Bot. Bull. Academia Sinica **17**: 185-191 (1976)

ISOELECTRIC POINTS OF THE POLYPEPTIDE COMPONENTS OF TOBACCO FRACTION 1 PROTEIN

S. D. KUNG

Department of Biological Sciences
University of Maryland Baltimore County
Catonsville, Maryland 21228, U.S.A.

(Accepted for publication April 12, 1976)

Abstract

Isoelectric focusing of *S*-carboxymethylated Fraction 1 protein from *Nicotiana tabacum* in polyacrylamide gel has resolved the subunits into their component polypeptides. There are three polypeptides in the large subunit and two in the small subunit. The isoelectric points are 6.10, 6.05 and 6.00 for the large subunit polypeptides and 5.35 and 5.31 for the small subunits.

Introduction

Fraction 1 protein, comprising more than 50 percent of the soluble leaf protein, is the most abundant protein in nature. This protein is found in all organisms containing chlorophyll a, including the prokaryotic blue-green algae. Fraction 1 protein is identical to ribulose Bisphosphate (RuBP) carboxglase-oxygenase (Marsho & Kung, 1976), a unique enzyme having dual functions that either fix or lead to the evolution of CO_2 (Kung, 1976). This protein has a molecular weight of 5.6×10^5 and can be dissociated into large and small subunits with molecular weights of 5.5×10^4 and 1.2×10^4 respectively (Kawashima and Wildman, 1970).

Recently, isoelectric focusing of *S*-carboxymethylated Fraction 1 protein from *Nicotiana tabacum* in polyacrylamide gel has resolved the subunits into their component polypeptides. The 8 large subunits were resolved into three polypeptides, each having a molecular weight of 5.5×10^4 and the 8 small subunits were resolved into two polypeptides, each having a molecular weight of 1.2×10^4 (Kung *et al.*, 1974). Examination of Fraction 1 protein from over sixty species of *Nicotiana* and 10 plant species ranging from green algae to ginkgo reveals that all large subunits consist of three polypeptides whereas the small subunits may vary from one to four polypeptides (Wildman *et al.*,

1975). The three polypeptides of the large subunit are inherited together via the maternal line whereas the polypeptides of the small subunit are inherited separately via pollen (Sakano *et al.*, 1974). This unique property of Fraction 1 protein povides us with a unique genetic marker for both chloroplast and nuclear genomes and it has also been successfully used as a genetic marker for probing many biological and botanical problems in tobacco plants (Kung, 1976). This success depends entirely on the difference of the isoelectric points (P^I) of each polypeptide. Yet, there is no detailed information available concerning their P^Is. This paper reports the isoelectric points of each polypeptide component of Fraction 1 protein prepared from *N. tabacum*, *N. glauca*, and their reciprocal hybrids. Also, evidence is provided demonstrating that the single polypeptide of the small subunit of *N. glauca* is distinctively different from the two polypeptides of the small subunit of *N. tabacum*.

Materials and Methods

The seeds of *N. tabacum* cultivar Turkish Samsun, *N. glauca*, and their reciprocal hybrids were kindly provided by Dr. S. G. Wildman of UCLA. Tobacco plants were grown in a greenhouse under controlled conditions. Fraction 1 protein was prepared by direct crystallization from leaf homogenates as described by Chan *et al.* (1971). Crystals were produced from leaf homogenates that had been centrifuged to remove particulate matter and passed through G-25 Sephadex to separate phenolic compounds.

The three times recrystallized Fraction 1 protein was first *S*-carboxymethylated (Kung *et al.*, 1974) and then dissociated in 0.5% SDS. The large and small subunits were separated according to the procedure of Rutner and Lane (1967).

Isoelectric focusing of Fraction 1 protein and its subunits were performed according to Kung *et al.* (1974). After isoelectric focusing, the disk gel was cut into 1 mm section and the isoelectric points were determined by measuring the pH of every 2 sections (Robinson, 1971).

Results

When Fraction 1 protein from *N. tabacum* was dissociated in 8 M urea, thiol groups undergo rapid oxidation. This is demonstrated by the formation of a complex banding pattern of polypeptides in the large subunits (Fig. 1A). However, the pattern in the small subunit is quite simple, being composed of two polypeptides of equal amount (Fig. 1C).

