

Size Exclusion-Fast-Protein-Liquid-Chromatography Separation of the Cytochrome b_6f -Complex Components from Spinach Chloroplast Membranes

(Received: 05.11.1998)

M.A. SHALLAN

Biochemistry Dept. Fac. Agriculture, Cairo Univ.
Giza, Egypt.

ABSTRACT

The cytochrome b_6f -complex was selectively solubilized from the thylakoid of spinach chloroplast membranes using octylglucoside detergent and purified on a sucrose density gradient in the presence of the detergent. Chromatographic separation of the complex by using size exclusion-Fast-Protein-Liquid-Chromatography (sec-FPLC) system on superose 12 column under reducing and nonreducing conditions was performed. One dimensional sodium dodecyl sulfate polyacrylamide gel-electrophoresis and staining with coomassie brilliant blue was carried out to analyze the eluted peaks containing proteins. The results presented in this work showed that the cytochrome complex may be found in an oligomeric form. A group of high molecular weight protein subunits with molecular masses ranging from approx. 37 to 95 kDa could be isolated and assumed to be in a close association with the complex in the thylakoid membrane. In addition, a binary subcomplex consisted of cytochrome b_6 and the so called subunit 17 kDa was obtained and separated. The functional capacity of these higher molecular weight protein subunits as well as the observed binary subcomplex is an open question.

Key words: Photosynthesis, Membrane proteins, Cytochrome b_6f -complex, sec-FPLC.

INTRODUCTION

The photosynthetic system of higher plants possesses a rather complex architecture. The electron flow from water to NADP is affected in thylakoid membranes by three integral membrane complexes consisting of a variety of polypeptide chains; the photosystem I and II responsible for the primary processes of light harvesting and charge separation and the cytochrome b_6f -complex mediating the electron flow between both systems with mobile

electron carriers like plastoquinone and plastocyanin as the molecular links. Thus, the cytochrome b_6f -complex can be described as a plastoquinol-plastocyanin-oxidoreductase that couple the electron transfer to proton translocation across the thylakoid membrane (Hurt and Hauska, 1981, Hauska *et al.*, 1983, Ort, 1986, Hauska, 1986 and O'keefe, 1988). In these functions, this complex is analogous to the cytochrome bc_1 complex of the mitochondrial and photosynthetic bacterial electron transport chains (Malkin, 1988 and Rich, 1990). It also generates ATP for CO_2 -