Biochimica et Biophysica Acta, 502 (1978) 269—275

BBA 47497

EVIDENCE THAT GENETIC INFORMATION FOR CHLOROPLAST COUPLING FACTOR I IS SHARED BY NUCLEAR AND CHLOROPLAST DNA

P. KWANYUEN and S.G. WILDMAN

Department of Biology, Molecular Biology Institute, University of California, Los Angeles, Calif. 90024 (U.S.A.)

(Received August 1st, 1977)

Summary

Using the method developed by Lien and Racker (Lien, S. and Racker, E. (1971) in Methods in Enzymology (San Pietro, A., ed.), Vol. 23, Part A, pp. 547—555, Academic Press, New York) for spinach leaves, coupling factor I was isolated from 2-kg quantities of *Nicotiana glutinosa*, *N. tabacum*, *N. excelsior*, *N. langsdorffii* leaves, and shown to consist of 5 subunits of comparable monomeric molecular weights to coupling factor I from spinach and also to be a trypsin-activated, Ca^{2+}-dependent ATPase. Limitation of hybrid plant material necessitated use of polyacrylamide electrophoresis to separate Fraction I protein (M_r = 550 000 with a monomeric large subunit of 56 000) from coupling factor I (M_r = 350 000; β-subunit of 56 000) so that approx. 50 μg of pure coupling factor I could be isolated from 50-g quantities of coupling factor I reciprocal hybrid plants and the coupling factor I subjected to electrofocussing in 8 M urea simultaneously with coupling factor I from the two parental species. Electrofocusing resolved 3 or 4 of the 5 subunits contained in the coupling factor I from leaves of the four *Nicotiana* species tested. All were like *N. tabacum* in regard to isoelectric points and staining intensity of 4 subunits except *N. glutinosa* coupling factor I which displayed 2 subunits which differed in staining intensity from 2 comparable subunits in coupling factor I of the other species. *N. glutinosa* coupling factor I also lacked one subunit resolved in the other species. The genetic information determining the difference in staining intensity of the 2 subunits of coupling factor I contained in *N. tabacum* × *N. glutinosa* reciprocal coupling factor I hybrids was inherited only by the maternal line and is therefore most likely contained in chloroplast DNA. Information determining the isoelectric point of a third subunit was transmitted by pollen to an coupling factor I hybrid and was

Abbreviation: CF_1, coupling factor I.

therefore contained in nuclear DNA. No difference in staining intensity or isoelectric points could be detected in a fourth subunit so the source of its coding information remains unknown.

Introduction

Chloroplast coupling factor I (CF_1) is a protein required for photosynthetic phosphorylation. It can be released in soluble form from thylakoid membranes of chloroplasts by treating them with low concentrations of EDTA [1]. The purified CF_1 from spinach leaves has been shown by Racker and associates [2] to contain latent Ca^{2+}-dependent ATPase activity which can be activated by trypsin or heating. It is composed of two major subunits and three others [3], all of different monomeric molecular weights. Two of the five subunits of CF_1 have been shown to be synthesized in the chloroplast [4]. Since CF_1 is an oligomeric protein like Fraction I protein [5], we have investigated whether the genetic material coding for CF_1 would be split between nuclear and chloroplast DNAs as is the case for the large and small subunits of Fraction I protein [6].

Materials and Methods

Plants. The several species and interspecific hybrids of *Nicotiana* plants were grown as previously described [5]. Leaves were harvested from plants which had grown for 2—3 months.

Assay of Ca^{2+}-dependent ATPase activity of Nicotiana CF_1. The assay was conducted by the method of Lien and Racker [2]. Diphenylcarbamyl chloride-treated trypsin was used for enzyme activation.

Electrofocusing. Isoelectric focusing of purified CF_1 and crude EDTA-extract of CF_1 in the pH range 5—7 was performed in slab gels containing 5% polyacrylamide and 8 M urea, details having been described by Kung et al. [6]. Electrofocusing was performed overnight at room temperature with an Ortec-pulsed power supply at a potential of 300 V and 95 pulses per second while the current was maintained below 5 mA.

