Coordinated Regulation of Ammonium Assimilation and Carbon Catabolism by Glyoxylate in *Saccharomyces cerevisiae*

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The activities of citrate synthase (EC 4.1.3.7) and NADP⁺-dependent glutamate dehydrogenase (GDH) (EC 1.4.1.4) of *Saccharomyces cerevisiae* were inhibited *in vitro* by glyoxylate. In the presence of glyoxylate, pyruvate and glyoxylate pools increased, suggesting that glyoxylate was efficiently transported and catabolized. Pyruvate accumulation also indicates that citrate synthase was inhibited. A decrease in the glutamate pool was also observed under these conditions. This can be attributed to an increased transamination rate and to the inhibitory effect of glyoxylate on NADP⁺-dependent GDH. Furthermore, the increase in the ammonium pool in the presence of glyoxylate suggests that NADP⁺-dependent GDH was being inhibited *in vivo*, since the activity of glutamine synthetase did not decrease under these conditions. We propose that the inhibition of both citrate synthase and NADP⁺-dependent GDH could form part of a mechanism that regulates the internal 2-oxoglutarate concentration.

INTRODUCTION

NADP⁺-dependent glutamate dehydrogenase (GDH) catalyses the synthesis of glutamate from ammonium and 2-oxoglutarate (Holzer & Schneider, 1957). Ammonium is derived from the metabolism of nitrogenous compounds, or from the medium, and 2-oxoglutarate from carbon metabolism. Thus, ammonium assimilation constitutes the metabolic process in which carbon and nitrogen metabolism meet, and NADP⁺-dependent GDH is the enzyme that links these two metabolic pathways. It has been proposed that in *Saccharomyces cerevisiae*, 2-oxoglutarate, or some other intermediate of the tricarboxylic acid cycle, modulates NADP⁺-dependent GDH activity (González *et al.*, 1985). Furthermore, it could be expected that fluctuations in the intracellular levels of intermediates of the cycle could in turn regulate the activity of the NADP⁺-dependent GDH. Thus, regulation of the tricarboxylic acid cycle could have a direct effect on NADP⁺-dependent GDH activity and therefore on ammonium assimilation. This mechanism may constitute a regulatory link between these two metabolic pathways, which could determine the amount of 2-oxoglutarate and ammonium that can be assimilated in a given physiological condition.

It has been shown that cells of *Escherichia coli* growing on glyoxylate contain much lower levels of citrate synthase than similar cells growing on acetate (Kornberg, 1966), suggesting that this compound could modulate the operation of the tricarboxylic acid cycle by regulating the levels of citrate synthase. Furthermore, since the metabolism of glyoxylate through the dicarboxylic acid cycle does not produce 2-oxoglutarate, it would be expected that in the presence of glyoxylate, intracellular 2-oxoglutarate levels would be decreased, and that this would affect the activity of the NADP⁺-dependent GDH and thus ammonium assimilation. We

Abbreviations: GDH, glutamate dehydrogenase; MM, minimal medium.

studied the effect of glyoxylate on citrate synthase and NADP⁺-dependent GDH in *S. cerevisiae* in order to determine whether this 2-oxoacid could play a role in the regulation of carbon and nitrogen metabolism.

METHODS

Strain and growth conditions. The wild-type strain S288C (*MAT α mal gal2*) was obtained from the Cold Spring Harbor Laboratory, NY, USA. Cells were routinely grown on minimal medium (MM) containing salts, trace elements and vitamins following the formula of Difco Yeast Nitrogen Base. Glucose (2%, w/v) was used as carbon source and 40 mM-(NH₄)₂SO₄ as nitrogen source. Glyoxylate or pyruvate were added to the cultures, as appropriate, as aqueous autoclaved solutions. The cultures were incubated at 30 °C with agitation. Cells were grown on YPD medium comprising 1% (w/v) yeast extract (Difco), 2% (w/v) peptone (Difco) and 2% (w/v) glucose, until they reached stationary phase; they were then washed and resuspended in MM. These suspensions were used to inoculate cultures to an OD₆₅₀ of 0.05.

