Solubilization and enzymic activity of bacterial cytochrome *b*

We wish to report that trypsin hydrolysis of the particulate fraction of a pyruvate oxidase system releases heat-labile, non-dialyzable, enzymically-active material into a soluble form. Since the physical and enzymic properties of cytochrome-containing particulate fractions prepared from various species of aerobic bacteria share common characteristics, the particle solubilization described in this paper may be of general use in the study of electron transport in these organisms.

In addition to the well documented pathway of pyruvate oxidation mediated by DPN, CoA and lipoic acid, *Escherichia coli*, and to a greater extent *Proteus vulgaris*, contain a pyruvate oxidase which oxidatively decarboxylates pyruvate to acetate and CO_2 with the uptake of molecular O_2 according to equation (1)[1-4].

$$CH_3\text{-}CO\text{-}COO^- + \tfrac{1}{2}\,O_2 \longrightarrow CH_3\text{-}COO^- + CO_2 \qquad (1)$$

Ferricyanide or 2,6-dichlorophenolindophenol will substitute for O_2 as the electron acceptor for this enzyme system according to equation (2).

$$CH_3\text{-}CO\text{-}COO^- + 2Fe^{+++}(CN)_6 + H_2O \longrightarrow CH_3\text{-}COO^- + CO_2 + 2Fe^{++}(CN)_6 + 2H^+ \qquad (2)$$

The enzyme system catalyzing both these reactions has been separated into a soluble enzyme fraction and a cytochrome-containing particulate fraction which sediments as a brownish transparent gel at high gravitational forces[3-5].

Studies in this laboratory have shown that in an acetate-requiring mutant of *E. coli* the genetic block lies in the DPN, CoA, lipoic acid pathway[6], and in this instance the mutant produces high levels of the particulate system in response to the accumulation of pyruvate in the growth medium. The soluble fraction of the mutant pyruvate oxidase system has been obtained in a high state of purity (> 90 % pure as judged from the schlieren pattern in the ultracentrifuge). The purified soluble enzyme is a non-autooxidizable flavoprotein and contains 1 mole FAD per 200,000 g protein[7]. However, the particulate fraction is not amenable to classical protein-purification procedures, and various attempts to prepare soluble protein or lipoprotein fractions from the particulate fraction using lipid solvents have led to complete or almost complete loss of enzymic activity. On the other hand, following brief exposure to trypsin approx. 50 % of the total dry weight of the particulate fraction is converted to a soluble form which is no longer sedimentable at 144,000 $\times$ *g* for 1 h. When coupled with the purified soluble flavoprotein, the soluble material prepared in this manner is enzymically active in catalyzing both reactions (1) and (2) (Fig. 1). The results shown in Fig. 1 form the basis of the assay method to measure the activity of the particulate fraction. In the presence of an excess of the soluble flavoprotein the rate of O_2 or ferricyanide reduction is proportional to the concentration of the particulate fraction or of the soluble material derived from it.

The solubilization of the particulate fraction by trypsin treatment, as indicated by a decrease in absorbancy, parallels the release of enzymic activity which is no longer sedimentable at 144,000 $\times$ *g* for 1 h. The solubilized material obtained from the trypsin-hydrolyzed particulate fraction is denatured when subjected to $(NH_4)_2SO_4$ fractionation but has been further purified by acid precipitation. Reduction of the solubilized material with $Na_2S_2O_4$ or enzymically with the soluble flavoprotein and pyruvate clearly indicate the presence of a cytochrome b_1 as evidenced by the

Abbreviations: DPN, diphosphopyridine nucleotide; CoA, coenzyme A.

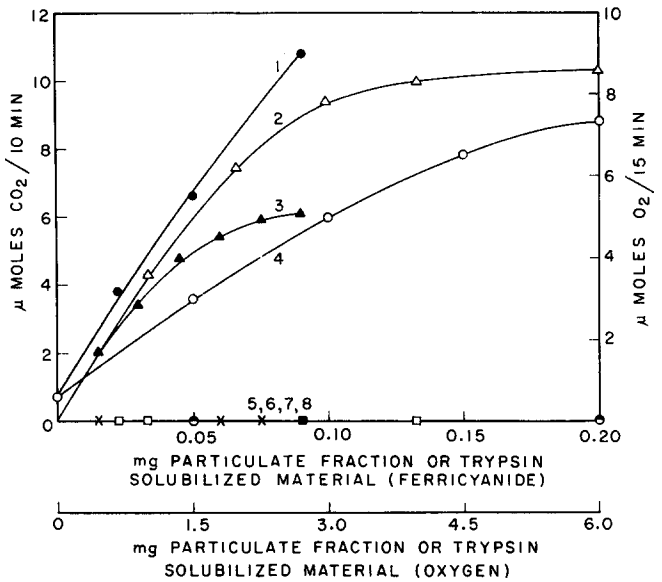

Fig. 1. The requirement for the soluble flavoprotein and the particulate fraction or soluble material derived from the particulate fraction for activity with either O_2 or ferricyanide as the electron acceptor. With ferricyanide as electron acceptor the complete system contained 100 μmoles potassium phosphate buffer, pH 6.0, 10 μmoles $MgCl_2$, 0.1 μmole thiamine pyrophosphate, 50 μmoles potassium pyruvate, 25 μmoles $K_3Fe(CN)_6$ and 0.48 mg soluble flavoprotein with increasing amounts of the particulate fraction (○—○, curve 4), or the trypsin-solubilized particulate fraction (●—●, curve 1). With O_2 as the electron acceptor the complete system contained the same components as the ferricyanide assay with ferricyanide omitted and 750 μmoles KOH in the center well to absorb CO_2. In the O_2 assay 1.45 mg of the soluble flavoprotein was added together with increasing amounts of the particulate fraction (△—△, curve 2), or the trypsin-solubilized particulate fraction (▲—▲, curve 3). Curves 5 and 6 show that the particulate fraction in the absence of the soluble flavoprotein has no activity with O_2 (□—□) or ferricyanide (■—■) as electron acceptor. Curves 7 and 8 (×—×, O_2; ⊖—⊖, ferricyanide) likewise show that the trypsin-solubilized fraction has no activity in the absence of the soluble flavoprotein. The ferricyanide reactions were carried out in N_2 and the rate of reaction was determined manometrically by CO_2 evolution. The O_2 assays were incubated in air. All incubations were at 30° in Warburg cups in a total volume of 1 ml for the time indicated.

appearance of reduced α and β bands at 556 and 523 mμ and a shift in the Soret band from 412 to 427 mμ. Preliminary runs in the ultracentrifuge indicate that the solubilized material is heterogeneous and is composed of two major components.

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