The Effect of Increased Phosphoglucose Isomerase on Glucose Metabolism in Saccharomyces cerevisiae*

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Comparison of microbial strains with normal and high content of single enzymes is coming into use for metabolic analysis and in vitro assessment of enzyme function. We present an example for phosphoglucose isomerase and glucose metabolism in the yeast Saccharomyces cerevisiae. We use cell suspensions in conditions of inhibited protein synthesis and respiration, with low assimilation, rapid and linear glucose utilization, fermentation almost quantitative, and high enough cell density for direct preparation of extracts for metabolite analysis. The mass action ratio and fitting of fructose-6-P and glucose-6-P concentrations and kinetic parameters of the enzyme are not inconsistent with near equilibrium of the reaction in the wild-type strain and small if any change in the high level strain. However, this conclusion would require that the V_{max} values underestimate the activity in the cell. On the other hand, the specific activities of glucose-6-P and fructose-1,6-P_{2} during metabolism of [2-3H]glucose are quite high which, together with knowledge of tritium exchange and isotopic effects for the reaction in vitro, would point to the reaction in the wild-type strain being far from equilibrium; the specific activities are lower in the high level strain, indicating that extra enzyme is functional. One way to reconcile the latter results would be for tritium exchange to be considerably lower in vitro than known in vitro.

One aim of metabolic analysis is to reconcile enzyme properties in vitro with functioning of the enzyme in vivo, i.e. metabolic fluxes. The subject can be experimentally and theoretically exacting and even controversial (1–4), particularly regarding enzyme to enzyme channeling of intermediates (5, 6). In general, theory (7, 8) is more advanced than its application, and genetics has contributed little (9).

One relatively simple application of genetics, at least for microbes, is coming into use: comparing of strains differing only the enzyme in vivo,i.e. meta-
tabolic fluxes. The subject can be experimentally and theoretically exacting and even controversial (1–4), particularly regarding enzyme to enzyme channeling of intermediates (5, 6). In general, theory (7, 8) is more advanced than its application, and genetics has contributed little (9).

One relatively simple application of genetics, at least for microbes, is coming into use: comparing of strains differing only in the presence of a multicopy plasmid carrying a gene and hence with the gene product, the enzyme of interest, in normal or much higher amount, respectively. This paper addresses this case for phosphoglucose isomerase in Saccharomyces cerevisiae, and concerns glucose flux, substrate and product concentrations, and their labeling during metabolism of [2-3H]glucose. We employ a system of analysis of non-growing cells which may have general use.

MATERIALS AND METHODS

Strains, Media, and Resting Cell Incubations—Minimal medium M61 (10) is buffer B61 (0.1 M KH_{2}PO_{4}, 15 mM (NH_{4})_{2}SO_{4}, 0.8 mM MgSO_{4}, 2 mM (Fe_{2})(SO_{4}), adjusted to pH 6.1 with KOH), supplemented with 0.4 mg/l each of thiamine-HCl, pyridoxine, and pantothenate, and with 0.4% peptone, 0.2% yeast extract. The specific activity was 2.3% glucose. It was used for growth of strain DFY425 (a, leu2-1) carrying either multicopy vector YEp13 (LEU2) or this vector with the phosphoglucoisomerase gene PGI1 (11). The initial experiments with strain DFY1 (a lys1-1), the usual wild-type strain employed in this laboratory, and congenic with DFY425, employed growth in enriched medium R61 (buffer B61 supplemented with 10 g/liter Bacto-tryptone (Difco) and 4 g/liter Bacto-yeast extract (Difco)) (10). The added carbon sources are specified.

In the standard resting cell incubation, cells harvested by centrifugation after growth from small inocula (OD_{580} values of 0.02 or less) were washed once in B61 and resuspended in B61 also containing 10 μg/ml cycloheximide and 2 μg/ml antimycin A. After 30–60 min of preincubation at 30 °C with gentle mixing, glucose was added (zero time), and the culture was sampled periodically. Other than experiments only measuring glucose or ethanol (Table I), which used culture supernatants, sampling was as described (12): 0.5 ml portions of tubes containing 0.028 ml of 11.7 μL per chloric acid, 20 μL Na_{2}EDTA, vigorously mixed for 30 s, and, after at least 30 min neutralized with a solution of 3 mM KHCO_{3}, and, within 1 h, centrifuged to remove salts and cell debris.

