A Crystalline Flavin Pyruvate Oxidase*

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Previous communications from this laboratory have reported the recognition and partial purification of a flavoprotein pyruvate oxidase which catalyzes the oxidative decarboxylation of pyruvate to acetaldehyde and CO₂ (1, 2). This enzyme is obtained from an acetate auxotroph of Escherichia coli and is formed in response to the accumulation of pyruvate in the growth medium (3). We now wish to report the isolation of this flavoprotein in crystalline form and to describe some of the properties of the purified enzyme.

The purified flavoprotein has the typical behavior of a globular protein. It is insoluble in aqueous solutions of low ionic strength but readily dissolves in solutions of ionic strength of 0.2 and higher. Purification and crystallization of the flavoprotein is accomplished by the following steps: (a) rupture of the Escherichia coli cells by grinding with glass beads in 0.02 M potassium phosphate buffer, pH 7, in a colloid mill (4); (b) preparation of a 0.25 to 0.75 saturated ammonium sulfate fraction; (c) protamine sulfate precipitation and elution of the precipitate fraction or in the presence of a soluble cytochrome b₅ preparation derived from the particulate fraction. When fully saturated with soluble cytochrome b₅, the flavoprotein has a turnover number of 10,000 (mole of pyruvate oxidized per minute per mole of enzyme). The incubation of reduced flavoprotein with thiamine pyrophosphate as coenzyme, the purified flavoprotein will also react sluggishly with electron acceptors such as 2,6-dichlorophenolindophenol and ferricyanide (turnover number = 1800, ferricyanide). Maximal activity (turnover number = 53,000, ferricyanide) is achieved in these reactions by a variety of methods. Partial hydrolysis of the flavoprotein by trypsin (11) or chymotrypsin (12) produces an altered protein which is activated by these various agents is under investigation.

When supplemented with pyruvate as substrate and thiamine pyrophosphate as coenzyme, the purified flavoprotein will also react sluggishly with electron acceptors such as 2,6-dichlorophenolindophenol and ferricyanide (turnover number = 1800, ferricyanide). Maximal activity (turnover number = 53,000, ferricyanide) can be achieved in these reactions by a variety of methods. Partial hydrolysis of the flavoprotein by trypsin (11) or chymotrypsin (12) produces an altered protein which is activated by these various agents is under investigation.

The sedimentation constant (s₂₀,₀) of the enzyme in 0.2 M potassium phosphate buffer, pH 5.7, is 11.5 S. Flavin analyses (6–8) performed on the enzyme indicate that FAD is the sole flavin component and is present in a ratio of 4 moles of FAD per mole of enzyme. The crystalline enzyme does not contain thiamine pyrophosphate but has an absolute thiamine pyrophosphate requirement for reduction of the enzyme-bound FAD.

The reduced flavoprotein-substrate complex is nonautoxidizable. When supplemented with pyruvate as substrate and thiamine pyrophosphate as coenzyme, the purified flavoprotein will also react sluggishly with electron acceptors such as 2,6-dichlorophenolindophenol and ferricyanide (turnover number = 1800, ferricyanide). Maximal activity (turnover number = 53,000, ferricyanide) can be achieved in these reactions by a variety of methods. Partial hydrolysis of the flavoprotein by trypsin (11) or chymotrypsin (12) produces an altered protein which is activated by these various agents is under investigation.

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The crystalline flavoprotein has a molecular weight of approximately 265,000 as determined by the Archibald method (5). The sedimentation constant (s₂₀,₀) of the enzyme in 0.2 M potassium phosphate buffer, pH 5.7, is 11.5 S. Flavin analyses (6–8) performed on the enzyme indicate that FAD is the sole flavin component and is present in a ratio of 4 moles of FAD per mole of enzyme.
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