Experimental Results and Discussion

Identification of CF_1 in N. glutinosa leaves

Using the procedure of Lien and Racker [2] for obtaining purified CF_1 from spinach leaves, the product from *N. glutinosa* leaves remained contaminated by Fraction I protein as shown by cross reaction with anti-Fraction I protein serum. The contamination could be removed by a third pass through a DEAE-Sephadex A-50 column. The final product was found to be composed of 5 subunits (Fig. 1) with mobilities and molecular weights similar to those to spinach CF_1. However, the more involved purification procedure required 2 kg of *N. glutinosa* leaves as starting material. Absence of Fraction I protein in our contemplated experiments was of crucial importance since its large subunit coded by chloroplast DNA has a molecular weight of 56 000, the same as the

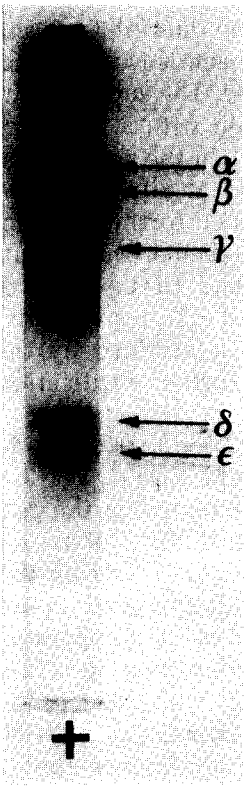

Fig. 1. SDS-polyacrylamide gel electrophoresis of purified *N. glutinosa* CF_1. The subunits are designated α, β, γ, δ and ε according to Lien et al. [3] and have similar mobilities and relative molecular weights as the purified spinach CF_1.

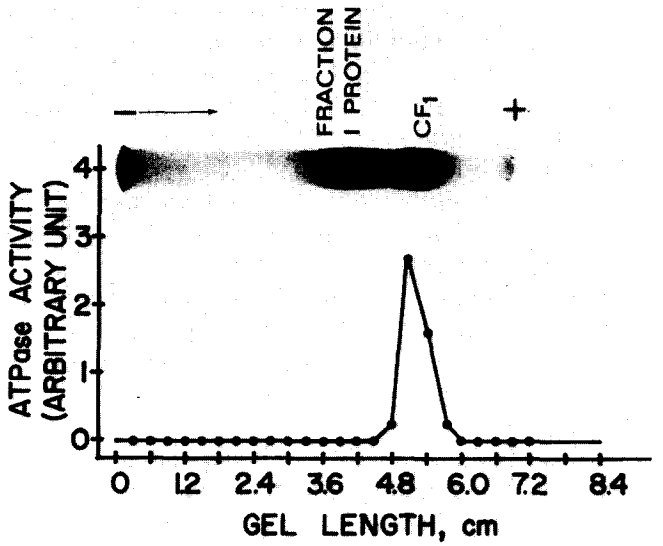

Fig. 2. Gel electrophoretic behavior of *N. glutinosa* crude CF_1 extract indicating that only one of the two protein bands contains Ca^{2+}-dependent ATPase activity.

β-subunit of CF_1, and its small subunit coded by nuclear DNA has a molecular weight of 12 500, very close to that of the ε-subunit of CF_1. Since we were limited to quantities of from 50—100 g of leaves from the reciprocal, interspecific CF_1 hybrids that were to be used for the genetical analysis, it was necessary to develop another method for obtaining CF_1 from *Nicotiana* plants that would be free from Fraction I protein.

