Glyoxylate Reductase and Glycollate Oxidizing Enzyme System in Moulds and Bacteria

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Recent studies[1-10] on microbial metabolism of glyoxylate have led to the nature of the physiological function of glyoxylate ⇄ glycollate system, a combined reaction of glyoxylate reductase and glycollate oxidizing enzyme system. Bacterial glyoxylate reductase[1,2,9,10] reveals very much higher specificity towards TPN** than towards DPN. The oxidation of glycollate to glyoxylate is catalyzed by FMN-linked glycollate oxidase (dehydrogenase) of microorganisms[1,3,5-8]. Present communication describes this oxidation-reduction system in moulds and bacteria.

Glyoxylate reductase of moulds: Fig. 1 shows the enzymic oxidation of TPNH and DPNH by a dialyzed cell-free extract of glucose-grown *Penicillium purpurogenum*. Mould glyoxylate reductase was highly specific towards TPNH; there was little activity with DPNH. It is of interest to note that the cell-free extracts of *Penicillium* species have little activity of pyridine nucleotides enzyme such as DPN-linked glyoxylate reductase, TPNH oxidase and DPNH oxidase. Results of experiments carried out with the dialyzed cell-free extract of *Penicillium purpurogenum* showed that the Michaelis constant of TPN-linked glyoxylate reductase was approximately 1.5×10^{-4} *M* for glyoxylate. When the dialyzed cell-free extracts (40 mg protein) of glucose-grown moulds such as *Neurospora* and *Penicillium* species were anaerobically incubated at 37°C for 7 hours with a reaction mixture (10 ml) containing 500 μ moles of phosphate buffer, pH 7.4, 500 μ moles Na-citrate, 50 μ moles of $MgSO_4$ and 500γ of TPN, 7-28 μ moles of α-ketoglutarate and 7-32 μ moles of glycollate were obtained as the fermentation products. However, a remarkable accumulation of glyoxylate took place against little or no formation of both α-ketoglutarate and glycollate, when the anaerobic degradation of citrate proceeded in the presence of semicarbazide. The authors have furthermore demonstrated[9,10] that the reduction of glyoxylate by microbial glyoxylate reductase is coupled to the dehydrogenation of hexose monophosphate via the pentose phosphate pathway. Thus, it has strongly indicated that glyoxylate reductase functions as a TPNH-consumer in carbon-metabolisms of moulds. TPN-linked glyoxylate reductase has been found in *Penicillium chrysogenum*, *Penicillium purpurogenum*, *Penicillium notatum* and *Neurospora sitophila*.

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** The following abbreviation are used: DPN (TPN)=di (tri) phosphopyridine nucleotides; DPNH (TPNH)=reduced di (tri) phosphopyridine nucleotides; FMN=riboflavin-5′-phosphate.

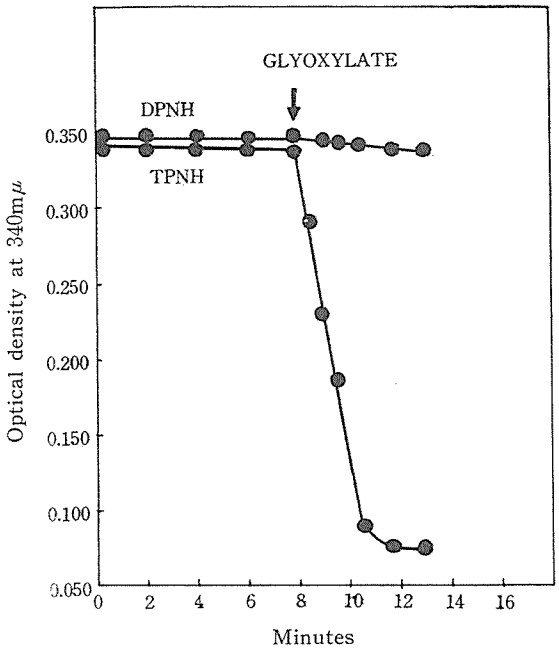

Fig. 1. Oxidation of TPNH and DPNH by a dialyzed cell-free extract of *Penicillium purpurogenum*, in presence or absence of glyoxylate.

