Origin of Sequence Heterogeneity of the Small Subunit of Fraction 1 Protein from *Nicotiana tabacum*

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The partial amino acid sequences of the small subunit of Fraction 1 protein from *N. sylvestris*, *N. tomentosiformis* and *N. tabacum* were determined. The sequence of *N. sylvestris* is NH_2-Gln-Val-Trp-Pro-Pro-Ile-Asn-----Tyr-COOH. In the sequence up to the 7th amino acid and C-terminus, differences were only found at the 6th position in the three species, where *N. sylvestris* and *N. tomentosiformis* show Ile and Tyr, respectively. *N. tabacum* show both Ile and Tyr in almost equal amount at this position. These results confirmed a previous hypothesis that *N. tabacum* had been evolved through the hybridization of *N. sylvestris* and *N. tomentosiformis*.

Fraction 1 protein consists of two kinds of subunits which differ in size (1). Each subunit has been known to be homogeneous by gel electrophoresis, gel chromatography and analytical centrifugation (2). Recently, however, Kung *et al.* found that the small subunit from *Nicotiana tabacum*, a commercial tobacco, is composed of two kinds of polypeptides which are same in molecular weight but different in isoelectric point (3). Gray *et al.* confirmed the results and further proposed that the two kinds of small subunits from *N. tabacum* had been derived from its putative parent species, *N. sylvestris* and *N. tomentosiformis* (4). Despite of the interesting results reported by those researchers, it has still remained ambiguous as to whether the difference in isoelectric points is a direct reflection of the difference in DNA structure coding for the primary structure of the small subunits. To clarify the problem, partial amino acid sequences of the small subunits from *N. sylvestris*, *N. tomentosiformis* and *N. tabacum* were determined and compared. The present report deals with

Tab. 1 Edman degradation of the small subunit of N. tabacum

Step	Amino acid	Yield of PTH-amino acid (μ moles)[a]	Identification of PTH-amino acid
1	Gln	0.44	T.L.C.[b]
2	Val	0.44	T.L.C., G.C.[c]
3	Trp	0.40	T.L.C.
4	Pro	0.26	T.L.C., G.C.
5	Pro	0.18	T.L.C., G.C.
6	Ile, Tyr*	0.17	T.L.C.,G.C., A.A.A.[d]
7	Asn	0.16	T.L.C.

[a]The amount of PTH-amino acid was determined from the ultra violet absorption at 269 nm. [b]Thin layer chromatography (7). [c]Gas chromatography (8). [d]Amino acid analysis (9). * The molar ratio of Ile/Tyr was detected to be 1.1 by amino acid analysis.

similarities and differences of the partial amino acid sequences among the three species.

Materials and Methods

Fraction 1 protein was purified from N. sylvestris, N. tomentosiformis and N. tabacum by a crystallization method previously reported (5). Carboxymethylation of Fraction 1 protein was performed according to the procedure of Kung et al. (3) but on a 20 times larger scale. The carboxymethlated Fraction 1 protein was seperated into large and small subunits by Sephadex G-200 column chromatography in alkali (5). The small subunit was precipitated by adjusting the solution to pH 5.5 with 1 N HCl. The precipitate was dissolved in 30 % acetic acid and further purified by Sephadex G-75 column equilibrated with 5 % acetic acid to remove the contaminating large subunit. The pure small subunit was lyophilized and kept in cold until use.

N-terminal amino acid sequence was determined by both automated and manual Edman degradation method. Automated Edman degradation was performed on a JEOL JAS 47K sequence analyzer. Manual Edman degradation was performed according to the method of Edman (6) as follows; 5 mg of the small subunit in 1 ml of 5 % N,N-dimethylallylamine-trifluoroacetic acid buffer containing 60 % pyridine (pH 9.5) was coupled with 10 μl of phenylisothiocyanate at 50°C for 30 min. For step 4 and 5 where Pro was expected, the peptide was coupled with phenylisothiocyanate at 54°C for 40 min. The yield and identification method of PTH-amino acid from the small subunit of N. tabacum are shown in Tab. 1. The similar results were obtained in other species.

N. sylvestris	Gln-Val-Trp-Pro-Pro-Ile-Asn----------Tyr.
N. tabacum	Gln-Val-Trp-Pro-Pro-(Ile/Tyr)-Asn----------Tyr.
N. tomentosiformis	Gln-Val-Trp-Pro-Pro-Tyr-Asn----------Tyr.

Fig. 1 Comparison of partial amino acid sequence of the small subunit of Fraction 1 protein among the three species.

The C-terminal amino acid was determined enzymatically with carboxypeptidase A (10).

Results and Discussions

Gray et al. showed that the isoelectric point of the small subunit of N. sylvestris was slightly different from that of N. tomentosiformis (4). Previously, we suggested that the difference might be due to difference in sequence, because 4 out of 25 chymotryptic peptides derived from the small subunit were different in the small subunit derived from both species (5). The present experiments confirmed this hypothesis. As shown in Fig. 1, a difference in sequence was found at the 6th position where N. sylvestris contained Ile whereas N. tomentosiformis contained Tyr. On the other hand, N. tabacum showed a sequence heterogeneity at the 6th residue where both Ile and Tyr were detected in almost equal amount. From the present result as well as the previous results on chymotryptic peptides and isoelectric focusing, we concluded that N. tabacum contains small subunits with two different polypeptide sequences. Furthermore, the present result confirmed the hypothesis that interspecific hybridization of N. sylvestris and N. tomentosiformis was involved in the evolution of N. tabacum.

An alternative interpretation of the sequence heterogeneity of N. tabacum would be that this is due to intraspecific variation. However, the possibility could be ruled out in this case, since the small subunit prepared from single individual of N. tabacum was always resolved into two bands by isoelectric focusing.

With respect to the N-terminal amino acid of the small subunit of N. tabacum, Gibbons et al. favoured placing Met before the N-terminal Gln (11), because they occasionally detected Met in addition to Gln at the first step of the Edman degradation. However, we have never detected Met in more than ten experiments using different batches including different varieties. Sometimes we detected small amount of an unknown compound in addition to Gln, but it was not Met. To eliminate the possibility that N-terminal Met might be split off during the purification of the small subunit, we subjected Fraction 1 protein crystals which were fully active for RuDP carboxylase directly to Edman degradation. Even in this case, only Gln, but not Met, was detected at the first step of the degradation. Consequently, we are convinced that the N-terminal amino acid of the small subunit of Fraction 1 protein from N. tabacum is Gln.

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