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Down-regulation of the hexose transport system: Metabolic basis studied with a fibroblast mutant lacking phosphoglucose isomerase

(metabolic schism/transport curb/release by malonate/glucosephosphate isomerase)

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ABSTRACT **Down-regulation ("curb") of hexose transport in Chinese hamster lung fibroblasts has been studied in a metabolic mutant highly defective in phosphoglucose isomerase (PGI; glucosephosphate isomerase; D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9). In the parental strain (PGI^+) glucose as well as glucosamine and mannose were able to elicit a curb of the hexose transport system. In the PGI mutant, only glucose was able to mediate a transport curb. The inability of glucosamine and mannose to promote a transport curb in the PGI^- strain must be ascribed to the fact that the 6-esters of these aldohexoses are converted by their own specific deaminase and isomerase to fructose 6-phosphate, which initiates the pyruvate–tricarboxylate energy-yielding pathway but cannot be converted to glucose 6-phosphate in the mutant. The latter ester can be metabolized, but its metabolism in the mutant is confined to the pentose shunt. It is shown that inhibitors such as 2,4-dinitrophenol and malonate exert only slight inhibition of the pentose shunt yet release the glucose-mediated curb elicited by glucose and glucosamine in the parental PGI^+ strain and also the glucose transport curb persisting in the PGI mutant.**

It has recently been observed in cultured hamster fibroblasts that glucose-mediated down-regulation of the hexose transport system (abbreviated "transport curb") was released by addition of 2,4-dinitrophenol (DNP), oligomycin, and malonate (1, 2). Because these inhibitors also interfere with oxidative phosphorylation and the tricarboxylate cycle, one is led to believe that oxidative energy metabolism may be crucial for the preservation of the transport curb (2).

The role of lactic acid generation for the establishment of the transport curb was considered dubious, especially after it was disclosed that the amino sugar D-glucosamine exerts a marked transport curb of the hexose transport system, yet only traces of lactic acid are generated (1, 2).

Fructose, a furanoid ketohexose, is recognized neither by the hexose transporter from erythrocytes (3) nor by that of hamster fibroblasts (see table 1 of ref. 2). Yet the catabolism of fructose, via fructose 6-phosphate (Fru-6-*P*), is presumably much like that of D-glucosamine.

The glucose-mediated transport curb and its release by glucose starvation has recently been studied on a tumor line, 023, from Chinese hamster lung fibroblasts (4). In contrast to other fibroblast lines, in which the presence of cycloheximide at the onset of glucose starvation interferes with the release of the transport curb (5, 6), no interference was observed in 023 (4).

In 1980, Pouyssegur *et al.* (7) isolated and characterized a metabolic mutant called DS-7. It was shown (7) to be highly defective in the enzyme phosphoglucose isomerase (PGI; glucosephosphate isomerase; D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9).

This strain and the parental strain 023 are tumorigenic lines, although DS-7 is nonglycolytic (8). For the sake of clarity, we shall call the DS-7 strain with the defect in phosphoglucose isomerase PGI^- and the parental strain 023 PGI^+. Not only would the PGI^- state interfere with the conversion of glucose 6-phosphate (Glc-6-*P*) to Fru-6-*P*, but also glucosamine catabolism would be affected; glucosamine 6-phosphate can be enzymatically deaminated, but only via Fru-6-*P* (9). Hence in the PGI^- mutant, in which the conversion Fru-6-*P* → Glc-6-*P* is not operative, glucosamine and mannose cannot be efficiently metabolized via the pentose-phosphate shunt.

MATERIALS AND METHODS

Cells and Culture Conditions. Chinese hamster lung fibroblasts free of mycoplasma were grown in Dulbecco's modified Eagle's medium (DME medium) containing 4.5 g of glucose per liter with 10% fetal calf serum and 4 mM L-glutamine.

When the cells were near confluent, the cultures were washed twice with sugar-free medium and maintained for 16–20 hr in modified DME medium [without $Fe(NO_3)_3$, glycine, serine, and pyruvate]. In some samples glucose was omitted or replaced by other sugars as indicated. Malonate and DNP were added as stated previously (1, 2).