The P^I of polypeptides present in Fig. 1A was measured and indicated in Fig. 2. Only the range of P^Is of the complex bands of large subunits is given because the banding pattern varies from experiment to experiment depending

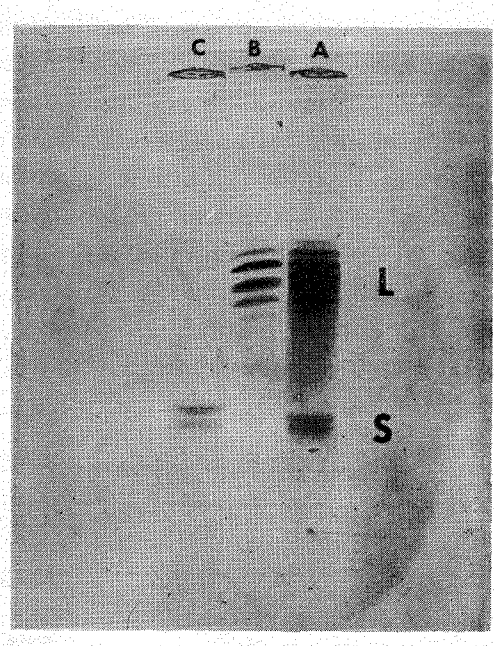

Fig. 1. Isoelectric focusing of *N. tabacum* Fraction 1 protein in 8 M urea. A. Fraction 1 protein; B. isolated large subunit; C. isolated small subunit. pH 10 at top: pH 3 at bottom.

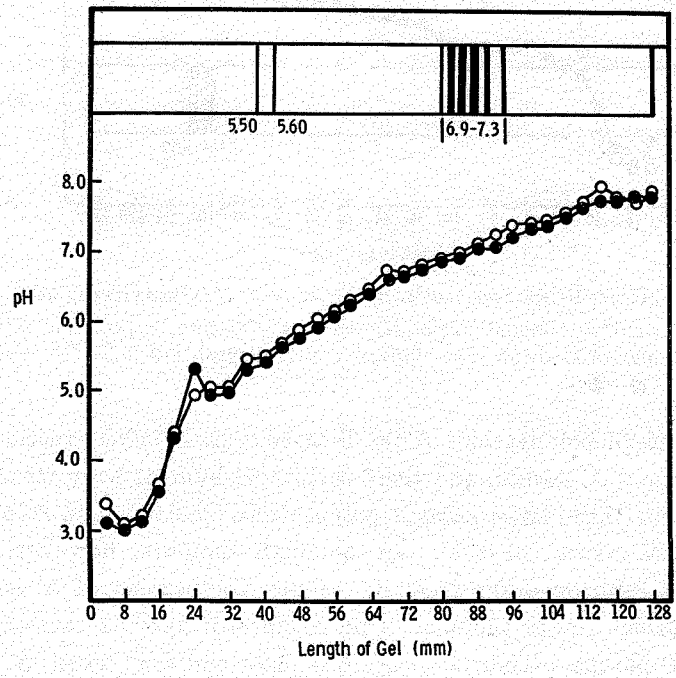

Fig. 2. pH gradients and isoelectric points of *N. tabacum* Fraction 1 protein in 8 M urea (pH 3-10). ●—● and ○—○ represent two different experiments.

on the degree of oxidation of the thiol groups. The P^Is of the two polypeptides from an isolated small subunit (Fig. 3) appears to be higher than that of an intact small subunit (Fig. 2). These values can be considered valid due to the consistency of results even when tested under two different pH ranges (Fig. 3).

Fig. 4 clearly illustrates that the pattern of polypeptides in the large subunit is greatly simplified by *S*-carboxymethylation. After *S*-carboxymethylation only three equally stained polypeptides are present in the large subunit, whereas the polypeptides of the small subunit still remain at two. This pattern is reproducible and not affected by protein concentration. *S*-carboxymethylation also shifts the P^I values. This shift in P^I is much greater for the large subunit than for the small subunit (Fig. 2-4).

