Communication

Polymorphism of a Photosystem I Subunit Caused by Alloploidy in Nicotiana

Junichi Obokata*, Kohki Mikami, Nobuaki Hayashida², and Masahiro Sugiiura

Research Center for Molecular Genetics, Hokkaido University, Sapporo 060, Japan (J.O., K.M.); Department of Botany, Faculty of Science, Hokkaido University, Sapporo 060, Japan (K.M.); and Center for Gene Research, Nagoya University, Nagoya 464, Japan (N.H., M.S.)

ABSTRACT

The photosystem I complex from Nicotiana tabacum, which has an alloploid genome, contains subunits of 17.5 and 18.5 kilodaltons whose N-terminal amino acid sequences are highly homologous. Comparative analysis of photosystem I subunits among N. tabacum and its ancestral plants, N. tomentosiformis and N. sylvestris, revealed that the 17.5 kilodalton subunit of N. tabacum derives from N. sylvestris, and the 18.5 kilodalton subunit from N. tomentosiformis.

The PSI complex in higher plants contains two large subunits of 82 kD and several small subunits of less than 20 kD (2, 3, 15–17, 25). The two large subunits are the products of chloroplast genes, psaA and psaB (4, 8, 9, 12, 19, 22), and characteristically bear P700 and electron acceptors A0, A1, and Fx (5). These two subunits are highly conserved over a wide range of plant species. In contrast to this, the number and molecular mass of the smaller subunits reported by different laboratories varies, raising the question whether these discrepancies result from the interspecies variation or from the differences in experimental procedures.

Tobacco is one of the most suitable plants for the analysis of photosystem proteins because its chloroplast genome is completely sequenced (22) and there are many mutants and interspecific hybrids (23). In this paper, we report a polymorphism in Nicotiana tabacum of a PSI low molecular mass subunit, the psaD gene product (6, 11, 14, 21). Comparative analysis of the PSI proteins among alloploid and pure line tobacco species revealed that this polymorphism results from alloploidy.

MATERIALS AND METHODS

Preparation of PSI

Nicotiana tabacum, Nicotiana sylvestris, and Nicotiana tomentosiformis were grown in a green house. Chloroplasts were prepared from leaves (17), and PSI was purified as previously described (16).

Determination of N-Terminal Amino Acid Sequence

PSI proteins were separated by LDS-PAGE (16), blotted onto PVDF membranes, and subsequently applied to a gas-phase protein sequenator, ABI model 470A, according to the method of Matsudaira (13).

RESULTS AND DISCUSSION

LDS-PAGE analysis showed that the PSI complex from Nicotiana tabacum contains proteins of 18.5 and 17.5 kD (Fig. 1). N-Terminal amino acid sequences determined for the first 30 residues of each protein showed striking homology. Within this area, the only difference between them lies in the respective presence or absence of three alanine residues (Fig. 1). These proteins are not encoded by the tobacco chloroplast genome, indicating that they are of nuclear origin. Judging from their N-terminal amino acid sequences, these proteins are homologous to the psaD gene product (6, 11, 14, 21) which is a putative ferredoxin-binding protein (26).

Why are these two proteins so homologous? We considered the following three alternative hypotheses to explain this homology. (a) The first hypothesis is that the proteins of 18.5 and 17.5 kD are derived from different ancestral genomes of N. tabacum. N. tabacum is alloploid, containing two types of nuclear genomes which are derived from different ancestral plants (10). (b) The second possibility is that these two proteins are products of a multigene family as is the case for apoproteins of the light-harvesting complex (20). (c) The third possibility is that they are partially homologous subunits and both are necessary for PSI function. This situation would be similar to the psaA and psaB proteins of PSI (4, 8), and D1 and D2 proteins of PSII (1, 7).

In this study, we examined the first hypothesis in more detail. N. tabacum, which has 24-paired chromosomes, is thought to be the progeny of a cross between N. sylvestris and N. tomentosiformis, both of which have 12-paired chromosomes (10). We compared the PSI proteins among these three species. As shown in Figure 2, LDS-PAGE analysis revealed

---

1 This work was supported in part by Grant-in-Aid for Scientific Research on Priority Areas of the Molecular Mechanism of Photo-reception (62621501, 63621001, 01621501) from the Ministry of Education, Science and Culture in Japan.

2 Present address: Laboratory of Gene Structure, The Institute of Physical and Chemical Research (Riken), Tsukuba Life Science Center, Koyadai 3-1-1, Tsukuba 305, Japan.

3 Abbreviations: LDS, lithium dodecyl sulfate; PVDF, polyvinylidene difluoride.
Figure 1. LDS-PAGE analysis of the PSI proteins from N. tabacum. Protein bands were detected by silver staining. N-Terminal amino acid sequences of the proteins of 18.5 and 17.5 kD are shown in the right hand of the lane.

Figure 2. A comparison of the PSI proteins among N. tomentosiformis, N. tabacum, and N. sylvestris. N. tabacum is allopl oid, having two types of genomes, one of which derives from N. tomentosiformis, and the other from N. sylvestris. Arrows indicate the 18.5 kD (left hand) and 17.5 kD (right hand) proteins.

N. tomentosiformis  AEEAAAATKEAEAP
N. tabacum 18.5 kD  AEEAAAATKEAEAPVQTPPQLDPNTPSXIFG
N. tabacum 17.5 kD  AEEAAA---TKEAEAPVQTPPQLDPNTPSXIFG
N. sylvestris  AEEAAA---TKEEA

Tomato psaD  AEEAPAA-TEKPAAPAGFTPQLDPNTPSF1FG

Figure 3. N-Terminal amino acid sequences determined for the PSI proteins from N. tomentosiformis, N. tabacum, and N. sylvestris, compared with the sequence of a psaD protein of tomato (6). Asterisks indicate the amino acid common between the 18.5 kD protein of N. tabacum and the psaD protein of tomato.

that the 18.5 kD protein of N. tabacum shares the same mobility with a PSI protein from N. tomentosiformis (the left arrow), and the 17.5 kD protein shares mobility with a protein from N. sylvestris (the right arrow). Furthermore, the N-terminal amino acid sequence of the protein from N. tomentosiformis is identical with that of the 18.5 kD protein of N. tabacum, and also the N-terminal sequence of the protein from N. sylvestris is identical with that of the 17.5 kD protein of N. tabacum (Fig. 3). These results indicate that the 18.5 kD protein of N. tabacum is derived from N. tomentosiformis, and the 17.5 kD protein is from N. sylvestris.

This finding indicates that insertion and/or deletion of the three alanine residues occurred during the evolution of this protein within the genus Nicotiana. Considering that photosystem proteins of chloroplast origin are highly conserved over a wide range of plant species (18), this variability in amino acid sequence even within the genus seems to be a characteristic of nuclear-encoded proteins. This implies that the difference in reported number and molecular mass of the PSI small subunits is probably due in many cases to the variability of these proteins among plant species, since most of the PSI small subunits are of nuclear origin (3, 18).

Since an allopl oid genome brings about the polymorphism of nuclear-encoded proteins as shown in this study, pure line plants would be preferable to allopl oid ones in the analysis of photosystem subunits.

ACKNOWLEDGMENTS

The authors thank Prof. S. Tanifuji for his encouragement during this study, and Dr. R. F. Whittier for critical reading of this manuscript. The seeds of tobacco were kind gifts from Japan Tobacco, Inc.

LITERATURE CITED

24. Deleted in proof