Activation Studies by Phospholipids on the Purified Cytochrome \(c_4:o\) Oxidase of \textit{Azotobacter vinelandii}\(^1\)

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Abstract

A modified procedure is described that was used to solubilize and purify the TMPD-dependent cytochrome \(c_4:o\) oxidase from \textit{Azotobacter vinelandii}. Two functional components (Fractions I and V) were obtained after DEAE-cellulose chromatography. Fraction V contained both cytochrome \(c_4\) (3.6 nmol/mg protein) and cytochrome \(o\) (1.6 nmol/mg protein). This cytochrome oxidase complex oxidized TMPD at "moderate" rates. Fraction I, a clear greenish-yellow fraction, contained primarily phosphatidylethanolamine with some phosphatidylglycerol. Fraction I itself could not oxidize TMPD, but when it was preincubated with Fraction V, a 2-4-fold stimulation in TMPD oxidase activity occurred. Other "authentic" micellar phospholipids also readily activated TMPD oxidase activity in Fraction V. The \textit{maximum} activation effect obtained with Fraction I was in essence duplicated with purified phosphatidylethanolamine.

Key Words: Cytochrome oxidase; \textit{Azotobacter vinelandii}; phospholipid activation; cytochrome \(c_4:o\) oxidase; phosphatidylethanolamine; TMPD oxidase.

Introduction

Much like the mammalian mitochondrial cytochrome \(a + a_3\) oxidase, most bacterial cytochrome oxidases are particulate entities, tightly bound to the inner cytoplasmic membrane. The difficulties encountered in studying bacterial oxidases are: (1) the lack of a suitable assay for measuring cytochrome oxidase activity, (2) no established detergent solubilization procedures for isolating such oxidases, and (3) usually two or three multiple types of terminal oxidases are found in a single organism.

\(^1\)Dedicated to the memory of David E. Green, a fine gentleman, an excellent scientist, and a true scholar. He will be missed by many of his former colleagues.
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The *Azotobacter vinelandii* cytochrome oxidase is of particular interest as this organism has the highest respiratory rate of any known cell (Burk, 1930). Photochemical action spectra have revealed three cytochrome oxidases in *A. vinelandii*, i.e., cytochrome *a*, *d*, and *o* (Castor and Chance, 1959; Jones and Redfearn, 1967a). Repske and Josten (1958) isolated an active NADH oxidase from this organism. This complex contained large concentrations of cytochrome *b*, *c₄*, and *d*. Subsequently Jones and Redfearn (1967b) solubilized a red particle fraction from *A. vinelandii*. This “red particle” fraction contained “enriched” concentrations of nonheme iron, cytochrome *b₁*, *c₄* + *c₅*, and *o*, and it oxidized succinate and ascorbate-DCIP fairly rapidly. A concomitantly solubilized “green particle” fraction had low ascorbate-DCIP oxidase activity, but contained high concentrations of cytochrome *d* and *a₁*, as well as cytochrome *b* and ubiquinone. Jurtshuk and associates (Mueller and Jurtshuk, 1972; Jurtshuk et al., 1981) solubilized a highly active membrane-bound TMPD⁴ oxidizing enzyme complex from *A. vinelandii*. This solubilized cytochrome oxidase was readily precipitated by ammonium sulfate; it contained predominantly cytochrome *c₄* and cytochrome *o*.

In this report, we present a modified procedure which can be used to purify the cytochrome *c₄:o* oxidase of *A. vinelandii*. The enzymatic properties and spectral characteristics of this cytochrome oxidase are the main theme of this report. We also show that a specific fraction (Fraction I), isolated from the same purified oxidase complex, was required for maximal TMPD oxidase activity.

**Materials and Methods**

*Preparation of Electron Transport Particle*

*A. vinelandii* strain 0 was grown in a 200-liter capacity fermenter (New Brunswick Scientific Co.) under conditions previously described (Jurtshuk et al., 1967). Late-log phase cells were harvested and washed twice with 0.02 M phosphate buffer, pH 7.5. The electron transport particle, designated R₃, was isolated from sonically disrupted cells by differential centrifugation (Jurtshuk et al., 1967). The R₃ fraction represents the membrane pellet after high-speed centrifugation at 114,000 × *g*, and the resultant supernatant cytosol fraction.

⁴Abbreviations used: TMPD, *N,N,N',N'-tetramethyl-p-phenylenediamine*; Triton X-100, octylphenoxypolyethoxyethanol; DEAE, diethylaminoethyl; HPTLC, high-performance thin-layer chromatography; R₃ fraction, sonic ETP (electron transport particle); S₄ fraction, supernatant remaining after removing the R₃ or sonic ETP fraction by ultracentrifugation; Fraction V, the active cytochrome *c₄:o* (or *c:o*) oxidase. Abbreviations used for phospholipids: PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PS, phosphatidylinerine.