A procedure for separating Nicotiana CF_1 from Fraction I protein

50 g of *Nicotiana* leaves were homogenized in a rotary leaf chopper [7] equipped with chilling system, with 62.5 ml of 0.5 M sucrose medium containing 20 mM Tricine/NaOH and 2 mM EDTA, pH 7.8. Following the Lien and Racker procedure [2], the chloroplast pellet was suspended twice in 0.75 mM EDTA, pH 7.6, to 0.1 mg chlorophyll per ml at room temperature to release CF_1 from the membranes which were removed as a green pellet by centrifugation at 20 000 × *g* for 30 min. All further steps were conducted at room temperature. The combined supernatants were found to contain Ca^{2+}-dependent ATPase activity. In comparison with an activity of 8.5 μmol ATP

hydrolyzed per min per mg chlorophyll calculated from the data of Lien and Racker [2] for their crude EDTA-extract of CF_1 from spinach leaves, approx. 8 μmol were obtained in supernatants from *Nicotiana* leaves. The supernatants containing ATPase activity were combined and concentrated with a PM-30 or XM-50 membrane in an Amicon ultrafiltration assembly. When the volume was approx. 5—10 ml, the protein concentrate was centrifuged at 20 000 × *g* for 10 min to remove a small turbidity. The supernatant was then further concentrated with a collodion ultrafiltration apparatus (25 000 molecular weight cut-off). When the volume was approx. 1 ml, Tricine/NaOH and EDTA pH 7.8, were added to bring the final concentrations to 20 mM and 2 mM, respectively. Protein concentration of the concentrated solution was determined by the method of Lowry et al. [8]. This concentrate was used for further purification of CF_1 by gel electrophoresis.

50 μl portions of CF_1 concentrate were subjected to 3% polyacrylamide disc gel electrophoresis in 20 mM Tricine/NaOH and 2 mM EDTA, pH 7.8 at a constant current of 5 mA per gel for 105 min. After removing the gels from their tubes, one gel was stained for at least 2 h with 0.25% Coomassie brilliant blue in 5 : 1 : 5 methanol/glacial acetic acid/water. As shown by the photograph at the top of Fig. 2, two prominent protein bands appeared on the gel after staining a CF_1 concentrate from *N. glutinosa* leaves. A comparable unstained gel was sliced into 3-mm pieces, and each piece crushed to a fine suspension and used for Ca^{2+}-dependent ATPase activity with the results shown by the graph in Fig. 2. Only one protein band contained CF_1 activity which was the one of lower molecular weight thus giving confidence that a clear separation of CF_1 from Fraction I protein had been accomplished.

The CF_1 band in several gels was sliced away from Fraction I protein contaminant, the CF_1 eluted by squeezing the gels through a syringe with a 20-gauge needle and soaking the fine fragments in 20 mM Tricine/NaOH and 2 mM EDTA, pH 7.8, overnight. The gel fragments were removed by centrifugation and the solubilized CF_1 concentrated by collodion membrane ultrafiltration. When a 1-ml volume was obtained, it was further concentrated with an A-75 Minicon concentrator. The final volume was approx. 50 μl. The purified CF_1 was electrophoresed again in the same manner as before with the results shown in Fig. 3. Only a single band of protein was obtained whose mobility corresponded with CF_1 in the crude extract. A similar result was obtained with CF_1 concentrates from leaves of *N. langsdorffii*, *N. excelsior*, and *N. tabacum*. All the purified species of CF_1 were indistinguishable in molecular weights of the five subunits from the *N. glutinosa* CF_1 shown in Fig. 1. The above described procedure made it possible to obtain enough CF_1 from parents of potential reciprocal hybrid plants to see whether electrofocusing would reveal any differences in the isoelectric points of the subunits of CF_1 when different species were compared that thereby provide material for genetical analysis.

