

Rapid HPLC Separation of the Protein Mr.=17000 of Spinach Thylakoid Membranes

(Received: 07. 04.1999)

M.A. Shallan

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

ABSTRACT

A methodological approach is described which allows the isolation of the integral membrane protein subunit IV of cytochrome *b₆f*-complex from chloroplasts. The technique consists of (1) selective solubilizing of the cytochrome complex from chloroplasts using octylglucoside detergent and purification on a sucrose density gradient, (2) removal of salts and SDS through selective and facile extractibility of the complex subunits using cyanopropyl cartridge and step-gradient using an organic solvent mixture composed of isopropanol/tri-fluoro acetic acid, and (3) purification of proteins by Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) on Nucleosil CN column using the above mentioned organic solvent mixture as eluant. The results showed that removal of SDS efficiency was 95.65%. A simple and rapid purification of subunit IV in pure form could be achieved. This sample was dissolved in an organic solvent compatible with subsequent amino acid microsequencing and can serve as antigen to produce monoclonal antibodies that can be used as probes to survey the location and arrangement of this subunit along thylakoid membranes. This is the first example that a single component of cytochrome *b₆f*-complex has been purified using HPLC on reverse phase.

Key words: Photosynthesis, membrane proteins, Cytochrome *b₆f*-complex, RP-HPLC

INTRODUCTION

The cytochrome *b₆f*-complex called plastoquinol -plastocyanin-oxidoreductase operates in photosynthetic electron transfer in linear electron flow between photosystems II and I or in cyclic flow around photosystem I and couple the electron transfer to proton translocation across the thylakoid membrane to produce ATP (Hauska *et al.*, 1983, Ort, 1986, Malkin, 1988, O'keefe, 1988, Rich, 1990, Cramer and Knaff, 1991, Bendall and Manasse, 1995 and Kubicki *et al.*, 1996). In these functions, this complex is analogous to the cytochrome *bc₁* complex of the mitochondrial and

photosynthetic bacterial electron transport chains (Hauska *et al.*, 1988 and Trumpower and Gennis, 1994). Recently, an important role of the complex in chlorophyll quenching processes was discovered (Gal *et al.*, 1990, Knaff, 1991, Vallon *et al.*, 1991, Frid *et al.*, 1992, Dwivedi and Bhardwaj, 1994, Vener *et al.*, 1995, Price *et al.*, 1995, Meurer *et al.*, 1996 and Krendelewa *et al.*, 1996). While the mitochondrial complex contains approximately 10 protein subunits (Furbacher *et al.*, 1996), the chloroplast cytochrome *b₆f*-complex is the simpler subunit composition with four major peptides of mol. wt.= 17,000-33,000, of which three are organelle encoded and one (the mol. wt.=20,000 Rieske iron-