Galactose Uptake Test. Cultures were rinsed three times with 37°C phosphate-buffered saline, pH 7.2 (P_i/NaCl). They were then incubated with [^{14}C]galactose (0.1 mM, 0.2–0.5 μCi/ml; 1 Ci = 3.7×10^{10} becquerels) for 10 min at 37°C. L-[^{3}H]Glucose was added to the labeled galactose as usual to test for completeness of washing. After the uptake test, cultures were rinsed three times with ice-cold P_i/NaCl. The cultures were then extracted with 70% (vol/vol) ethanol for 15 min or more. The radioactivity of an aliquot of the ethanol extract was measured in a scintillation counter. The cell protein remaining on the dish was dissolved in 0.1 M NaOH and determined by a modified Lowry method, tailored to the various amounts of protein remaining (2).

3-*O*-Methylglucose (3-*O*-MeGlc) Transport. Before the transport test the cells were loaded with nonradioactive 3-*O*-MeGlc. Cells were rinsed three times with sugar-free and serum-free DME medium. They were then incubated for 30 min at 37°C with serum-free and glucose-free DME medium

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Abbreviations: PGI, phosphoglucose isomerase; DNP, 2,4-dinitrophenol; Fru-6-*P*, fructose 6-phosphate; Glc-6-*P*, glucose 6-phosphate; DME medium, Dulbecco's modified Eagle's medium; P_i/NaCl, phosphate-buffered saline; 3-*O*-MeGlc, 3-*O*-methylglucose.

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Table 1. Catabolic down-regulation of 3-*O*-MeGlc transport by glucose and glucosamine in the PGI mutant and its parental strain

Conditioning medium	3-*O*-[^{14}C]MeGlc transported, pmol/mg cell protein per 20 sec	
	DS-7 (PGI$^-$)	023 (parental)
No hexose	13.0	17.8
No hexose + malonate	23.9	20.3
Glucose	3.6	4.6
Glucose + malonate	15.3	12.0
Glucosamine	10.7	5.7
Glucosamine + malonate	10.7	17.5
Fructose	15.2	18.2
Fructose + malonate	21.7	20.8

Concentrations in medium: glucose, 22 mM; glucosamine, 5 mM; fructose, 22 mM; malonate, 25 mM; DME medium with 4 mM L-glutamine. Dialyzed fetal calf serum, 10%. Transport was measured at 23°C.

containing 50 mM 3-*O*-MeGlc. The cultures were then rinsed rapidly, at least three times at 22°C with P_i/NaCl and then given 3-*O*-[^{14}C]MeGlc (1 μCi/ml, uniformly labeled in the glucose moiety) containing L-[^{3}H]glucose, usually for 20 sec at 22°C. The cultures were finally rinsed rapidly (10 sec) with ice-cold P_i/NaCl and extracted with ethanol.

CO_2 Determination. Cells were grown on the bottom surface of upright 25-cm^2 tissue culture flasks in the usual standard DME medium. The near-confluent cells were rinsed twice with sugar-free medium, then incubated at 37°C with medium containing 25 mM Hepes buffer substituted for $NaHCO_3$, 5 mM glucose, and 1 μCi of [1-^{14}C]glucose (New England Nuclear) for various times in a serum-stopper-sealed flask into which a plastic center well containing a glass-fiber filter paper had been inserted. To test for contamination, L-[^{3}H]glucose was added to the incubation medium. After the incubation flasks were chilled in ice, 0.2 ml of 0.1 M $NaHCO_3$ was injected into the sample as a carrier, and 0.2 ml of phenethylamine was injected into the center well. Lastly 0.2 ml of 10% trichloroacetic acid was injected into the sample. The flasks were shaken for 30 min at room temperature. The filter paper was then removed from the center well and transferred to a counting vial containing 10 ml of Aquasol (New England Nuclear), and its radioactivity was measured in a scintillation counter overnight. Lactic acid was determined by assay with lactate dehydrogenase (Sigma) as described in the Sigma catalogue.