Electrofocusing of Nicotiana CF_1

Sufficient crude CF_1 was prepared from leaves from *N. excelsior*, *N. langsdorffii*, *N. tabacum* and *N. glutinosa* so that 20 gels each containing 50 μl of a particular CF_1 protein could be simultaneously electrophoresed in the

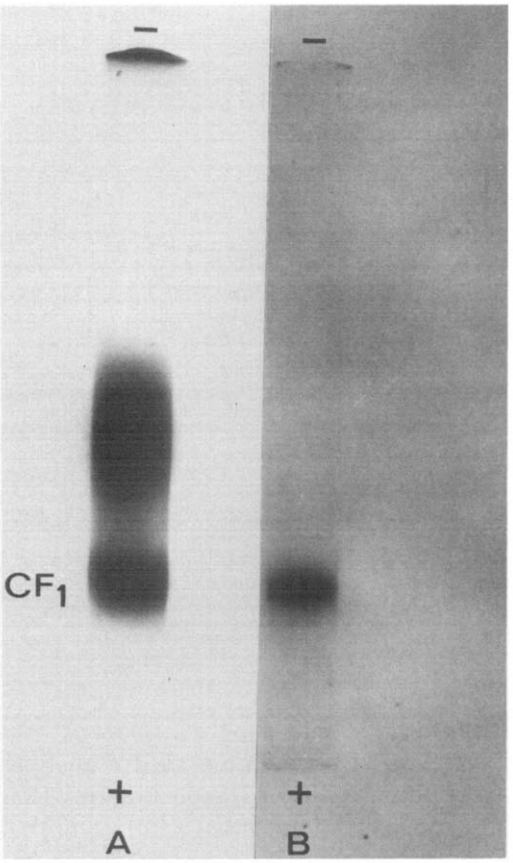

Fig. 3. Gel electrophoretic behavior of purified *N. glutinosa* CF_1 (gel B) compared to the crude CF_1 extract (gel A).

same apparatus to separate CF_1 from Fraction I protein as described. One gel was stained to locate the CF_1 band and used as the reference for slicing out the CF_1 region in the other 19 gels. After elution and concentration as described, 20 µl aliquots of protein were applied to a slab gel containing 8 M urea and ampholine to create a 5—7 pH gradient. Only the CF_1 from *N. glutinosa* and *N. tabacum* were different in electrofocusing behavior. The CF_1 from *N. excelsior* and *N. langsdorffii* could not be distinguished from *N. tabacum* CF_1.

As shown by the photograph in Fig. 4, the CF_1 from *N. tabacum* was resolved into 4 bands with different isoelectric points. This is in contrast to the 3 bands which could be resolved for CF_1 from *N. glutinosa*. Therefore, the CF_1 was isolated from leaves of reciprocal *N. tabacum* × *N. glutinosa* F_1 hybrids. In *N. tabacum* and *N. tabacum* ♀ × *N. glutinosa* ♂ CF_1, the band marked "A" in Fig. 4 stains intensely whereas in *N. glutinosa* and *N. glutinosa* ♀ × *N. tabacum* ♂, band "A" stains lightly. Conversely, band "C" in *N. tabacum* and *N. tabacum* ♀ × *N. glutinosa* ♂ stains lightly whereas it is heavily stained in *N. glutinosa* and *N. glutinosa* ♀ × *N. tabacum* ♂. Because the staining intensity of bands "A" and "C" from other CF_1 species are like those of *N. tabacum* CF_1, it seems likely that the difference in staining intensity