Reaction mixture contained: 200 μmoles of phosphate buffer, pH 6.0, approximately 0.17 μmole of either TPNH or DPNH, and 0.1 ml (300γ protein) of a dialyzed cell-free extract of *Penicillium purpurogenum*: after 8 minutes' incubation, 10 μmoles of Na-glyoxylate were added; final volume, 3.2 ml. Oxidation of reduced pyridine nucleotides was measured at room temperature at 340 mμ using a Hitachi Spectrophotometer, light path of 1.0 cm. Preparation of the cell-extract: The mould was grown on a shaker at 30°C for 70 hours in a medium (pH 5.7) containing 3% glucose, 0.1% $(NH_4)_2SO_4$, 0.1% each KH_2PO_4 and Na_2HPO_4, 0.03% $MgSO_4 \cdot 7H_2O$, 0.3% yeast extract, 0.5% peptone, 500γ% each $FeSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$ and NaCl, and 1.5% $CaCO_3$. After cultivation, the cells were harvested by a centrifuge and washed with distilled water. The washed cells were ground with powdered glass for 30 minutes, extracted with 0.1 *M* phosphate buffer, pH 7.3. The insoluble matter was removed by centrifuge and the resulting supernatant fluid was dialyzed against distilled water at 5°C for 20 hours.

Glycollate oxidizing enzyme system in bacteria and moulds: The presence of either methylene blue or pyocyanine brought about the accelerating effect on the oxidation of glycollate to glyoxylate by the cell-free extracts of various microorganisms including yeast,[6] moulds[8] and a bacterial strain; *Achromobacter* species. However, this effect of dyes was scarcely observed with cell-free extracts of other bacteria including *coli-aerogenes*, *Corynebacterium sepedonicum* and *Pseudomonas fluorescens*. It should here be noted that the oxidation of lactate to pyruvate in *coli-aerogenes* is catalyzed by the two kinds of enzyme systems, one containing methylene blue-independent lactate oxidase and another containing the dye-dependent lactate dehydrogenase. Typical results of experiments are shown in Table 1. It will be seen that relative oxidizing activities of bacterial preparations towards both glycollate and lactate are changeable according to the kinds of organisms.

The localization of enzymes in bacterial cells was investigated with both *Es-*

Table 1. Oxidation of glycollate and DL-lactate by dialyzed cell-free extracts of several bacterial strains.

Bacteria and growth media (shaking culture at 30°C for 1-2 days)	Substrates added		Addition of methylene blue	Ketonic acids formed (μmoles)	
	Glycollate	Lactate		Glyoxylate	Pyruvate
Corynebacterium sepedonicum grown on 2% glucose-1% peptone.	+	−	+	0.6	
	+	−	−	0.5	
	−	+	+		2.3
	−	+	−		2.1
Escherichia coli grown on 1% bouillon-3% Na-acetate	+	−	+	3.4	
	+	−	−	2.8	
	−	+	+		365.0
	−	+	−		218.0
Achromobacter species grown on 2% peptone-0.5% Na-glycollate	+	−	+	36.8	
	+	−	−	13.5	
	−	+	+		50.2
	−	+	−		23.1
Pseudomonas fluorescens grown on 2% peptone-3% Na-acetate.	+	−	+	6.0	
	+	−	−	5.9	
	+	−	+ (pyocyanine)	6.9	

Incubations were carried out at 28°C for 60 minutes on a shaker in the presence or absence of dyes ($1.4\times10^{-4}M$) with the mixtures (15 ml) containing 750 μmoles of phosphate buffer, pH 7.4, 500 μmoles of either glycollate or DL-lactate, 500 μmoles of semicarbazide and dialyzed cell-free extracts (100 mg protein) of stated bacteria. Preparation of cell extracts and analytical procedure were performed by the methods mentioned in the previous papers.