RESULTS

In order to distinguish between inhibition effects and down-regulatory (curb) effects, cells incubated in medium containing the conditioning hexoses were examined with and without inhibitors of oxidative energy metabolism. A genuine metabolic transport curb should be released by the prolonged coincubation with such inhibitors (1, 2). Table 1 shows that the hexose transport system of the PGI$^-$ strain is strongly curbed by glucose, a curb that was released when malonate was also present. In contrast D-glucosamine [added in amounts that may be optimal for its catabolism (10)] was unable to promote any genuine transport curb in the PGI$^-$ strain. The parental PGI$^+$ strain responded to incubation with D-glucosamine as well as to glucose by a typical transport curb, which could be released (or prevented) by coincubation with malonate. Absence of hexoses or of aldohexoses (i.e., exposure to a ketohexose such as fructose) gave rise to a release of the hexose transport curb in the absence of any metabolic inhibitors.

Table 3. Lactic acid generation in the PGI mutant and its parental strain

Strain	Hexose in medium	Lactic acid,* nmol/ml medium	Relative hexose uptake
DS-7 (PGI$^-$)	Glucose	464	(1.00)
	Mannose	6,706	2.6
	Glucose + mannose	550	1.0
	None	324	3.3
	Glucosamine	680	2.3
023 (parental)	Glucose	12,413	(1.00)
	Mannose	12,663	0.73

Concentrations: glucose, 22 mM; mannose, 22 mM; and glucosamine, 5 mM; DME medium with 4 mM L-glutamine. Dialyzed fetal calf serum, 10%.
* Lactic acid excreted over 20 hr.

This pattern is also illustrated in Table 2, in which the uncoupler DNP has been used. The PGI$^+$ strain showed a typical transport curb by glucose as well as by glucosamine; both responses were released or counteracted by DNP. The same type of incubation with the PGI$^-$ strain showed once more that this mutant has lost its transport-curb response mediated by glucosamine but preserved the glucose response. Incubation of mannose with the mutant, although generating lactic acid by itself, did not mediate any transport response, whereas the parental strain responded as well to mannose as to glucose (see Table 3). A response to mannose has also been recorded in NIL strains (11).

Malonate and DNP interfere with oxidative energy metabolism following the Fru-6-*P*–phospho*enol*pyruvate–tricarboxylate cycle. The 6-esters of mannose and glucosamine are converted to Fru-6-*P* by a specific isomerase and a deaminase, respectively. In the PGI$^-$ strain, glucose is unable to become converted to Fru-6-*P* and hence to phospho*enol*pyruvate and the energy-generating cycle. The latter can presumably also be

Table 2. DNP releases hexose transport curb mediated by aldohexoses

Hexose in medium	3-*O*-[^{14}C]MeGlc transported, pmol/mg cell protein per 20 sec					
	DS-7 (PGI$^-$)			023 parental		
	Without DNP	With DNP	With DNP/ without DNP	Without DNP	With DNP	With DNP/ without DNP
None	16			13.4		
Fructose	13.8	22.0	1.6	9.5	19.4	2.0
Glucose	3.2	9.3	2.9	3.5	9.7	2.9
Glucosamine	11.4	10.7	0.9	2.8	15.8	5.6

Concentrations in medium: fructose, 22 mM; glucose, 22 mM; glucosamine, 5 mM; DNP, 0.2 mM. Transport was measured at 23°C.

Table 4. $^{14}CO_2$ production from [1-^{14}C]glucose by the PGI$^-$ mutant

Incubation		Total
Time, hr	Medium	$^{14}CO_2$, nmol
1	Control	17.6 ± 2.0
	Malonate	13.4 ± 1.1
3	Control	71.9 ± 15.9
	Malonate	57.8 ± 2.2

$^{14}CO_2$ production was measured by incubating near-confluent cultures with 5 mM [1-^{14}C]glucose in the presence or absence of 25 mM sodium malonate. Results are mean ± SD.