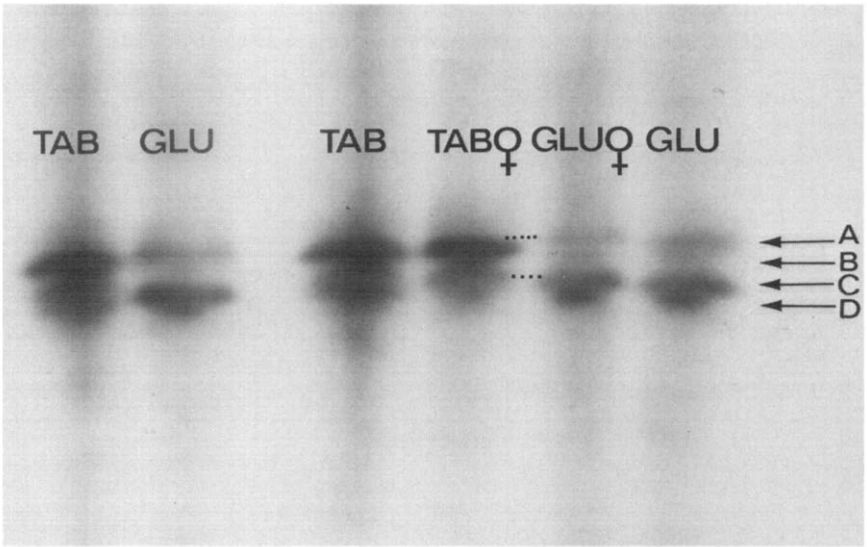

Fig. 4. Photograph of a slab gel after electrofocusing of CF_1 from two *Nicotiana* species and reciprocal, interspecific F_1 hybrids. TAB = *N. tabacum*; GLU = *N. glutinosa*; TAB ♀ = *N. tabacum* ♀ × *N. glutinosa* ♂; GLU ♀ = *N. glutinosa* ♀ × *N. tabacum* ♂.

represents a difference in composition of amino acids making up these bands. Therefore, the genetic information which appears to determine a difference in composition is inherited as a maternal factor and is most likely contained in chloroplast DNA. As indicated by dotted lines, a slight difference in isoelectric points between the bands in question can be seen which also appears to be inherited as a maternal factor and is consistent with the interpretation that a difference exists in amino acid composition.

The "B" band is found in *N. tabacum* CF_1, but is absent from the same position in the *N. glutinosa* CF_1. A 4th band for *N. glutinosa* CF_1 could not be positively identified in another position on the gel. The "B" band is however found in both hybrids but reduced in amount in *N. tabacum* ♀ × *N. glutinosa* ♂ compared to *N. tabacum*. It is a faint component in *N. glutinosa* ♀ × *N. tabacum* ♂ and somewhat difficult to photograph but readily seen by direct observation of the gels. Its presence in the latter hybrid shows that genetic information for this protein came from *N. tabacum* pollen and therefore was contained in the nuclear DNA of pollen.

The "D" band is present in CF_1 of both species and both reciprocal hybrids, has the same isoelectric point, and stains with the same intensity so that no information was obtained as to whether nuclear or extranuclear DNA is the source of its coding information. Further comparative analysis of CF_1 from other *Nicotiana* species might uncover differences necessary for this determination.

We do not know which bands resolved by electrofocusing correspond with the subunits resolved by SDS gel electrophoresis although it seems likely that A and C being major components as judged by degree of staining may be α and β with D being γ and B being either δ or ϵ. Test of this notion is

impractical with current methodology since at least 20 isoelectric focusing gels would have to serve as a source for cutting out the bands in question so that enough of the proteins could be obtained for determination of their molecular weights by gel electrophoresis.

Acknowledgements

Research supported by Contract E(04-3)34, Project 8 from Energy Resource and Development Admistration, National Science Foundation grant 75-97368 and a grant from Monsanto Commercial Products Corporation.

References

1 Vambutas, V.K. and Racker, E. (1965) J. Biol. Chem. 240, 2660—2667
2 Lien, S. and Racker, E. (1971) in Methods in Enzymology (San Pietro, A., ed.), Vol. 23, Part A, pp. 547—555, Academic Press, New York
3 Lien, S., Berzborn, R.J., and Racker, E. (1972) J. Biol. Chem. 247, 3520—3524
4 Mendiola-Morgenthaler, L.R., Morgenthaler, J.J., and Price, C.A. (1976) FEBS Lett. 62, 96—100
5 Kawashima, N. and Wildman, S.G. (1972) Biochim. Biophys. Acta 262, 42—49
6 Kung, S.D., Sakano, K., and Wildman, S.G. (1974) Biochim. Biophys. Acta 365, 138—147
7 Wong, F.Y. (1972) Doctoral Dissertation, pp. 35—37, University of California, Los Angeles
8 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275