The Glutathione-dependent Glyoxalase Pathway in the Yeast
*Saccharomyces cerevisiae*

*A VITAL DEFENSE LINE AGAINST METHYLGLYOXAL PRODUCED DURING GLYCEROL CATABOLISM*

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Glyoxalase I (EC 4.4.1.5), which catalyzes the reaction methylglyoxal + GSH → S-lactoylglutathione, is a ubiquitous enzyme for which no clear physiological function has been shown. In the yeast *Saccharomyces cerevisiae*, methylglyoxal may derive from the spontaneous decay of intracellular glyceraldehyde-3-P, which may accumulate during growth on glycerol as the carbon source. The half-life time for the triose phosphate was found to be 4.6 h under physiological conditions (pH 6.2, 0.05 M phosphate at 30 °C). Glyoxalase I is induced by growth on glycerol or by the addition of methylglyoxal to the growth medium. The enzyme is also subject to carbon catabolite repression. A mutant strain, fully defective in glyoxalase I and bearing only one nuclear mutation, was obtained. The strain, which is killed by exposure to glycerol, excretes methylglyoxal into the medium. Growth of the mutant on glucose as carbon source appears to be similar to that of the wild type strain. This investigation has clearly demonstrated a physiological role of glyoxalase I in an eucaryotic cell.

For many years, methylglyoxal (2-oxopropanal) was considered to be a possible intermediate in normal glycolysis (1). However, further studies led to the idea that the compound is not an intermediate of the major glycolytic pathway (2). The 2-oxoaldehyde may be formed nonenzymatically from trioses under physiological conditions (3), but this step is enzyme catalyzed in some prokaryotic cells (4-5). The hydration and rearrangement of methylglyoxal to D-(−)-lactic acid are catalyzed by the glutathione-dependent system glyoxalase (6) consisting of two distinct enzymes: glyoxalase I (recommended name: lactoyl-glutathione lyase, EC 4.4.1.5), CH3-C(=O)-OH + GSH → CH3CH(OH)-C(=O)-SG and glyoxalase II (recommended name, hydroxyacylglutathione hydrolase, EC 3.1.2.6), CH3CH(OH)-C(=O)-SG + H2O → CH3CH(OH)-C(=O)-OH + GSH. In this paper, we present chemical, enzymological, and genetic evidence showing that, in the yeast *Saccharomyces cerevisiae*, the glyoxalase pathway is a defense mechanism against methylglyoxal produced by the spontaneous decay of accumulating triose phosphates during glycerol catabolism.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

Nonenzymatic Formation of Methylglyoxal from Glycolytic Intermediates—A complete glyoxalase bypass to the glycolytic sequence includes an enzyme system for methylglyoxal formation, the glyoxalases, and enzymes for the transformation of D-(−)-lactate into pyruvate (4). As shown in Table I, glyoxalase I and D-(−)-lactate dehydrogenase (cytochrome) (29) activities were found in yeast growing on various growth media. In contrast, no methylglyoxal synthase and D-(−)-lactate dehydrogenase (NAD) activities were detected in the Σ 1278 b *S. cerevisiae* strain growing under aerobic conditions. Two methods (see "Experimental Procedures") were used for the estimation of methylglyoxal, after incubation at 37 °C of crude extracts in the presence of dihydroxyacetone-P2 or glyceraldehyde 3-P (30).

Both direct estimation and analysis after enzymatic coupling failed to demonstrate the enzyme-catalyzed formation of methylglyoxal. Several experimental conditions were subsequently tried but without success: desalting of the crude extracts, use of permeabilized cells, variations of the pH (between 6 and 9), nature of the buffer (Hepes, phosphate, Tris, imidazole, sodium citrate), and variation of the ionic strength (1/2 = 0.01 to 1/2 = 0.5 ~ KCl).

It was previously reported (31) that D-(−)-lactate dehydrogenase (NAD) is present under aerobic conditions in the Σ 1278 b strain. This result was further proved to be erroneous because of the presence of significant amounts of L-(+)-lactic acid in the commercial batch of D-(−)-lactic acid used during the preliminary investigation.

Reinvestigation with authentic D-(−)-lactic acid, prepared in the laboratory, did not show the presence of D-(−)-lactate dehydrogenase (NAD) in the yeast which was grown under aerobic conditions (Table II). In contrast, as previously reported by Labeyrie et al. (32), this enzyme was found after growing *S. cerevisiae* under strict anaerobiosis.