"rekindled" by L-glutamine (see *Discussion*), a major constituent of DME medium, and hence oxidative energy metabolism is always available, even to the PGI$^-$ strain. However, in the mutant, only glucose can initiate the first steps of the Glc-6-*P* pentose shunt. The preservation of the glucose-mediated transport curb in the PGI$^-$ strain testifies to the additional involvement of the shunt in the composite type of hexose transport curb. Hence, it seems that both oxidative pathways, the energy-generating one as well as the shunt, are needed. The interference of the former by inhibitors such as malonate does not apply to CO_2 generation from the pentose shunt. We have found by the use of [1-^{14}C]glucose that in the PGI$^-$ strain, the shunt remained highly active, even in the presence of malonate (see Table 4).

DISCUSSION

As pointed out in the Introduction, the glucose-mediated transport curb requires the uptake of a hexose that can be metabolized beyond the 6-phosphoric ester state and is also a ligand of this transport system. Neither the hexose analogues 3-*O*-MeGlc and 2-deoxy-D-glucose (which qualify with respect to the second requirement but not in regard to the first) nor fructose [which fulfills the first requirement but does not behave like a ligand (2)] is able to down-regulate hexose transport in PGI$^+$ strains such as NIL (2, 10) or 023 (Tables 1 and 2; unpublished data). In contrast, glucose, mannose, and D-glucosamine, which satisfy both requirements (2), exerted a marked transport curb in 023 (PGI$^+$).

It is noteworthy that DS-7 responded so strongly to glucose, which in this mutant generates little energy metabolism (7). Conversely, D-glucosamine and mannose, which remain good energy sources in the mutant, were unable to mediate a significant transport curb. A genuine transport curb should be counteracted by DNP or by malonate (1, 2), but, as seems evident, the effect of D-glucosamine on the PGI mutant was unaltered in the presence of inhibitors of oxidative energy metabolism. In contrast, the glucose curb of the transport system of the mutant was lifted by DNP or by malonate. Because we found that malonate does not significantly inhibit the generation of labeled CO_2 from [1-^{14}C]glucose (Table 4), i.e., via the Glc-6-*P* pentose shunt, the effect of malonate on the only remaining transport curb in the mutant must be an effect on oxidative energy metabolism from another source. We suspect that the other source from which the mutant can generate oxidative energy stems from L-glutamine, which is present in abundant amounts in the maintenance medium. Besides its many biosynthetic functions, L-glutamine also contributes to oxidative energy metabolism (12, 13). This in turn proceeds via the tricarboxylate cycle, which is known to be sensitive to malonate. Hence, the metabolic transport curb relies on two oxidative pathways, the Glc-6-*P* pentose shunt[§] and the pyruvate–tricarboxylate cycle, fed either from L-glutamine or from Fru-6-*P* via pyruvate. As mentioned, Fru-6-*P* can be formed in the mutant either from glucosamine 6-phosphate by enzymatic deamination or from mannose 6-phosphate by a specific isomerase, which apparently is well developed in both strains, because mannose generated an abundance of lactic acid. In 023, which is PGI$^+$, mannose and D-glucosamine as well as glucose are able to participate in both types of oxidative metabolism. The requirement of the curb for two oxidative pathways could be disclosed only by our systematic tests on the PGI mutant. We also suspect that the role of L-glutamine in the regulation of transport in 023 and DS-7 is quite different than that observed in the NIL strains. In the NIL strains absence of L-glutamine (combined with presence of glucose) may affect the transport system much like cycloheximide combined with glucose (14)—i.e., curtailing the population of hexose transporters in the membrane. In contrast, in the 023 and DS-7 strains, the role of L-glutamine seems largely dominated by its role in oxidative energy metabolism. In our further studies of regulation of hexose transport, we have tried to maintain 023 and DS-7 in media devoid of L-glutamine for 20 hr and found this feasible. This seems to enable us to demonstrate more clearly the peculiar schism in metabolic regulation of hexose transport in the PGI mutant (15).

§ A small fraction of the ribulose 5-phosphate might reach the stage of triose phosphate and pyruvate. However, because the mutant also synthesizes RNA, most of the ribulose 5-phosphate is probably committed to the formation of ribose 5-phosphate.

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