Glucose utilization in resting cells, v_{glu}, was essentially linear with time (e.g. Fig. 1), and fairly constant over a considerable range of OD_{580} values in the incubation. For example, in a comparison of the same cells incubated at 250, 150, 75, and 25 OD_{580}/ml, the specific rates of glucose consumption (μmol/OD_{580} h) were 2.48, 2.26, 2.11, and 1.53, respectively. Comparison of different strains or conditions of incubation or previous growth employed the same cell density.

Experiments with [2-3H]- or [U-14C]Glucose—Cells from 500-ml cultures in growth on minimal medium were collected at an OD_{580} of 0.5–1.8, washed, and the drained pellets were resuspended in 1–2 ml of B61 and kept on ice. One sample of these cells was diluted to OD_{580} of 50 in 50 ml KH_{2}PO_{4} (pH 7.4), 2 mM Na_{2}EDTA, 2 mM 2-mercaptoethanol for assay of phosphoglucoisomerase. With another sample the usual preincubation with cycloheximide and antimycin A was set up at OD_{580} of 0.3–1.0, and at zero time diluted to OD_{580} of 250 by addition of 2% glucose containing [U-14C]glucose (ICN 11047, 3.48 mCi/mmol) or [2-3H]glucose (Amersham Corp. TRK.36, 18 mCi/mmol); final specific activities are shown in Table IV. At the appropriate time(s) determined by assessment of v_{glu} with unlabeled glucose, a 0.5-ml sample was acidified and extracts prepared as above and frozen until use.

The thawed extracts were re centrifuged, and the supernatants filtered (Gelman 0.45 μm Acrodisk LC13), diluted with 5 volumes of water, and chromatographed by anion-exchange using Beckman Ultrasil AX columns and a 26-ml linear gradient of potassium phosphate (12). Fractions containing glucose-6-P were combined and the glucose-6-P converted to 6-phosphate, by addition to a 1-ml sample of 0.1 mM NADP and 2 mM MgCl_{2} and 2 μl of glucose-6-P dehydrogenase (Boehringer Mannheim 127 035, 1 mg/ml). The reaction at 30 °C was followed to completion at A_{414}. After a 5-min treatment at 100 °C and refiltration, the 6-phosphogluconate was obtained by the same regime of anion-exchange chromatography. The appropriate fractions were assayed by direct addition (final volume 0.6 ml) of 5 mM potassium phosphate (pH 6.7), 0.1 mM NADP, and 2 μl of 6-phosphogluconate dehydrogenase.

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RESULTS

Glucose Metabolism in Resting Cells—We have previously described (17) the metabolic characteristics, which are exceptional, of the wild-type S. cerevisiae strain, DFY1, employed in this laboratory. During growth in the usual enriched medium, its rate of glucose metabolism, \( v_{\text{Glu}} \), the product of the reciprocal of the yield times the first order growth rate constant, \( \mu \), was \(-2.5 \text{ mmol/h/m}^2 \text{ OD}_{660} \) of cells, and metabolism largely fermentative, with a yield of \(-1.5 \text{ ethanol and 0.1 glycerol/glucose consumed. After glucose exhaustion under aerobic conditions, with derepression of respiratory metabolism, growth continues at a lower rate at the expense of the ethanol made earlier. As long known, sugar metabolism in microbes may be rapid in non-growing cells (18, 19). When cells obtained from exponential growth in the enriched medium with glucose (i.e. at relatively low cell density when glucose is largely unconsumed) are resuspended in mineral salts buffer B61 (as used for growth but without supplements), and containing cycloheximide and antimycin A to prevent protein synthesis and respiratory ATP formation, glucose utilization was linear with time, as in Fig. 1, with a \( v_{\text{Glu}} \) about three-fourths that in growth and a similar ethanol yield (Table I, line 1). In such conditions \(-90\% \) of glucose used can be accounted for, chiefly by the fermentation products, with assimilation, assessed as trichloroacetic acid-insoluble material from incubations in \([6-^{14}\text{C}]\text{glucose, being only a few percent (Table II). We therefore use the } v_{\text{Glu}} \text{ value as an estimate of the net flux through phosphoglucoisomerase, } v_{\text{pdi}}. \) (\( v_{\text{pdi}} \) will be referred to as } v \text{ and is the difference between forward and reverse rates, } i.e. \( v_f - v_r \).) \( v_{\text{Glu}} \) values were about the same in resting cells incubated without antimycin A (Table I, line 2); however, constancy of products regardless of cell density would probably require special aeration. As expected for an organism which ferments aerobically, the presence of antimycin A in growth was without large effect (lines 3 and 4). On the other hand, \( v_{\text{Glu}} \) values were significantly lower for cells grown in galactose (lines 5–8) or

