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Functional activities of monomeric and dimeric forms of the chloroplast cytochrome b6f complex

R. K. Chain & R. Malkin*
Department of Plant Biology University of California Berkeley, 111 Koshland Hall, CA 94720-3102, USA;
*Author for correspondence

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Abstract

A monomeric form of the isolated cytochrome b6f complex from spinach chloroplast membranes has been isolated after treatment of the dimeric complex with varying concentrations of Triton X-100. The two forms of the complex are similar as regards electron transfer components and subunit composition. In contrast to a previous report (Huang et al. (1994) Biochemistry 33: 4401-4409) both the monomer and dimer are enzymatically active. However, after incorporation of the respective complexes into phospholipid vesicles, only the dimeric form of the cytochrome complex shows uncoupler sensitive electron transport, an indication of coupling of electron transport to proton translocation. The absence of this activity with the monomeric form of the cytochrome complex may be related to an inhibition by added lipids.

Abbreviations: CCCP—carbonyl cyanide m-chlorophenylhydrazone; mega-9-nonanoyl-N-methylglucamide

Introduction

Cytochrome bcl and b6f complexes are involved in the oxidation of quinols and the reduction of high-potential electron carriers, such as cytochrome c or plastocyanin. (Cramer et al. 1987; Malkin 1992; Anderson 1992; Hope 1993; Trumpower and Gennis 1994). Accompanying this electron transfer is proton translocation across the respective biological membrane such that these complexes play an important role in the establishment of the proton motive force that is ultimately used for the synthesis of ATP.

While the mechanism of electron transfer and proton translocation through cytochrome complexes is not fully understood, it is widely accepted that the Q-cycle, originally proposed by Mitchell (1975, 1976), is the most accurate model that describes these events (Rich 1986; Trumpower 1990). A wealth of kinetic information is now available on a number of systems that support this mechanism, and this includes recent molecular studies in both eukaryotic and prokaryotic systems (Daldal et al. 1989; Ding et al. 1992; Graham et al. 1992; Hacker et al. 1993; Gray et al. 1994).

The protein subunit composition of these complexes is rather well defined although there remain questions concerning the function of many of the individual subunits that apparently are not involved in the binding of prosthetic groups (Haley and Bogorad 1989; see Schmidt and Malkin 1993 and Pierre and Popot 1993 for recent work on the cytochrome b6f complex). In recent years, questions concerning the multimeric state of the complex have also emerged (reviewed by von Jagow and Sebald 1980; Cramer et al. 1987; O’Keefe 1988). It has been shown that both the cytochrome bcl complex from mitochondria and the cytochrome b6f complex from chloroplasts can exist in both monomeric and dimeric states. In the case of the mitochondrial cytochrome bcl complex, it is generally accepted that the functional unit of the complex is dimeric in form (von Jagow et al. 1977; Weiss and Kolb 1979). This has
led to models for the functioning of the cytochrome \textit{bcl} complex based on this dimeric structure (De Vries et al. 1982; Schmitt and Trumpower 1990). There are varying reports as to whether the monomeric form of this complex is enzymatically active, and a recent report by Musatov and Robinson (1994) has documented the preparation of a monomeric form of the bovine heart cytochrome \textit{bcl} complex that is fully active in electron transport. In the case of the cytochrome \textit{bhf} complex, there are also recent reports of a monomeric form of the complex (Chain and Malkin 1991), and a recent paper by Huang et al. (1994) has concluded that only the dimer is active in the membrane and that monomeric cytochrome \textit{bhf} complex is not functional. However, titrations with specific inhibitors of this complex have also been interpreted as indicating that the cytochrome \textit{bhf} complex can function in its monomeric form (Rich et al. 1991 but see also Graan and Ort 1986).

In this work, we have examined the dim- monomer conversion of the chloroplast cytochrome \textit{bhf} complex. We find that low amounts of Triton X-100 convert the active dimer into a monomer form that retains full activity. We have also considered the coupling of electron transport to proton translocation with these respective forms of the cytochrome complex and our results indicate that only the dimeric form shows coupled activity and that the monomeric form of the complex is inhibited by lipids.

Materials and methods

\textit{Preparation of the chloroplast cytochrome \textit{bhf} complex}

The cytochrome \textit{bhf} complex was isolated from spinach chloroplasts by the procedure of Hurt and Hauska (1982) that uses octyl-glucoside and cholate as detergents, with the modification that the detergent used in the final sucrose density gradient centrifugation step of the purification was 6 mM dodecyl-\(\beta\)-D-maltoside. The cytochrome complex was localized as a brownish band at approximately 30% sucrose on this gradient. The collected complex was diluted to a final sucrose concentration of 5% with 6 mM dodecyl-\(\beta\)-D-maltoside in 50 mM Tris-HCl buffer (pH 7.8) and concentrated to a final concentration of 20–40 \(\mu\)M cytochrome \(f\) with an Amicon YM-100 diaflow membrane. The concentration of cytochrome \(f\) was based on an extinction coefficient of 18 mM\(^{-1}\) cm\(^{-1}\) at 554–540 nm and 20 mM\(^{-1}\) cm\(^{-1}\) for cytochrome \(b6\) at 563–575 nm.

\textit{Separation of monomer and dimer forms of the complex}

The conversion of the dimeric form of the cytochrome \textit{bhf} complex to the monomer was achieved on a second sucrose density gradient. The basic gradient was 10–30% sucrose in 25 mM Tris-HCl (pH 7.8) which was centrifuged in an SW-60 Beckman rotor at 50,000 rpm for approximately 20 hours. The dimeric form of the complex was maintained on this second gradient by using 6 mM dodecyl-\(\beta\)-D-maltoside + 0.5% sodium cholate + 5 mM MgCl\(_2\) in the gradient. The monomer was formed on gradients prepared in the presence of Triton X-100 (0.05–0.25%). Approximately 4–6 nmol of the cytochrome complex (based on cytochrome \(f\) concentration) was added to each gradient. The two forms of the complex were collected by pumping the 4.4 ml gradient through a flow cell in an Aminco DW-2000 spectrophotometer and monitoring the cytochrome absorbance at 414–449 nm. Samples were collected from the bottom of the centrifuge tube so higher molecular weight forms of the complex have lower elution times than forms nearer to the top of the tube. No attempt was made to separate the two forms of the complex at the lower concentrations of Triton where both were apparent.

\textit{Electron transport activity measurements}

The assay for the cytochrome complex was based on the quinol-plastocyanin oxidoreductase activity. Decyl-plastoquinol was used as the electron donor and the ferricyanide-plastocyanin system was used as the acceptor system. In this acceptor system, plastocyanin serves as the intermediate electron acceptor with ferricyanide, the terminal acceptor, being used to monitor the reaction (Rich et al. 1987). The assay medium consisted of 50 mM Tris-HCl (pH 7.8) plus 10 mM NaCl and 5 mM MgCl\(_2\) in a final volume of 3.0 ml. The concentrations (\(\mu\)M) of ferricyanide, decyl-plastoquinol, plastocyanin and the cytochrome complex were 500, 83, 6.5 and 10–12. The reaction was initiated by the addition of decyl-plastoquinol and followed the reduction of ferricyanide at 420–500 nm using an Aminco DW-2000 spectrophotometer in the dual wavelength mode with a stirred cuvette. All activities were corrected for the non-catalyzed rate of ferricyanide reduction measured in the absence of the cytochrome com-