During our investigations, we always noted a spontaneous degradation of the triose phosphates, into methylglyoxal. A

1 Portions of this paper (including "Experimental Procedures," Figs. 1, 2, and 4-6, and Tables II, IV, and V) are presented in miniprint at the end of this article. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda MD 20814. Request Document No. 82M-25855, cite authors, and include a check or money order for $4.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

2 The abbreviations used are: -P, phosphoric residue; -PO2, diphosphoric residue; TLC, thin layer chromatography; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

A preliminary account of this work was presented at the 119th meeting of the Belgian Society of Biochemistry held in Louvain-en-Woluwe (Bruxelles) February 20, 1982 (31). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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intracellular amount of glyceraldehyde-3-P was present when glycerol was used as carbon source (Table III). The remainder of our investigation was devoted to a study of the role of glyoxalase I in the detoxification of methylglyoxal in the yeast.

Regulation of Glyoxalase I—Table I shows that the cellular levels of glyoxalase I depended on the carbon source supplied to S. cerevisiae. Glucose-grown cells contained low levels of glyoxalase I. Glucose-glycerol-, pyruvate-, and ethanol-grown cells contained substantial levels of the enzyme.

Glycerol-grown cells contained greater levels of the enzyme. In contrast, D(-)-lactate dehydrogenase (cytochrome) was not affected by the carbon source. This result suggested that glyoxalase I is subjected to some form of catabolite repression and induction controls. Indeed, in the presence of pyruvate and ethanol, two substrates which give only low concentrations of certain key metabolites, with respect to those prevailing when grown on glucose (35), we observed intermediate levels of glyoxalase I. Furthermore, glycerol and methylglyoxal exerted some induction effect on the enzyme.

Kinetics of repression-induction of glyoxalase I was studied in order to gain more information on the regulation of the system (Fig. 2, A-C). Transfer of cells growing on glycerol to a medium containing glucose as a sole carbon source (Fig. 2B) resulted in an instantaneous inhibition of the enzyme synthesis. The new differential rate of biosynthesis was nearly identical with the value determined on glucose-grown cells (Fig. 2A). No catabolite inactivation was observed in our experiments. Transfer from glucose to glycerol (Fig. 2C) resulted in a severe reduction in protein synthesis and a lag phase in the growth (Fig. 3A).

In the course of this transient phase, the glyoxalase I differential rate of synthesis was 8-fold that of a glycerol-grown balanced culture (Fig. 2A). A continuous diminution of the differential rate of synthesis was observed after growth resumption (not shown). This pattern is consistent with a system subjected to a glucose effect through a key regulatory metabolite, rapidly consumed during the glucose to glycerol shift and that cells resynthesize in the course of growth resumption. The precise nature of the metabolites implicated in the sequence of regulatory events remains to be specified but of probable relevance is the fact that methylglyoxal, a compound derived from glycerol dissimilation, exerted an effect identical with glycerol on the induction of glyoxalase I (Table I). What emerged at this stage of the investigation was the picture of a highly regulated system, potentially operative.
in the detoxification of methylglyoxal. Further support of this hypothesis was the isolation of a mutant strain defective in glyoxalase.

Isolation of a Mutant Defective in Glyoxalase I—Methylglyoxal affects numerous metabolic functions in microorganisms (36, 37). Therefore, if glycerol is a potential source of the 2-oxaldehyde, a mutant strain defective in glyoxalase I should not be able to grow when the triol is supplied as the sole source of carbon and energy. However, such mutants should be capable of growth when other substrates such as glucose, pyruvate, and ethanol are supplied as carbon and energy sources. Furthermore, such mutant strains would be expected to exhibit an unusual sensitivity to exogenous methylglyoxal.

A haploid wild type strain of *S. cerevisiae* (Σ 1278 b) was mutagenized as described under “Experimental Procedures” and the cells obtained were used for plating on 250 agar plates containing glucose as carbon source (about 200 colonies/plate).

Replicas were made on media containing, respectively, glycerol, ethanol, or pyruvate as carbon source. Several presumptive glycerol utilization mutants were obtained and, after purification, the sensitivity of the strains to methylglyoxal was tested. Among 42 strains not growing on glycerol but growing on pyruvate or ethanol, one strain (P 27) did not grow on agar plates containing glucose + 3 mM methylglyoxal.

The strains capable of growth when supplied with glucose, pyruvate, or ethanol, but not glycerol, as carbon source were selected for biochemical analysis.

Biochemical Characterization of the Mutant Strains: Absence of Glyoxalase I in the P 27 Strain—Sprague and Cronan (38) have clearly shown that glycerol kinase and sn-α-glycerophosphate dehydrogenase constitute the major pathway of glycerol catabolism in *S. cerevisiae*. Extracts of presumptive mutant strains and of parental strain Σ 1278 b were prepared. Each extract was assayed for glycerol kinase, α-glycerophosphate dehydrogenase, and glyoxalase I. GSH intracellular concentration was determined as previously described (21). As shown in Table IV, several mutant strains were only affected in glycerol kinase or in α-glycerophosphate dehydrogenase. In contrast, the P 27 mutant strain which exhibited a clear phenotype of hypersensitivity to methylglyoxal (see above) was found to be defective only in glyoxalase I. In fact, the enzyme was not detectable in crude extracts obtained from the yeast growing on glucose, pyruvate, or ethanol.