TABLE I

| Growth medium | Ant | \( v_{\text{Glu}} \) | Ethanol/glucose |
|---------------|-----|-----------------|----------------|
| 1) Glucose    | +   | 1.70 (9)        | 1.6            |
| 2) Glucose    | -   | 1.56 (7)        | 1.1            |
| 3) Glucose (+Ant) | +   | 1.73 (1)        |                |
| 4) Glucose (+Ant) | -   | 1.76 (1)        |                |
| 5) Galactose  | +   | 0.87 (3)        | 1.75           |
| 6) Galactose  | -   | 0.53 (3)        | 0.33           |
| 7) Galactose (+Ant) | +   | 0.80 (3)        |                |
| 8) Galactose (+Ant) | -   | 0.77 (3)        |                |
| 9) Ethanol    | +   | 0.45 (3)        | 1.5            |
| 10) Ethanol   | -   | 0.28 (3)        | 0.33           |
| 11) Glucose (high OD_{660}) | +   | 0.97 (3)        |                |
| 12) Glucose (high OD_{660}) | -   | 0.92 (3)        |                |
ethanol (lines 9 and 10), and intermediate with cells from cultures grown to saturation in glucose (lines 11 and 12). Although the various differences might in part reflect different optical properties of cells from different conditions of growth, this strain tested from a similar range of growth conditions gave the narrow range of values of 180–230 μg dry weight/OD₆₅₀. All the experiments which follow used cells obtained from exponential growth on glucose, and the “standard” incubation in the presence of cycloheximide and antimycin A.

With high cell densities, metabolites of interest may be obtained in adequate amounts by direct extraction. After 30 min of preincubation without glucose, glucose-6-P, fructose-6-P, and fructose-1,6-P₂ are in low concentration. Upon glucose addition they rise and, after a substantial overshoot, reach a quasi-steady state, returning to low values after glucose exhaustion (Fig. 1). The overshoot, often reported (e.g., 20, 21), will not be discussed further; short term oscillations of metabolites after metabolic perturbation are also known (e.g., 22).

The Effect of High Phosphoglucone Isomerase on \( \nu_{\text{Glu}} \) and Metabolites—We compared a wild-type strain congeneric with DFY1, DFY425, either carrying the multicopy vector YEpl3 or this vector with the phosphoglucone isomerase gene, i.e., pPGI (Table III). Phosphoglucone isomerase was in ~11 times normal amount in the latter strain; as shown for pyruvate kinase, the plasmid does not affect amounts of other glycolytic enzymes (11). By assay, the \( V_{\text{max}} \) forward value of 21.5 for the strain with normal amount of enzyme is in ~20-fold excess to the actual glucose flux value (\( \nu_{\text{Glu}} \)) of 1.15 (same units). One would not, therefore, expect the latter value to change in the strain with high level of enzyme, and indeed it was about the same (1.34). Glucose-6-P and fructose-6-P concentrations were barely high level of enzyme, and indeed it was about the same (1.34). Glucose-6-P-specific activities were not exceptional (23) and, as above, underestimate the \( \nu_{\text{Glu}} \) of 1.2; 6-phosphogluconate inhibition, known in vitro (10) might further depress the expected rates. However, we note that, aside from the usual possibility that the enzyme parameters are slightly different in the cell, and the reported range of \( K_m \) values being large anyway (13), the calculation is very sensitive to small errors. (For example, if glucose-6-P concentration were 2.2 mM instead of 1.8 mM and the other values were unchanged, \( \nu_r - \nu_c \) would be 1.2.) Thus, as with the mass action ratio, the data on concentration of metabolites together with known kinetic constants are not quite of line with the reaction being close to equilibrium in the wild-type strain and having more enzyme making little difference.