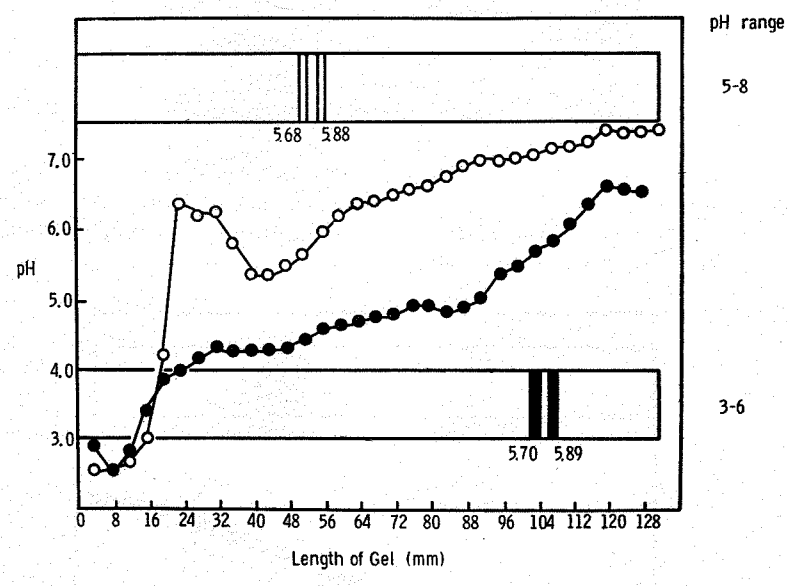

Fig. 3. pH gradients and isoelectric points of *S*-carboxymethylated small subunit isolated from *N. tabacum* Fraction 1 protein. P^Is are measured from two different pH ranges, 5-8 (○—○) and 4-6 (●—●).

The P^Is of all polypeptides of the *S*-carboxymethylated Fraction 1 proteins from *N. tabacum*, *N. glauca*, and their reciprocal hybrids were measured. The P^I of one of the three large subunit polypeptides (pl 6.00) of *N. tabacum* differs from that of *N. glauca* (pl 6.15). In the small subunits, however, both polypeptides of *N. tabacum* differ from the single polypeptide of *N. glauca*. The three polypeptides (large subunit) of the reciprocal hybrids correspond to that of the female parent. Their P^Is are 6.10, 6.05, and 6.00 when *N. tabacum* is the female parent whereas the P^Is are 6.15, 6.10, and 6.05 when *N. glauca* is the female parent. The reciprocal hybrids all have three polypeptides in their

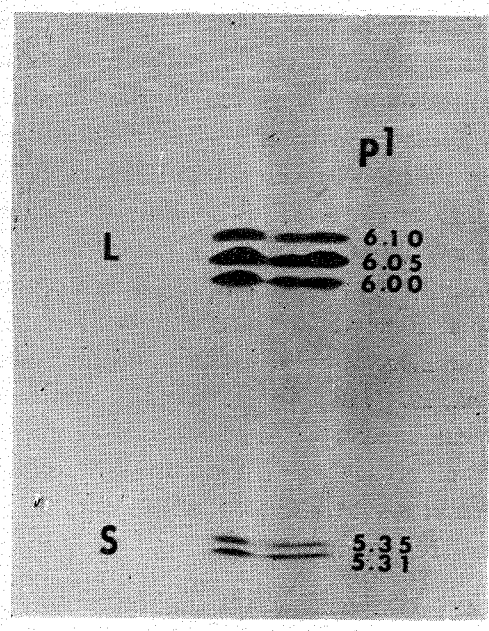

Fig. 4. Isoelectric points of *S*-carboxymethylated large and small subunits of *N. tabacum* Fraction 1 protein.

small subunits, two from *N. tabacum* (pI 5.35 and 5.31) and one from *N. glauca* (P^I, 5.30).

Conclusion and Discussion

The formation of disulphide bonds of the large subunits seems to be at least one of the reasons for the complexity of banding patterns of polypeptides of tobacco Fraction 1 protein. *S*-carboxymethylation has effectively prevented oxidation of the thiol groups and thereby reduced the bands from many to three. Whether the remaining polypeptide components represent different polypeptide chains or modification of a single polypeptide chain is still unknown. The arginine-lysine composition (Kawashima *et al.*, 1971) and the tryptic peptides analysis (Kung *et al.*, 1974) of *N. tabacum* Fraction 1 protein offered no clue of the origin of these bands. It is possible that multiple molecular forms of similar size may exist in the large subunit. This could be due to post-translational modification such as deamidation of glutaminyl- or asparaginyl-residues (Robinson and Rudd, 1974). This possibility is currently under investigation.

The introduction of carboxymethyl groups by *S*-carboxymethylation, increases the negative charge of this protein. Thus, the P^I of each polypeptide has shifted toward the acidic range. The greater shift of P^I by the large subunit is consistent with the findings that the majority of the thiol groups

resides in the large subunit (Kawashima and Wildman, 1970).