Effect of Glycerol on the Growth and Viability of the P 27 Mutant Strain: Excretion of Methylglyoxal—When transferred from the medium containing glucose as a sole carbon source to the glycerol-containing medium (Fig. 3A), strain Σ 1278 b exhibited a lag phase of about 5 h before growth resumption. In contrast, a decline in the viable count was observed for the P 27 strain, suggesting suicide catabolism of glycerol.

Methylglyoxal, the presumed toxic metabolite derived from glycerol, also strongly affects the viability of the mutant strain (Fig. 3B). In further experiments, strains Σ 1278 b and P 27 were challenged with glycerol to detect a possible release of methylglyoxal into the incubation fluid (see “Experimental Procedures”). Using the specific 2-oxaldehyde assay of Klotzsch and Bergmeyer (39), we found for the P 27 strain an average production of 2.5 μg 2-oxaldehyde/mg yeast, dry weight, in a 12-h incubation (three separate assays, results expressed in methylglyoxal equivalents). Control filtrates from glycerol-challenged cells of strain Σ 1278 b contained only very minor traces of the 2-oxaldehyde.

Further evidence that the excreted 2-oxaldehyde was methylglyoxal was obtained from the following experiments. TLC characterization of the 2,4-dinitrophenylhydrazones of the carbonyl compounds present in culture filtrates of the P 27 strain showed the presence of an osazone migrating at the same position as authentic methylglyoxal bis-2,4-dinitrophenylhydrazone (Fig. 4). In contrast, the corresponding spot was not detected in the derivatives prepared from the wild type strain Σ 1278 b culture filtrate. Confirmation that the derivative was in fact methylglyoxal bis-2,4-dinitrophenylhydrazone was gained by physicochemical analyses.

Preparative column chromatography of a benzene extract of the hydrazones over silica gel, with benzene as an eluent (see “Experimental Procedures”), yielded a fraction containing two compounds, one of which migrated on the plates as the synthetic derivative of methylglyoxal. Preparative TLC

### Table III

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Intracellular concentration*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dihydroxyacetone-P</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.28 ± 0.06</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.24 ± 0.03</td>
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</table>

* Data are mean ± range about the mean (three independent assays).
Despite numerous efforts of interpretation (40-42), the role and the fate of methylglyoxal in living cells yet remain largely unexplained. In yeast, Meyerhof (43) and Kiessling (44) suspected that the 2-oxoaldehyde may come from glycolytic intermediates.

In fact, we have shown that glyoxalase I is the principal element of detoxification of methylglyoxal produced nonenzymatically when substantial amounts of glyceraldehyde-3-P accumulate. In essence, the system shows some resemblance to catalase (45) and superoxide dismutase (46) which function in a similar relationship to oxidative metabolism. Glyoxalase I from yeast is not absolutely specific for methylglyoxal and may utilize numerous 2-oxoaldehydes (46, 47).

Nothing is now known about potential endogenous precursors of 2-oxoaldehydes in yeast except for methylglyoxal. It appears likely that a search for such reactions would be difficult but rewarding (49). Related to this question is the fact that the mutant strain, devoid of glyoxalase I, has a phenotype of slightly reduced growth rate on glucose as compared to the wild type strain. This suggests that the basal level of glyoxalase I, which is found in wild type cells growing on glucose, could function in the detoxification of 2-oxoaldehyde coming from sources other than triose phosphate. Although the present investigation establishes a precise role for glyoxalase I, no data warrant that the biotransformation of 2-oxoaldehydes in the yeast may partly be afforded by a different mechanism. For example, pyridine nucleotide-dependent dehydrogenases, catalyzing the reduction of 2-oxoaldehydes, are present in animal tissues (50). One of the most intriguing problems which we faced was the justification of detoxification when glycerol dissimilation-assimilation is operative in S. cerevisiae. Glycerol catabolism has only received attention in recent years (37, 52) seemingly because baker’s yeast is better adapted to carry out a glycerol fermentation on glucose (50, 51).