Extraction and determination of intracellular metabolites. For the estimation of 2-oxoglutarate, pyruvate and glyoxylate, extracts were prepared as described by Kang *et al.* (1982). Ammonium was extracted by the method of Tempest *et al.* (1970) and Tachiki *et al.* (1981). The concentrations of ammonium and 2-oxoglutarate were determined with beef GDH by following NADH oxidation at 340 nm (Dubois *et al.*, 1974). Pyruvate concentration was determined with beef lactate dehydrogenase by following NADH oxidation at 340 nm (Bergmeyer, 1963). Glyoxylate was determined by the method of Tribels & Vogel (1966).

Amino acids were extracted and determined as described by González *et al.* (1983). For enzyme determinations, soluble extracts were prepared by grinding whole cells, suspended in their corresponding extraction buffer, with glass beads in a Braun cell disruptor. Citrate synthase (EC 4.1.3.7), NADP⁺-dependent GDH (EC 1.4.1.4) and glutamine synthetase (EC 6.3.1.2) were assayed by the methods of Parvin (1969), Doherty (1970) and Ferguson & Sims (1974) respectively. Protein was determined by the Lowry method, using bovine serum albumin as standard.

Chemicals. All 2-oxoacids, beef GDH, beef lactate dehydrogenase and bovine serum albumin were obtained from Sigma.

RESULTS AND DISCUSSION

Effect of glyoxylate on citrate synthase activity

Citrate synthase from cells grown on MM for 6 h was inhibited by glyoxylate but not pyruvate (Fig. 1). In order to study whether the inhibition of citrate synthase could alter the carbon flow through the tricarboxylic acid cycle we determined the intracellular content of 2-oxoglutarate. In glyoxylate-treated cells 2-oxoglutarate levels were 4-fold higher than in cells incubated in MM without glyoxylate; glyoxylate-treated cells had almost 30-fold higher glyoxylate levels than those from the untreated culture (Table 1). This could be explained by assuming that the utilization of 2-oxoglutarate by NADP⁺-dependent GDH had diminished. It has been proposed that the catabolism of glyoxylate through the dicarboxylic acid cycle can result in the production of pyruvate (Kornberg, 1966). If this were the case, the increase in the pyruvate pools could shift the endogenous glutamate pools towards transamination, which would result in increased levels of 2-oxoglutarate. The pyruvate level in glyoxylate-treated cells was 14-fold higher than cells in MM (Table 1).

Effect of glyoxylate on nitrogen assimilation

Since a decrease in NADP⁺-dependent GDH activity would result in ammonium accumulation, we measured the amount of ammonium present in glyoxylate-treated and untreated cells. Ammonium levels were 2.8-fold higher in glyoxylate-treated cells (Fig. 2*a*). This accumulation could be attributed to a decrease in ammonium assimilation through NADP⁺-dependent GDH or through glutamine synthetase. Since the activity of the latter enzyme reached values 4-fold higher in glyoxylate-treated cells than in control cells (Fig. 3), ammonium accumulation can only be the result of decreased NADP⁺-dependent GDH activity. Furthermore, the rise in glutamine synthetase activity (Fig. 3) was accompanied by a decrease in the ammonium pool that had accumulated, suggesting that under these conditions ammonium assimilation proceeded through glutamine synthetase. As a control, we determined the effect of the addition of pyruvate instead of glyoxylate. The ammonium pool in the pyruvate-treated cells was very similar to that in the control culture (Fig. 2*b*). 2-Oxoglutarate levels were similar in cells

Fig. 1

Fig. 1. Effect of glyoxylate (●) and pyruvate (○) on citrate synthase. Activity was assayed *in vitro*. The activity corresponding to 100% was $4.7 \text{ U (mg protein)}^{-1}$. Results shown are representative of at least three determinations; variation was 5 to 10%.

Fig. 2

Fig. 2. Effect of glyoxylate and pyruvate on ammonium ion pools. (a) Cells were grown in MM for 2 h; the culture was then divided and 20 mM-glyoxylate (○) was added to one half; the rest of the culture was left untreated (●). Ammonium pools were then determined. (b) Cells were treated as in (a), but half the culture was treated with 20 mM-pyruvate (○) instead of glyoxylate; the other half was again untreated (●). Results shown are representative of at least three determinations; variation was 5 to 10%.