The P^I of the large subunits differs between *N. tabacum* and *N. glauca*. This is clearly a reflection of differences in their amino acid composition. The wide range of differences in the P^I of the small subunits between these two species is also consistent with the marked difference in their amino acid composition (Kawashima *et al.*, 1971). The difference between the two polypeptides in the small subunits of *N. tabacum* has been demonstrated by finger printing technique (Kung *et al.*, 1974) and amino acid sequencing (Gibbons *et al.*, 1976). The study of P^I of the reciprocal hybrids of *N. tabacum*×*N. glauca* not only confirm the mode of inheritance of the subunits as previously reported (Sakano *et al.*, 1974), but also reveals new information that the single polypeptide of the small subunit of *N. glauca* is different from both of those in *N. tabacum*. This difference was not resolved when it was analyzed in a 5–8 pH range (Sakano *et al.*, 1974). The availability of a more refined pH range of 5–7 made this resolution possible.

Acknowledgment

A grant from Baltimore Federated Garden Club and the technical assistance of Mr. R. Bufano are deeply appreciated.

Literature Cited

CHAN, P.H., K. SAKANO, S. SINGH and S.G. WILDMAN. 1972. Crystalline Fraction 1 protein: Preparation in large yield. Science **176**: 1145-1146.

GIBBONS, G.C., S. STROBACK, B. HASLETT and D. BOULTER. 1976. The N-terminal amino acid sequence of the small subunit of RuDP carboxylase from *Nicotiana tabacum*. Experientia **31**: 1040-1041.

KAWASHIMA, N. and S.G. WILDMAN. 1970. Fraction 1 protein. Ann. Rev. Plant Physiol. **21**: 325-358.

KAWASHIMA, N., S.Y. KWOK and S.G. WILDMAN. 1971. Studies of Fraction 1 protein III. Comparison of the primary structure of the large and small subunits obtained from five species of *Nicotiana*. Biochim. Biophys. Acta **236**: 578-586.

KUNG, S.D. 1976. Tobacco Fraction 1 protein: a unique genetic marker. Science **191**: 429-435.

KUNG, S.D., K. SAKANO and S.G. WILDMAN. 1974. Multiple peptide composition of the large and small subunits of *Nicotiana tabacum* Fraction 1 protein ascertained by fingerprinting and electrofocusing. Biochim. Biophys Acta. **365**: 138-147.

KUNG, S.D., J.C. GRAY, S.G. WILDMAN and P.S. CARLSON. 1975. Polypeptide composition of Fraction 1 protein from parasexual hybrid plants in the Genus *Nicotiana*. Science **187**: 353-355.

MARSHO, T.V. and S.D. KUNG. 1976. Oxygenase properties of crystallized Fraction 1 protein from tobacco. Arch. Biochem. Biophys. **173**: 341-346.

ROBINSON, A.B. and C.J. BUDD 1974. Deamidation of glutaminyl and asparaginyl residues in peptides and proteins. Curr. Top. Cellul. Regul. **8**: 247-295.

ROBINSON, H.K. 1972. Comparison of different techniques for isoelectric focusing on polyacrylamide gel slabs using bacterial asparaginase. Anal. Biochem. **49**: 353-366.

RUTNER, A.C. and M.D. LANE. 1967. Nonidentical subunits of ribulose diphosphate

carboxylase. Biochem. Biophys. Res. Comm. **28**: 531-537.

SAKANO, K., S. D. KUNG and S. G. WILDMAN. 1974. Identification of several chloroplast DNA genes which code for the large subunit of *Nicotiana* Fraction 1 protein. Mole. gen. Genet. **130**: 91-97.

WILDMAN, S. G., K. CHEN, J. C. GRAY, S. D. KUNG, P. KWANYUEN and K. SAKANO. 1975. Evolution of Ferredoxin and Fraction 1 protein in the Genus *Nicotiana*. *In* Genetics and Biogenesis of Mitochondria and Chloroplasts. (C. W. Birky, Jr., P. S. Perlman and T. J. Byers, eds.), pp. 309-329. Ohio State Univ. Press, Columbus.

Fraction 1 Protein 的等電點

孔 憲 鐸

馬利蘭大學巴特摩爾分校生物學系

Fraction 1 Protein 是自然界存量最豐富的蛋白質。其功用不祇是在光合作用中很重要，而在光呼吸作用中亦不能缺少。最近由等電點分析結果顯示其構造至爲複雜。本文略述 Fraction 1 Protein 大小 Subunit 的等電點的分析與測定之結果