Formation of Large Thioredoxin *f* Accompanies Chloroplast Development in *Scenedesmus obliquus*

Petra Langlotz and Hartmut Follmann

Fachbereich Chemie der Philipps-Universität, Biochemie, Hans-Meerwein-Straße, D-3550 Marburg, Bundesrepublik Deutschland

Z. Naturforsch. **42c,** 1364–1366 (1987); received August 18, 1987

Algae, Chlorophyll Synthesis, Chloroplast Development, Light, *Scenedesmus obliquus*, Thioredoxins

Chloroplast-free mutant cells C-2A′ of the green algae *Scenedesmus obliquus* lack thioredoxin *f*, which functions in the light activation of chloroplast enzymes, but do contain the regular thioredoxins I and II. When dark-grown algae are transferred to light, thioredoxin *f* activity appears rapidly and increases in parallel with photosynthetic activities; however it precedes chlorophyll biosynthesis. The formation of thioredoxin *f* is inhibited by cycloheximide indicating that it occurs on the cytoplasmic ribosomes, in accord with the lack of thioredoxin genes on the chloroplast genomes.

Introduction

The functional differentiation of thioredoxins in plants is difficult to analyze because these SH proteins occur in multiple forms of different subcellular origin in both photosynthetic and non-photosynthetic tissues [1–3] and because they are interchangeable in the common enzyme activation assays *in vitro* [4]. Unicellular green algae appear to possess a simpler thioredoxin profile in that they have only one chloroplast thioredoxin *f* which is distinguished from the regular thioredoxins with cytoplasmic functions by its unusual size (M_r = 28,000), and is probably related to an analogous cyanobacterial protein [5, 6].

We have recently purified and characterized the four thioredoxins from *Scenedesmus obliquus* [6, 7]. Thioredoxin *f* was completely missing in dark-grown cells of the mutant, C-2A′, which lack chlorophyll and photosynthetic activities but develop a functional chloroplast after transfer to light [8, 9]. Since algal thioredoxin *f*, besides its function in the light stimulation of Calvin cycle enzymes, specifically activates the light- and thiol-dependent formation of chlorophyllide from protochlorophyllide [10, 11] it was of interest to follow in detail the appearance of this protein in greening *Scenedesmus* cultures.

Reprint requests to Prof. Dr. H. Follmann.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/87/1100–1364 $ 01.30/0

Experimental

Materials and Methods

Biochemicals and other reagents were purchased from Merck, Serva, or Boehringer, Mannheim. DEAE cellulose (DE 52) was a product of Whatman, and Sephadex G-50 of Pharmacia. NADP-Malate dehydrogenase and fructose-bisphosphatase from spinach leaves and ribonucleotide reductase from *Escherichia coli* were purified as described [1, 12, 13]. Protein was determined by the method of Lowry [14] and chlorophyll was determined spectrophotometrically in a methanol extract of 1–3 ml algal culture [15].

Algal cultures

Scenedesmus obliquus, strain D3, wild type and mutant C-2A′ cells were cultured as previously described [6, 8]. After three days of growth in the dark at 33 °C the mutant cells were transferred to light (12,000 lux) and the culture continued for 2–24 h at 28 °C. Inhibitors were added before the onset of illumination.

Thioredoxin purification and assay

Thioredoxins were extracted from the different *S. obliquus* cultures (20–25 ml packed cells) under identical conditions. Purification [6, 7] was carried up to the Sephadex G-50 chromatography step which permits complete separation of algal thioredoxin *f* and I + II; the latter mixture was not resolved further because of their identical activities [7]. Thioredoxin *f* activity was determined using spinach chloroplast fructose-bisphosphatase and *E. coli* ribonucleotide reductase as indicator enzymes [4, 6], and the thioredoxin I + II fraction was measured by its activation of spinach chloroplast NADP-malate dehydrogenase [7].