In Escherichia coli, which uses a network similar to S. cerevisiae for glycerol metabolism (53), glycerol kinase has the unusual feature of being subject to a feedback inhibition by fructose-1,6-P2 (54). This “pacemaker” effect (53), connected with efficient regulation at the level of gene expression, avoids the overproduction of methylglyoxal which is derived from triose phosphate through the action of methylglyoxal synthase (4). In E. coli, methylglyoxal synthase and glyoxalase I belong to a constitutive pathway (50), which is yet enigmatic and apparently inadequate for the channeling of large amounts of 2-oxoaldehydes. In S. cerevisiae, glycerol kinase is not feedback inhibited by fructose-1,6-P2 (see Ref. 55 for other yeast genera).

The absence of a pacemaker effect in yeast may result from the necessity for that microorganism to maintain a high potential of glycerol degradation. That growth rate and yield of S. cerevisiae on glycerol depend strongly on the external supply of amino acids in the culture medium4 seems to be related to the above hypothesis. It may reflect a limitation in the rate of biosynthesis of carbon precursors of some amino acids when glycerol is the sole carbon and energy source. Under those conditions, the metabolic flooding of glycerol may appear as a solution to minimize a downstream metabolic peculiarity, the price being paid by an increased amount of methylglyoxal produced from accumulating glyceraldehyde-3-P. Finally, apart from the function of glyoxalase I, a physiological role for GSH in the yeast has been indirectly displayed (56-58) and have been debated (21, 59-62).

**REFERENCES**


4 M. Penninckx, unpublished observations.
Yeast Glyoxalase I: Metabolic Function

Longmans, Green and Co., London


The glyoxylase I metabolic function of the yeast Saccharomyces cerevisiae is a vital defense line against methylglyoxal production during growthcarbohydration.

Figure 1: Metabolism of Methylglyoxal in Yeast. 

The glycolytic dependent glyoxylase I is expressed in the presence of methylglyoxal, as shown in the figure. The reaction mixture contains methylglyoxal, NADH, oxygen, and glyoxylase I. The product, glyceraldehyde, is then measured.

Figure 2: Effects of various buffers on the activity of glyoxylase I. 

The activity of glyoxylase I is measured in the presence of various pH buffers. The enzyme activity is highest in a pH buffer of 7.0.

Figure 3: Effects of various temperatures on the activity of glyoxylase I. 

The enzyme activity decreases with increasing temperature. The optimal temperature for the enzyme is around 37°C.

Figure 4: Time course of glyoxylase I activity. 

The activity of glyoxylase I decreases over time in the absence of methylglyoxal.

Figure 5: Western blot analysis of glyoxylase I expression. 

The expression of glyoxylase I is detected by Western blot analysis using an antibody specific to glyoxylase I.

Figure 6: Effect of methylglyoxal on glyoxylate reductase activity. 

The activity of glyoxylate reductase decreases in the presence of methylglyoxal.

Figure 7: Effect of glyoxylate reductase on glyoxylate metabolism. 

The addition of glyoxylate reductase to the reaction mixture decreases the accumulation of methylglyoxal.
Yeast Glyoxalase I Metabolic Function

Table 1: Specific activity of enzymes and glyoxalase activity in intracellular and in extract

<table>
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<tr>
<th>Strain</th>
<th>Glycerol</th>
<th>Glycerol-3-P</th>
<th>Glyoxalase I</th>
<th>D-gluconate</th>
<th>Glutathione intracellular concentration (mM)</th>
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<td>P 27</td>
<td>n.d.</td>
<td>0.43 ± 0.10</td>
<td>1.4</td>
<td>3.3 ± 0.5</td>
<td>4.6 ± 0.9</td>
</tr>
<tr>
<td>P 27</td>
<td>n.d.</td>
<td>0.51 ± 0.13</td>
<td>1.4</td>
<td>3.3 ± 0.5</td>
<td>3.3 ± 0.3</td>
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Glycerol solutions containing 10 mM dihydroxyacetone-P (DHAP) or glyceraldehyde-3-P (GAP) at pH 7.0 were incubated at 37°C. Periodically, 0.1 ml portions were assayed for glutathione. The initial rates of DHAP or GAP formation were obtained for the initial 10 min incubation of the reaction mixture. The average value for n = 4 is 0.010 ± 0.002 mM GAP and 0.005 ± 0.001 mM DHAP.

References containing 1 mg of glutathione per ml and 1 mg of dihydroxyacetone-P (DHAP) or glyceraldehyde-3-P (GAP) at pH 7.0 were incubated at 37°C. Periodically, 0.1 ml portions were assayed for glutathione. The initial rates of DHAP or GAP formation were obtained for the initial 10 min incubation of the reaction mixture. The average value for n = 4 is 0.010 ± 0.002 mM GAP and 0.005 ± 0.001 mM DHAP.

Fig. 5: Evolution of methylglyoxal intracellular concentration after shifting to glucose

Table 2: Specific activity of enzymes and glyoxalase activity in intracellular and in extract

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