cherichia coli and *Achromobacter* species. After sonic treatment in 0.25 *M* sucrose-0.01 *M* phosphate buffer of pH 7.4, the bacterial cells were centrifuged at approximately 7000×g for 20 minutes and the resulting precipitates discarded. The supernatant fluid (cell-free extracts) was again centrifuged at approximately 100.000×g in a Spinco refrigerated centrifuge for 60 minutes. By this procedure, the cell-free extracts were separated into two fractions: (1) particles which were sedimented at 100.000×g and (2) the soluble fraction (supernatant liquid after removal of particles). It was found that the particle fraction of *E. coli* contained most of the glycollate oxidase activity of the original extract, whereas glycollate dehydrogenase of *Achromobacter* species was mainly contained in the soluble fraction. The soluble fraction of *coli-aerogenes* contained the following enzymes: aconitase, isocitrate dehydrogenase, TPN-linked glutamate dehydrogenase, isocitritase and TPN-linked glyoxylate reductase.

The authors' attention has also been directed to an oxidation-reduction reaction between glycollate and cytochrome c. Spectrophotometric measurements were carried out and it was found that animal cytochrome c was instantly reduced by a small amount of glycollate or of lactate in the presence of the cell-free extract of glucose-grown *Neurospora sitophila*. On the other hand, animal cytochrome c was scarcely

reduced by a bacterial system containing glycollate and the enzyme preparation (ammonium sulfate 0.3 saturation fraction of cell-extract) of glycollate-grown *Achromobacter* species. However, the bacterial cytochrome in preparations of *Achromobacter* was reduced by the addition of either glycollate or lactate, revealing α-band of 550mμ and β-band of 520mμ (Fig. 2)

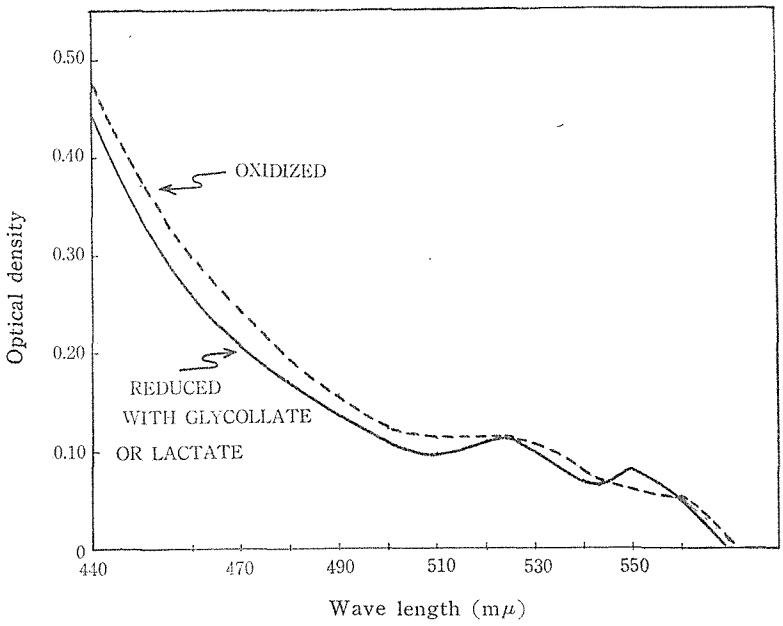

Fig. 2. The spectra of the oxidized and reduced states of *Achromobacter* cytochrome.

Reaction mixture contained: 50 μmoles of phosphate buffer, pH 7.2, 3 ml of ammonium sulfate 0.3 saturation fraction obtained from the cell-free extract of *Achromobacter* species grown aerobically at 30°C for 20 hours on a medium containing 0.5% Na-glycollate, 2% peptone, 1% yeast extract, 0.5% NaCl and 0.1% KH_2PO_4(pH 7.2). The oxidized cytochrome was reduced by the addition of approximately 50 μmoles of either Na-glycollate or Na-DL-lactate; final volume, 3.7 ml; room temperature. The spectra were measured, using spectrophotometer based on the opal glasses method.

Finally, data obtained in the previous and present papers, imply that TPN and cytochrome components serve as the electron carriers in microbial glyoxylate $\rightleftarrows$ glycollate system.

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