M $MgCl_2$. A portion of the Glc-6-*P* eluate was counted, and the Glc-6-*P* contained in the remainder converted to 6-phosphogluconate by the addition of NADP (0.16 mg) and glucose-6-phosphate dehydrogenase (0.5 units) in a total reaction volume of 1 ml. This reaction mixture was then separated by high voltage electrophoresis, in the same system, and the 6-phosphogluconate area of the electropherogram eluted and counted. The 6-phosphogluconate on the electropherogram could be derived only from Glc-6-*P*, since the first electrophoretic separation removed any other compounds with this mobility. Scintillation counting was performed, in these cases, by adding Aquasol to a portion of redissolved sample, and counting in a Packard Tri-carb with the windows set for ^{3}H and ^{32}P. The specific activity used was such that all cpm were greater than 1700.

Pool	Additions	Expt No.	Uptake (mM)	^{3}H/^{32}P Ratio			Percentage of extracellular Ratio
				Original Extract	Glc-6-*P* Area	6-*P*-gluconate Area	
Extracellular	None	1	—	4.38	5.06	4.97	(100)
		2	—	4.78	4.82	5.00	
Intracellular	None	1	14	4.67	4.31	4.48	94 ± 8
		2	12	4.50	4.30	4.68	
Intracellular	Glucose (10 mM)	1	25	4.80	4.66	5.36	104 ± 7
		2	16	5.05	4.76	5.14	
Intracellular	Fluoride (10 mM)	1	11	4.62	4.81	4.74	97 ± 4
		2	13	4.67	4.52	4.68	

within experimental error, the same as the extracellular, whether the experiment was done in the presence of glucose or fluoride, or their absence. The ratio was also independent of the degree of purification of the intracellular Glc-6-*P*, indicating that little or no hydrolysis and rephosphorylation of the [^{3}H]Glc-6-^{32}*P* had occurred. The mean value of the intracellular ^{3}H/^{32}P Glc-6-*P* ratio was 98 ±7 (n = 18, ±S.D.) percent of the extracellular ratio. Glucose 6-phosphate is, therefore, transported intact by the inducible DF2000 mutant with no exchange of either the glucose or phosphate portions of the molecule.

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References

1 Winkler, H.H. (1973) J. Bacteriol. 116, 1079—1081
2 Fraenkel, D.G., Falcoz-Kelly, F. and Horecker, B.L. (1964) Proc. Natl. Acad. Sci. U.S. 52, 1207—1213
3 Winkler, H.H. (1966) Biochim. Biophys. Acta 117, 231—240
4 Pogell, B.M., Maity, B.R., Frumkin, S. and Shapiro, S. (1966) Arch. Biochem. Biophys. 116, 406—415
5 Dietz, G.W. and Heppel, L.A. (1971) J. Biol. Chem. 246, 2881—2884
6 Heppel, L.A. (1969) J. Gen. Physiol. 54, 95—109
7 Winkler, H.H. (1970) J. Bacteriol. 101, 470—475
8 Winkler, H.H. (1971) J. Bacteriol. 107, 74—78
9 Dietz, G.W. and Heppel, L.A. (1971) J. Biol. Chem. 246, 2885—2890
10 Kornberg, H.L. and Smith, J. (1969) Nature 224, 1261—1262
11 Ferenci, T., Kornberg, H.L. and Smith, J. (1971) FEBS Lett. 13, 133—136
12 Kadner, Robert J. (1973) J. Bacteriol. 116, 764—770
13 Kadner, R.J. and Winkler, H.H. (1973) J. Bacteriol. 113, 895—900
14 Dietz, G.W. (1972) J. Biol. Chem. 247, 4561—4565
15 Kaback, H.R. (1972) Biochim. Biophys. Acta 265, 367—416
16 Winkler, H.H. (1973) J. Bacteriol. 116, 203—209
17 Roseman, S. (1969) J. Gen. Physiol. 54, 138s—180s
18 Fraenkel, D.G. (1968) J. Bacteriol. 95, 1267—1271
19 Fraenkel, D.G. (1968) J. Biol. Chem. 243, 6451—6459
20 Davis, B.D. and Mingioli, E.S. (1950) J. Bacteriol. 60, 17—28