Results and Discussion

Parallel cultures of *Scenedesmus obliquus* mutant C-2A′ were grown for 72 h in the dark, and the algae were then illuminated for various periods of time. The large thioredoxin *f* (M_r = 28,000) and the regular size thioredoxin I + II (M_r = 12,000) present in the heat-stable protein fractions of these and of wild-type cells were separated by gel permeation chro-

matography on Sephadex G-50 columns [6]. Three different enzyme activation assays were used to ensure reliable activity measurements for both types of thioredoxin. The results are presented in Fig. 1 and Table I. Thioredoxin *f* activity is not detectable in dark-grown, yellow mutant cells but appears and increases rapidly in greening cultures; a maximum in thioredoxin *f* activity is reached 6 h after the onset of illumination. The final level is of the same order of magnitude as that found in wild-type algae (Table I). In contrast, the major thioredoxins I and II of *S. obliquus* are already present in dark-grown cells and increase only 1.7-fold in illuminated cultures. Both thioredoxin levels do not change further between 12 and 24 h of growth in the light.

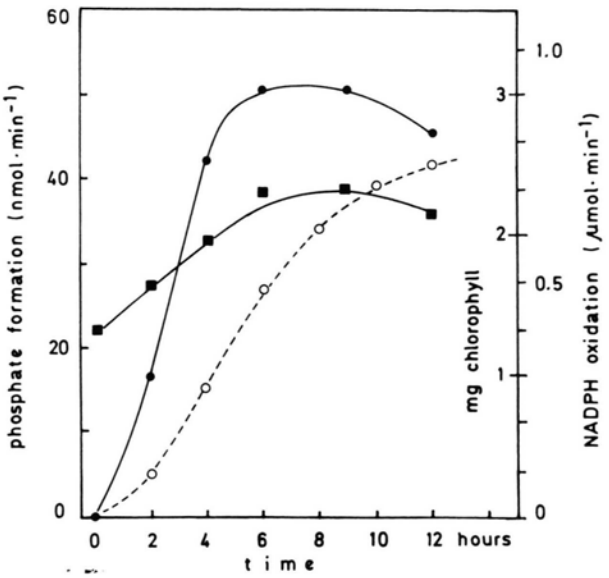

Fig. 1. Thioredoxin content in *Scenedesmus obliquus* mutant C-2A′ cells during light-induced greening and chloroplast development. Total thioredoxin activities per ml packed algae were measured as activation of chloroplast fructose-bisphosphatase (phosphate formation, left scale) by thioredoxin *f* and of NADP-malate dehydrogenase (NADPH oxidation, right scale) by thioredoxins I and II. ●: Thioredoxin *f*; ■: thioredoxins I + II; ○: chlorophyll formation (mg per ml packed cells).

The increase in thioredoxin *f* activity clearly precedes chlorophyll biosynthesis which is only complete after 24 h. However, thioredoxin *f* formation precisely parallels the onset of photosynthetic activities and formation of the apoproteins of the photosystem II complexes in the first 6 h of chloroplast biogenesis [8, 16]. Thus chloroplast thioredoxin *f* is available from the beginning for activation of Calvin cycle enzymes and of chlorophyll biosynthesis.

In a second experiment, thioredoxin biosynthesis in *Scenedesmus obliquus* was probed with inhibitors of chloroplastic and cytoplasmic protein synthesis (Table I). The addition of lincomycin had no significant effect on the thioredoxin content in C-2A′ and wild-type cell cultures. In contrast, 10 µg/ml cycloheximide in the growth medium strongly inhibited the appearance of thioredoxin *f* in the greening mutant. During 12 h of culture this inhibitor concentration had no effect on thioredoxin levels in wild-type cells (data not shown), presumably because thioredoxins have low turnover rates. Nevertheless it is obvious that chloroplast thioredoxin *f* is synthesized *de novo* on cytoplasmic ribosomes. This result is in accord with the lack of thioredoxin-coding sequences

Table I. Thioredoxin content of *Scenedesmus obliquus* wild-type and mutant cells under different growth conditions. Total thioredoxin activities per ml packed cells are expressed as in Fig. 1. Test enzymes: FbPase, fructose-bisphosphatase; RRase, ribonucleotide reductase; MDH, NADP-malate dehydrogenase.

