ACTIVATION OF 6-PHOSPHOGLUCONATE DEHYDRASE BY PYOVERDINE*

Frank H. Hulcher

Department of Biochemistry

The Bowman Gray School of Medicine

of Wake Forest University

Winston-Salem, North Carolina

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Pseudomonas mildenbergii, when grown without iron, produces a green fluorescent pigment, pyoverdine (Love & Hulcher, 1964), which has a molecular weight of 2,380 and consists of a peptide of threonine, serine, glutamic acid and lysine (4:2:1:1, molar ratio) with an N-methyl phenylacetyl hydroxamic acid group bound to the peptide (Hulcher, 1968). The present work defines a possible physiological role for pyoverdine as an activator of 6-phosphogluconate dehydrase.

Methods and Materials. Cells were grown on a chemically defined medium (Love and Hulcher, 1964) with or without ferric chloride, $1x10^{-5}$M, for 16 to 18 hours, centrifuged and washed three times in 0.05 M potassium phosphate buffer, pH 7.0. A cell suspension was prepared to yield measurable oxidative rates and aliquots were taken for protein determination. The cells were starved for three hours in buffer at 32°C prior to measurement of respiration. A Bronwill Warburg Apparatus Model NL-85 was used to measure oxygen utilization. Each experiment was performed 3 times in duplicate.

The cell extracts were prepared after suspending 2.0 gms of wet cells in 45 ml of 0.1 M potassium phosphate buffer, pH 7.0. Cells were sonicated 5 times for 1 minute at 3 minute intervals at 1-3°C, using a Bronson Sonifier Model MLS-75. The sonicate was centrifuged at 5,000 x g for 15 minutes with

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a Servall RC-2 refrigerated centrifuge. This preparation was centrifuged at 30,000 x g for 30 minutes followed by a centrifugation of the soluble fraction at 100,000 x g for 1 hour using a Spinco Model L ultracentrifuge.

Protein was determined by the biuret method (Layne, 1957), catalase was measured by the procedure described by Lück (1963) using a Zeiss model PMQ-II spectrophotometer. The 6-phosphogluconate dehydrase was assayed by the rate of pyruvate formed as measured by the 2,4-dinitrophenyl hydrazone (Kovachevich and Wood, 1955). It was also assayed as the KDP-gluconate semicarbazone (McGee and Dandonoff, 1958). This assay was used in the presence of excess 2-keto-3-deoxy-6-phosphogluconate aldolase.

Results. To illustrate the effect of iron-deficiency, the activity of catalase for cells grown with iron was 45.5 units/mg protein; for cells grown without iron the activity was 1.51 units/mg protein.

The effect of pyoverdine on the oxidation of glucose and phenylalanine by cell suspensions is seen in Figure 1. It stimulated the oxidation of both glucose by 34% and phenylalanine by 48% in 30 minutes, without a lag period. The effect was continuous throughout the assay period.

The effect of different concentrations of pyoverdine on the oxidation of glucose is shown was determined and gave a smooth parabolic curve reaching constant maximum activity with 1.4×10^{-4}M in the reaction mixture. Potassium cyanide (2×10^{-2}M) inhibited the oxidation of glucose by cells grown without iron, by 74.2% and completely eliminated the action of pyoverdine. Gluconate was oxidized rapidly by whole cells and its oxidation was stimulated by 24% with pyoverdine.

To determine if the stimulating effect occurred at the level of metabolism of the citric acid cycle, the effect of pyoverdine on the oxidation of acetate, citrate and succinate was studied. Acetate and succinate were oxidized but pyoverdine depressed the oxidation. Citrate was not oxidized.

