

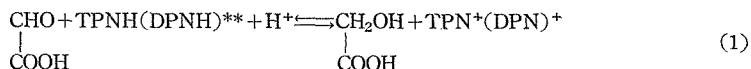
Glyoxylate-Glycollate System in Yeast

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Many investigators have established the importance of the tricarboxylic acid cycle for oxidative processes in yeast. All of the enzymes of the cycle have already been demonstrated in *Saccharomyces cerevisiae*. However, it has recently been found that isocitritase, the enzyme which degrades isocitrate to glyoxylate and succinate, is contained in baker's yeast^{1,2)}. The authors have more recently demonstrated glyoxylic reductase [Reaction I] in several bacteria including *Escherichia coli*, *Aerobacter aerogenes* and *Pseudomonas fluorescens*^{3,4)}.



It has moreover been pointed out by the authors that the oxidation of glycollate to glyoxylate is catalyzed by an oxidase, i.e., a flavoprotein (with FMN as prosthetic group and independent upon TPN or DPN) isolated from microorganisms such as *coli-aerogenes*. Some investigators have obtained flavoproteins from higher plant tissues^{5,6)} and mammalian liver⁷⁾ which catalyze the oxidation of glycollate. The present paper deals with the glyoxylate-glycollate system in yeast.

Fresh baker's yeast was incubated for one hour at 37°C in the presence of ethyl acetate. After incubation, cells were collected by a centrifuge, ground for one hour with powdered glass and thereafter suspended in 0.25 M phosphate buffer, the pH being adjusted to 8.6 with 5% ammonia solution. After being kept for 1-3 weeks at 5°C, the supernatant fluids were dialyzed against distilled water at 5°C for 40 hours.

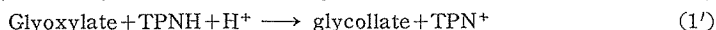
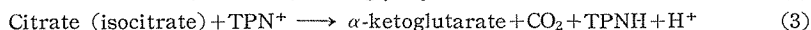
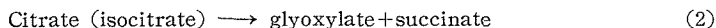
The anaerobic degradation of citrate was at first investigated with the dialyzed cell-free extract. Both glyoxylate and succinate were obtained as the degradation products of citrate when incubation was carried out in the absence of added TPN. It was, however, observed that when TPN was present, α -ketoglutarate and glycollate were formed in addition to succinate and carbon dioxide. This observation indicates that the degradation of citrate occurs by the combined action of aconitase, isocitric dehydrogenase, isocitritase and glyoxylic reductase

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** The following abbreviations have been used: DPN and TPN=di- and triphosphopyridine nucleotides; DPNH and TPNH=reduced di- and triphosphopyridine nucleotides; FMN=riboflavin-5'-phosphate; FAD=riboflavin adenine dinucleotide.

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as follows :



The formation of glycollate was extremely decreased in the absence of added TPN.

On the other hand, it has been found that the cell-free extracts mentioned above have the power of oxidizing glycollate to glyoxylate. Yeast glycollic oxidase was purified from the cell-free extract mentioned above according to the method similar to that applied for the preparation of yeast lactic dehydrogenase⁸⁻¹⁰), except that alumina C_γ gel was used in place of calcium phosphate

Table 1. Oxidation of glycollate and lactate by baker's yeast enzyme.

Substrates	Additions		Ketonic acids formed (μ moles)
	FMN (1 μmole)	Pyocyanine§ (2 μmoles)	
With purified enzyme preparation (63 mg as protein).			Glyoxylic acid
Glycollate	-	-	2.82
	+	-	4.58
	-	+	20.90
	+	+	25.20**
			Pyruvic acid
DL-Lactate	+	+	71.25*
With acid-treated enzyme (140 mg as protein), which was prepared from ammonium sulfate 0.4-0.8 saturation fraction of cell-free ext. by the method of Warburg et al.			Glyoxylic acid
Glycollate	-	-	0.50
	+	-	1.70
	-	+	0.60
	+	+	3.82