Culture	Thioredoxin *f*		Thioredoxins I + II
	FbPase assay [nmol $P_i \cdot min^{-1}$]	RRase assay [nmol $CDP \cdot h^{-1}$]	MDH assay [µmol $NADPH \cdot min^{-1}$]
Wild-type cells	19.1	10.9	0.3
C-2A′ mutant cells			
dark	0	0	0.6
light (12 h)	32.7	14.4	0.7
light, 110 µg lincomycin/ml	27.4	17.9	0.7
light, 10 µg cycloheximide/ml	8.0	4.0	0.7

in the chloroplast genomes of tobacco and the liverwort *Marchantia polymorpha* [17–19].

Our present data confirm that the large protein *f* of *S. obliquus* is the specific, light-mediating thioredoxin in the green algae and is a multifunctional equivalent of the more diversified small thioredoxins found in leaves.

Acknowledgements

This study has been supported by Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 305 (Ökophysiologie). We thank Dr. H. Senger, Mrs. I. Koss, and the staff of Fachbereich Biologie (Botanik) der Universität Marburg for their cooperation.

[1] J. P. Jacquot, J. Vidal, P. Gadal, and P. Schürmann, FEBS Lett. **96,** 243–246 (1978).
[2] R. A. Wolosiuk, N. A. Crawford, B. C. Yee, and B. B. Buchanan, J. Biol. Chem. **254,** 1627–1632 (1979).
[3] A. Berstermann, K. Vogt, and H. Follmann, Eur. J. Biochem. **131,** 339–344 (1983).
[4] W. Wagner, H. Follmann, and A. Schmidt, Z. Naturforsch. **33c,** 517–520 (1978).
[5] M. M. Whittaker and F. K. Gleason, J. Biol. Chem. **259,** 14088–14093 (1984).
[6] P. Langlotz, W. Wagner, and H. Follmann, Z. Naturforsch. **41c,** 275–283 (1986).
[7] P. Langlotz, W. Wagner, and H. Follmann, Z. Naturforsch. **41c,** 979–987 (1986).
[8] H. Senger and N. I. Bishop, Plant Cell Physiol. **13,** 633–649 (1972).
[9] N. I. Bishop and H. Senger, Plant Cell Physiol. **13,** 937–953 (1972).
[10] T. Griffiths, Biochem. J. **152,** 623–635 (1975).
[11] K. Kotzabasis, Ph.D. thesis, Philipps-Universität Marburg (1987); K. Kotzabasis, H. Senger, P. Langlotz, and H. Follmann, unpublished data (1987).
[12] B. B. Buchanan, P. Schürmann, and P. K. Kalberer, J. Biol. Chem. **246,** 5952–5959 (1971).
[13] N. C. Brown, Z. N. Canellakis, B. Lundin, P. Reichard, and L. Thelander, Eur. J. Biochem. **9,** 561–573 (1969).
[14] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and F. J. Randall, J. Biol. Chem. **193,** 265–275 (1951).
[15] G. MacKinney, J. Biol. Chem. **140,** 315–322 (1941).
[16] K. Humbeck and N. I. Bishop, Plant Cell Physiol. **27,** 1407–1417 (1986).
[17] K. Shinozaki *et al.*, EMBO J. **5,** 2043–2049 (1986).
[18] K. Ohyama *et al.*, Nature **322,** 572–574 (1986).
[19] We have searched the published genome sequences [17, 18] and found no region coding for the conserved active site peptide sequence Cys–Gly–Pro–Cys–Lys or -Arg present in all thioredoxins. We thank these authors for making the complete sequences available.