Metabolism of [2-\(^3\)H]Glucose—[2-\(^3\)H]Glucose has been employed to assess the phosphogluconate isomerase reaction in vivo (reviewed in Ref. 25), ever since demonstration of significant retention of tritium during in vitro catalysis: equilibration of [2-\(^3\)H]glucose-6-P or [1-\(^3\)H]fructose-6-P with enzyme gives complete exchange of tritium into water, while trapping of the product shows intramolecular proton transfer between C1 and C2 and only partial exchange with protons from the medium (27–29). In the present work we have done the resting cell incubations with [2-\(^3\)H]glucose and obtained the specific activities of intracellular glucose-6-P and of fructose-1,6-P₂.

As tested with [U-\(^1\)C]glucose (Table IV, lines 1–3), the isolated compounds had specific activities similar to the glucose employed, as expected (Fig. 1) for the pools being derived from external glucose. With tritiated glucose, experiments with cells from the steady state showed glucose-6-P-specific activities...
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TABLE IV
Specific activities of metabolites during resting cell incubation with radioactive glucose

| Experiment | Glucose | Glucose-6-P | Fructose-1,6-P₂ |
|------------|---------|-------------|----------------|
|            | specific activity (cpm/μmol) | specific activity (cpm/μmol) | specific activity (cpm/μmol) |
| 1) DF425/YEp13 | [U-14C] | 70 (304) | 70 (304) |
| 2) DF425/pPGI | [U-14C] | 72 (82) | 72 (82) |
| 3) DF425/pPGI | [U-14C] | 92 (86) | 106 (99) |
| 4) DF425/YEp13 | [2-3H] | 257 (99) | 117 (45) |
| 5) DF425/YEp13 | [2-3H] | 653 (132) | 246 (50) |
| 6) DF425/YEp13 | [2-3H] | 1,348 (186) | 468 (59) |
| 7) DF425/pPGI | [2-3H] | 140 (54) | 82 (32) |
| 8) DF425/pPGI | [2-3H] | 269 (54) | 149 (30) |
| 9) DF425/pPGI | [2-3H] | 614 (85) | 261 (36) |

* In experiments 6 and 9, the samples were obtained during the early phase of the incubations where metabolite concentrations peak before falling to a steady level (see Fig. 1).

Table IV is normalized to 1.0, and \( v \) is the rate of the phosphoglucone isomerase back reaction, normalized to \( v_{\text{max}} \) and \( r \) are the tritium retention fractions in the forward and reverse reactions, respectively, and \( df \) and \( dr \) the discriminations against tritium (rate with \( ^{3}H \) tritium in vivo). Values for \( F_{\text{Glu}} \) and \( F_{\text{Glu}} \) are shown in Table V. Fig. 2 shows \( G \), the normalized specific activity of glucose-6-P, as a function of \( v \), according to Equation 3, using the four parameters, tritium retention, and isotope discrimination for each direction pointed recently for the yeast enzyme (14, 22).

The expected specific activities should be related to retention of tritium in each single one way reaction and isotope discrimination against tritiated substrate (14, 28), and to the actual forward and reverse rates. A formulation analogous to that of Katz and Rognstad (26, 30) is shown in Table V, Fig. 2 shows \( G \), the normalized specific activity of glucose-6-P, as a function of \( v \), according to Equation 3, using the four parameters, tritium retention, and isotope discrimination for each direction pointed recently for the yeast enzyme (14, 22).

Because of the amounts of intermediates (Table III), \( F \), the specific activity of fructose-6-P, was not obtained and application of Equation 3 (which requires values of both \( F \) and \( G \)) employed, instead of \( F \), the specific activity of fructose-1,6-P₂. Fructose-1,6-P₂ coming from fructose-6-P should have the same specific activity, but later exchange reactions might reduce the value. Hence, use of Equation 4, which requires only the value of \( G \), is preferred. \( G \) is obtained from Equations 3 and 4 and the data of Table IV are shown in the lower part of Table V. Both calculations give \( v \), values for the wild-type strain of only ~4.