*Pyruvate = 2.83

**Glyoxylate

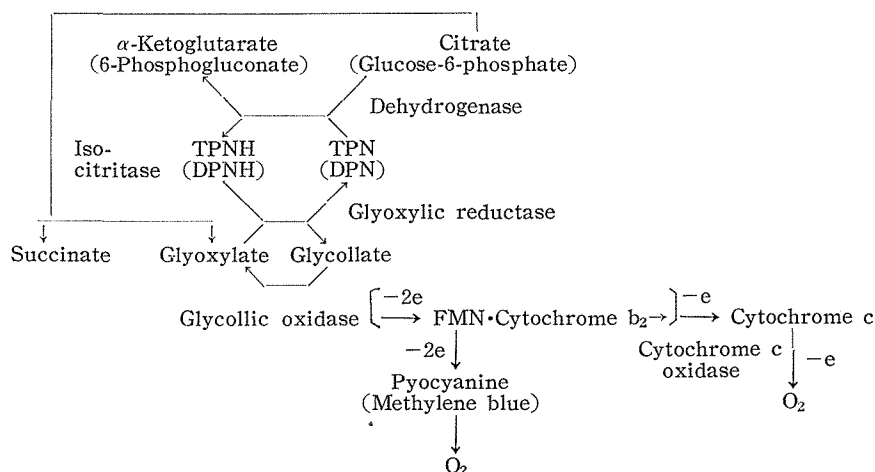
Reaction mixtures contained 750 μmoles phosphate buffer, pH 7.4, 500 μmoles semicarbazide, 500 μmoles substrates (Na-salts), the enzyme preparations as indicated and the requisite amounts of FMN and pyocyanine; total volume, 15 ml; 3 hours' incubation at 30°C on a shaker; gas phase, air. Analytical procedures were carried out by the methods reported previously¹¹).

§ Pyocyanine was generously supplied by Dr. M. KURACHI of this laboratory to whom the authors' thanks are due.

gel. Table 1 shows the oxidation of glycollate to glyoxylate by baker's yeast enzyme. The enzyme preparations were ascertained to catalyze the oxidation of glycollate to glyoxylate in the absence of TPN or DPN. The addition of pyocyanine or methylene blue brought about the activating effect on the oxidation of glycollate by the enzyme of baker's or brewer's yeast when molecular oxygen was the terminal electron acceptor. However, this effect of dyes was never observed in the case of the bacteria of *coli-aerogenes*. Glycollic oxidase of yeast was demonstrated to be a flavoprotein; the inactive apoenzyme (prepared according to the method of Warburg and Christian¹²) was reactivated by the ad-

dition of FMN or FAD. Interesting properties of yeast glycollic oxidase were moreover clarified by the spectrophotometric observations. When glycollate was added to baker's yeast enzyme solution containing phosphate buffer, pH 7.4, the bands of cytochrome b_2 in the reduced state instantly appeared: α -band, 557 $m\mu$; β -band, 527 $m\mu$. Cytochrome c was reduced by glycollate in the presence of the yeast glycollic oxidase. Yeast enzyme preparation used here was also able to oxidize malate in addition to lactate and glycollate. The rate of oxidation of lactate was faster than that of glycollate or malate when either pyocyanine or methylene blue was used as electron acceptor. Yamashita *et al.*⁹⁾ have recently reported that cytochrome b_2 involved in yeast lactic dehydrogenase preparation is reduced anaerobically by lactate, malate and TPNH. Inhibiting effects upon yeast glycollic oxidase were observed with quinine, quinacrine and *p*-chloromercuribenzoate. The authors have investigated the influence of pH upon the oxidations of both lactate and glycollate by the yeast enzyme, and it has been observed that the former reaction has the optimum pH at about 6.8 and the latter at about 7.7 in the presence of methylene blue. These facts may indicate that yeast enzyme used here contain glycollic (malic) oxidase in addition to lactic dehydrogenase.

Consequently, the following scheme would be possible :



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