

# Heterologous Overexpression of Membrane-Anchored Subunit II of Spinach Chloroplast ATP Synthase and Its Detergent-Free Purification as a Soluble Protein\*

Hans-Jürgen Tiburzy, Martin Zimmermann, Regina Oworah-Nkruma  
and Richard J. Berzborn

Lehrstuhl für Biochemie der Pflanzen, Fakultät für Biologie der Ruhr-Universität Bochum,  
D-44780 Bochum, Germany

Z. Naturforsch. **54c**, 230–238 (1999); received October 19/November 11, 1998

*Escherichia coli*, T7 RNA Polymerase/Promoter System, Immobilized Metal Ion Affinity Chromatography (IMAC), Membrane Protein

Subunit II is one of the four nonidentical subunits of the membrane integral, proton-transporting moiety ( $CF_o$ ) of the chloroplast ATP synthase. In chloroplasts of spinach leaves, it is the only nuclear-encoded  $CF_o$  subunit. It has been deduced that  $CF_oII$  is not an additional subunit typical for photosynthetic organisms with no counterpart in *E. coli*, but equivalent to *E. coli* subunit b (Tiburzy, H.-J. and Berzborn, R. J. (1997), Z. Naturforsch. **52c**, 789–798). Heterologous expression of subunit II was achieved by using the bacterial expression vector pT7-7. Recombinant subunit II ( $II_{rec}$ ) does not integrate into the bacterial membrane nor does it precipitate into inclusion bodies. Gel filtration chromatography indicates that  $II_{rec}$  forms higher order aggregates. In three chromatographic steps approx. 10 mg of soluble  $II_{rec}$  of electrophoretic homogeneity are obtained from one liter of bacterial culture without using detergents. Thus, a eukaryotic membrane-anchored protein has been overexpressed in *E. coli* and has been purified in a soluble form.

Reprint requests to Prof. Dr. R. J. Berzborn. Fax: (\*49) 234-70 94322, e-mail: richard.j.berzborn@ruhr-uni-bochum.de