**DISCUSSION**

As mentioned, strains with increased activity of single enzymes are coming into use for studies in vitro. For example, Brindle (33) concluded that there was acceptable fit, in *vitro*, for increased phosphoglycerate kinase in yeast. For another reversible reaction, aldolase in *Escherichia coli* the data could also be fitted, but required that in *vitro* \( V_{\text{max}} \) be higher than assayed (12). For irreversible reactions, with increased phosphofructokinase in yeast a compensatory decrease in fructose-2,6-P₂ was found to explain the absence of large effect on fructose-6-P concentration (34). And for high expression of fructose-1,6-bisphosphatase in yeast, a lack of futile cycling in glucose metabolism was suggested to reflect its inhibition by fructose-2,6-P₂ (35).

For these parameters, interpretations have been conservative, and unusual hypotheses (see below) were not needed to reconcile in *vivo* and in *vitro* results. In the present work, there are three findings to consider. First, the mass action ratios, which show the phosphoglucone isomerase reaction to be near equilibrium even in the wild-type strain, and hence high one way fluxes. Second, the retention of label from [2-3H]glucose in glucose-6-P, which points to lower flux values in the wild-type strain (0.5 of ~4, Table V). And third, the reduced labeling of glucose-6-P in the strain with high level of enzyme, which calculates (Table V) to a \( v \), of only three times wild-type even though the measured factor of enzyme increase was 11 and substrate and product concentrations were almost unchanged.

These results would be partially reconciled if, as well as the measured \( V_{\text{max}} \), underestimating the values in the cell, intrac-
molecular proton retention for the single one way reactions in vivo were higher than known in vitro. As an example, using the average of the two isootope discrimination values employed for Table V (i.e. \( df = dr = 0.33 \)) but increasing the proton retention fractions to 0.98 (for both \( rf \) and \( r \); they should be the same for the known enzyme mechanism (28)), then for the wild-type strain G value of 1.15 \( v \), would be 8.4, and for the high level strain G value of 0.54 it would be 83. Thus the factor of extra enzyme would be as measured and the actual one way rates also much higher.

Other, perhaps less likely, models should be mentioned. Retention of tritium in products from [2-3H]glucose has been often reported (see 26), although direct measurement of glucose-6-P is rare (36, 37). Dorrer et al. (38) suggested that in Chlorella the fact of similar specific activities to glucose, per hexose in sucrose, pointed to channeling of hexose-phosphates in a multienzyme complex. And Malaisse and Bodur (37) show calculations, based on data from pancreatic islet cells, interpreted as best fitting with partial channeling between phosphoglucone isomerase and phosphofructokinase. The same type of model could be applied here. Or, one might speculate that extra enzyme is at least partially not functional because of being in the wrong place or differently modified. Or, a regulatory circuit acting to establish hexose monophosphates at their apparent equilibrium ratio might be imagined.

Finally, the present method for assessing metabolism in non-growing yeast differs in some ways from custom, using inhibition of protein synthesis rather than implicitly relying on no new enzyme being made during an incubation, antimycin D for a non-respiratory condition, linear glucose use and proportionality to amount of cells, and some knowledge of assimilation. There is a similar protocol for E. coli (12). This type of incubation may have general use for in vivo studies of enzymes changed in amount or kinetic characteristics. It will be also be interesting to apply it to cases where enzyme amount can be set to lower than normal.