Table 1. Intracellular pools of 2-oxoacids in *S. cerevisiae* treated with glyoxylate or pyruvate

Cells were grown in MM for 2 h; the culture was then divided, and half was treated with 20 mM-glyoxylate; the other half was left untreated. In a separate experiment cells were treated with 20 mM-pyruvate instead of glyoxylate. After 20 min, samples were removed from both cultures, the cells were washed with MM and extracts were prepared and assayed as described in Methods. Results shown are means of three determinations; values in parentheses show variance of the mean.

2-Oxoacid	2-Oxoacid content [nmol (mg protein) ⁻¹]		
	MM	MM + glyoxylate	MM + pyruvate
Glyoxylate	11.61 (1.43)	326.60 (288.80)	9.00 (0.66)
Pyruvate	51.44 (10.52)	758.30 (972.20)	325.00 (416.67)
2-Oxoglutarate	9.20 (0.24)	39.43 (11.61)	9.73 (0.33)

in MM with and without pyruvate (Table 1). These data indicate that glyoxylate negatively modulates NADP^+ -dependent GDH, whereas pyruvate does not. Glyoxylate inhibited NADP^+ -dependent GDH activity *in vitro* while pyruvate did not (Fig. 4). This suggests that inhibition of NADP^+ -dependent GDH by glyoxylate provides a mechanism which compensates for the decrease in carbon flow due to the inhibition of citrate synthase by glyoxylate. This mechanism could play a role when poor carbon sources such as acetate are provided, since in these conditions, the activity of 2-oxoglutarate should be strictly regulated. In this regard, it has been reported that the enzymes which participate in glyoxylate synthesis are induced in acetate-

Fig. 3

Fig. 4

Fig. 3. Effect of glyoxylate on glutamine synthetase specific activity. Cells were grown in MM for 2 h; the culture was then divided and 20 mM-glyoxylate (○) was added to one half; the rest of the culture was left untreated (●). Glutamine synthetase activity was then determined. Specific activity is expressed as $\mu\text{mol } \gamma\text{-glutamyl hydroxamate produced min}^{-1} (\text{mg protein})^{-1}$ at 30 °C. Results shown are representative of at least three determinations; variation was 5 to 10%.

Fig. 4. Effect of glyoxylate (●) and pyruvate (○) on NADP⁺-dependent GDH. Activity was assayed *in vitro*. The activity corresponding to 100% was 5.9 U (mg protein)⁻¹. Results shown are representative of at least three determinations; variation was 5 to 10%.

Fig. 5. Effect of glyoxylate on amino acid pools. Cells were grown in MM for 2 h; the culture was then divided and 20 mM-glyoxylate was added to one half (a); the rest of the culture was left untreated (b). Amino acid pools (●, glutamate; ○, glutamine; ▲, alanine; △, glycine) were then determined. Results shown are representative of at least three determinations; variation was 5 to 10%.

grown cells (Gosling & Duggan, 1971). It has also been suggested (Bogonez *et al.*, 1985) that under certain physiological conditions, changes in the NADP⁺-dependent GDH activity provide control over the rate of 2-oxoglutarate utilization, resulting in the maintenance of a minimum pool of this 2-oxoacid. It is worth noting that strain S288C grew equally well with or without glyoxylate (data not shown), indicating that carbon and nitrogen flows were being coordinately regulated, thus allowing the maintenance of the same growth rate.

Effect of glyoxylate on nitrogen distribution

Amino acid analyses were done on glyoxylate-treated and untreated cells. The glutamate pool decreased very rapidly after glyoxylate addition, whereas in MM it remained almost constant (Fig. 5). At the same time, the alanine and glycine pools rose in the presence of glyoxylate. These results suggest that the glutamate amino nitrogen is being distributed through transamination. Furthermore, in glyoxylate-treated cells, the intracellular concentration of glutamine decreased and then increased slightly (Fig. 5*b*), suggesting that the increases in alanine and glycine pools could also be due to the functioning of glutamine transaminase, since this enzyme uses pyruvate and glyoxylate as substrates (Soberón & González, 1987). These results indicate that high intracellular concentrations of 2-oxoacids cause channelling of glutamate and glutamine towards transamination. This results in a decreased glutamine pool which allows glutamine synthetase activity to increase (Fig. 3) and increases ammonium assimilation through the glutamine synthetase–glutamate synthase pathway (Tempest *et al.*, 1970). As judged by amino acid accumulation and growth, total ammonium assimilation was not diminished in glyoxylate-treated cultures (Fig. 5). Our results indicate that the decrease in NADP⁺-dependent GDH activity is due to the inhibitory effect of glyoxylate and that this 2-oxoacid effects coordinate regulation of carbon and nitrogen metabolism.