Fractions prepared from cells grown without iron were examined for the

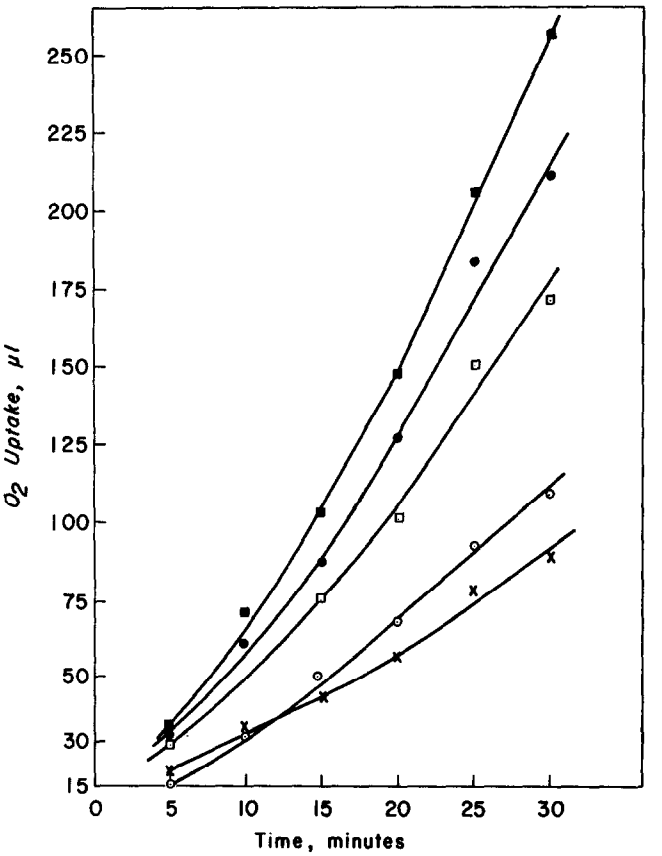

Figure 1. The effect of pyoverdine (PV) on the oxidation of glucose and phenylalanine by washed cells of Ps. mildenbergii.

Designations: X - endogenous rate; open circle - phenylalanine; solid circle - phenylalanine + PV; open square - glucose; solid square - glucose + PV.

Respirometer flasks contained 1.0 ml of 0.1 M potassium phosphate buffer, pH 7.0, 0.5 ml of 0.1 M phenylalanine or 0.1 M glucose (in one side arm), 0.5 ml of PV (2 mg/ml), and 1.0 ml of washed cells (14 mg protein/ml) to a volume of 3.0 ml. The oxidation was carried out at 32°C. The center wells contained 0.2 ml of 5% KOH fitted with Whatman papers.

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effect of pyoverdine in attempts to locate the site of action. Crude sonicated preparations showed results similar to those of whole cells, but with lower rates. However, some indication of the effect of combining the soluble fraction to the particulate fraction was seen where the oxidation of glucose was stimulated by 52% by pyoverdine.

The enzyme activities of sonicated cells grown with and without iron were

compared for glucose metabolizing enzymes with and without the addition of pyoverdine. No effect was observed except on 6-phosphogluconate dehydrase. The results in Table I show the stimulation of activity of the iron-grown and iron-deficient enzyme. Kovachwich and Wood (1955) showed the requirement of glutathione and ferrous iron. The activity from iron-deficient cells was stimulated 78% over the activity with glutathione and ferrous iron. Likewise the activity from iron-grown cells was increased 60%. No stimulation of 2-KDPG aldolase could be obtained.

TABLE I

Effect of glutathione, ferrous iron and pyoverdine (PV) on the activity of 6-phosphogluconate dehydrase.

From iron-grown cells					
specific activity					
no activator	GSH - -	- Fe^{++} -	GSH Fe^{++} -	GSH - PV	GSH Fe^{++} PV
0.09	0.078	0.152	0.150	0.240	0.193

From iron-deficient cells							
specific activity							
no activator	GSH - -	- Fe^{++} -	- - PV	- Fe^{++} PV	GSH Fe^{++} -	GSH - PV	GSH Fe^{++} PV
0.07	0.09	0.150	0.204	0.160	.190	.340	.243

The reaction mixture contained 0.2M tris (hydroxymethyl) amino methane -HCl buffer at pH 7.6, 3 μmoles sodium glutathione, 6 μmoles of $FeSO_4$, 7 μmoles Na 6-PG, 2.0 mg of pyoverdine as specified and 0.1 ml of enzyme in a volume of 1.0 ml. Control blanks were included with pyoverdine without substrate.

Discussion. The sparcity of available iron for iron-requiring enzymes was illustrated by the near absence of catalase activity. Pyoverdine stimulated the oxidation of glucose and phenylalanine by cells of Ps. mildenbergii grown without iron and directly stimulated some catabolic reaction of glucose and phenylalanine metabolism. The parabolic effect of concentration of

pyoverdine on the oxidation of glucose suggested the involvement of an enzyme. Cyanide reversed the stimulation of glucose oxidation, indicating that some reaction leading to a cyanide sensitive reaction was involved.

The inability of pyoverdine to stimulate the oxidation of acetate and succinate indicated that the reaction involved occurs prior to the citric acid cycle and acetate metabolism.

The stimulatory effect of pyoverdine on glucose oxidation appeared to be dependent on enzymes in the soluble fraction of the cells at the level of enzymes of glucose metabolism showed that pyoverdine activates 6-phosphogluconate dehydrase. Pyoverdine more than substitutes for iron in the reaction, yet, it has not been possible to detect iron in pyoverdine.

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