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REFERENCES
1. Garfinkel, L., Kohn, M. C., and Garfinkel, D. (1978) Eur. J. Biochem. 93, 183–192
2. Wright, B. E., and Kelly, P. J. (1981) Curr. Top. Cell. Regul. 19, 103–158
3. Shiraiishi, F., and Savageau, M. A. (1992) J. Biol. Chem. 267, 22934–22943
4. Sola, A. (1981) Curr. Top. Cell. Regul. 19, 77–101
5. Srivastava, D. R., and Bernhard, S. A. (1986) Curr. Top. Cell. Regul. 28, 1–68
6. Cornish-Bowden, A., and Cardenas, M. L. (1993) Eur. J. Biochem. 215, 67–92
7. Crabtree, B., and Newholme, E. A. (1985) Curr. Top. Cell. Regul. 25, 21–76
8. Kell, D. B., and Westerhoff, H. V. (1986) FEMS Microbiol. Rev. 30, 305–320
9. Franke, D. G. (1992) Annu. Rev. Genet. 26, 159–177
10. Franke, D. G. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4740–4744
11. Kawaaki, G., and Franke, D. G. (1982) Biochem. Biophys. Res. Commun. 108, 1107–1112
12. Bohle, J., Clifton, D., Kretschmer, M., and Franke, D. G. (1993) Biochemistry 32, 4682–4692
13. Neillmann, E. A. (1972) in The Enzymes (Boyer, P. D., ed.), 3rd ed., Vol. VI, pp. 271–354, Academic Press, New York
14. Lievens, V., Malaisse-Lagae, F., Willem, R., and Malaisse, W. J. (1989) Biochim. Biophys. Acta 998, 111–117
15. Clifton, D., Weinstock, S. B., and Franke, D. G. (1978) Genetics 88, 1–11
16. Lang, J. M., and Cirillo, V. P. (1987) J. Bacteriol. 169, 2393–2397
17. Walsh, R. B., Clifton, D., Horak, J., and Franke, D. G. (1991) Genetics 128, 521–527
18. Harden, A. (1923) Alcoholic Fermentation, Longmans, Green and Co., London
19. Gunsalus, I. C., and Shuster, C. W. (1970) The Bacteria: a Treatise on Structure and Function (Gunsalus, I. C., and Stanier, R. Y., eds) Vol. 2, pp. 1–58, Academic Press, New York
20. Barwell, C. Y., Woodward, B., and Brunt, R. V. (1971) Eur. J. Biochem. 18, 59–64
21. den Hollander, J. A., Brown, T. R., Uguib, K., and Shulman, R. G. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 6096–6100
22. Betz, A., and Chance, B. (1965) Arch. Biochem. Biophys. 109, 585–594
23. Newholme, E. A., and Start, C. (1973) Regulation in Metabolism, Wiley & Sons, New York
24. Wurster, B., and Schneider, F. (1970) Hoppe-Seyler’s Z. Physiol. Chem. 351, 901–906
25. Rose, I. A., and Rose, Z. B. (1969) in Comprehensive Biochemistry (Finkin, E. M., and Stolz, E. H., eds) Vol. 17, pp. 93–161, Elsevier, New York
26. Katz, J., and Rognstad, R. (1976) Curr. Top. Cell. Regul. 19, 237–289
27. Rose, I. A., and O’Connell, E. L. (1961) J. Biol. Chem. 236, 2086–3092
28. Rose, I. A. (1975) Adv. Enzymol. 43, 491–517
29. Seeholzer, S. H. (1995) Proc. Natl. Acad. Sci. U. S. A. 90, 1237–1241
30. Katz, J., and Rognstad, R. (1969) J. Biol. Chem. 244, 99–106
31. Kretschmer, M. (1992) Fit, WindowChemSoftware, Fairfield, CT
32. Malaisse-Lagae, F., Lievens, F., and Malaisse, W. J. (1989) Mol. Cell. Biochem. 89, 57–67
33. Brindle, K. M. (1988) Biochemistry 27, 6187–6196
34. Davies, S. E. C., and Brindle, K. M. (1992) Biochemistry 31, 4729–4735
35. Navas, M. A., Cerdan, S., and Gancedo, J. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1290–1294
36. Rose, I. A., and O’Connell, E. L. (1964) J. Biol. Chem. 229, 12–17
37. Lievens, V., and Malaisse, W. J. (1991) Med. Sci. Res. 19, 427–428
38. Dorrer, H.-D., Fedtke, C., and Trebst, A. (1966) Z. Naturforsch. 21B, 557–562
39. Malaisse, W. J., and Bodur, H. (1991) Int. J. Biochem. 23, 1471–1401