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REFERENCES

- BERGMEYER, M. J. (1963). *Methods of Enzymatic Analysis*, pp. 253–259. New York & London: Academic Press.
- BOGONEZ, E., SATRUSTEGI, J. & MACHADO, A. (1985). Regulation by ammonium of glutamate dehydrogenase (NADP⁺) from *Saccharomyces cerevisiae*. *Journal of General Microbiology* **131**, 1425–1432.
- DUBOIS, E., GRENSON, M. & WIAME, J. M. (1974). The participation of the anabolic glutamate dehydrogenase in the nitrogen catabolite repression of arginase in *Saccharomyces cerevisiae*. *European Journal of Biochemistry* **48**, 603–616.
- DOHERTY, D. (1970). L-Glutamate dehydrogenases (yeast). *Methods in Enzymology* **17**, 850–856.
- FERGUSON, A. R. & SIMS, A. P. (1974). The regulation of glutamine metabolism in *Candida utilis*: the role of glutamine in the control of glutamine synthetase. *Journal of General Microbiology* **80**, 159–171.
- GONZÁLEZ, A., TENORIO, M., VACA, G. & MORA, J. (1983). *Neurospora crassa* mutant impaired in glutamine regulation. *Journal of Bacteriology* **155**, 1–7.
- GONZÁLEZ, A., RODRÍGUEZ, L., OLIVERA, H. & SOBERÓN, M. (1985). NADP⁺-dependent glutamate dehydrogenase activity is impaired in mutants of *Saccharomyces cerevisiae* that lack aconitase. *Journal of General Microbiology* **131**, 2565–2571.
- GOSLING, J. P. & DUGGAN, P. F. (1971). Activities of tricarboxylic acid cycle enzymes, glyoxylate cycle enzymes, and fructose diphosphatase in bakers' yeast during adaptation to acetate oxidation. *Journal of Bacteriology* **106**, 908–914.
- HOLZER, H. & SCHNEIDER, S. (1957). Anreicherung und Trennung einer DPN-spezifischen und einer TPN-spezifischen Glutaminsäure Dehydrogenase aus Hefe. *Biochemische Zeitschrift* **329**, 361–367.
- KANG, L., KEELER, M., DUNLOP, P. C. & ROON, R. J. (1982). Nitrogen catabolite repression in a glutamate auxotroph of *Saccharomyces cerevisiae*. *Journal of Bacteriology* **151**, 29–35.
- KORNBERG, H. L. (1966). Anaplerotic sequences and their role in metabolism. *Essays in Biochemistry* **2**, 1–31.
- PARVIN, R. (1969). Citrate synthase from yeast. *Methods in Enzymology* **13**, 16–19.
- SOBERÓN, M. & GONZÁLEZ, A. (1987). Glutamine degradation through the ω -amidase pathway in *Saccharomyces cerevisiae*. *Journal of General Microbiology* **133**, 9–14.
- TACHIKI, T., TOBORO, K., HORINO, I. & TOCHIBURO, T. (1981). Assimilation of ammonia by *Gluconobacter suboxydans* through glutamine synthetase/glutamate synthase pathway. *Agricultural and Biological Chemistry* **45**, 1715–1717.
- TEMPEST, D. W., MEERS, J. L. & BROWN, C. M. (1970). Synthesis of glutamate in *Aerobacter aerogenes* by a hitherto unknown route. *Biochemical Journal* **117**, 405–407.
- TRIBELS, F. & VOGEL, G. A. (1966). Degradation of allantoin by *Pseudomonas acidovarans*. *Biochimica et biophysica acta* **113**, 292–320.