

REDOX REGULATION OF PLASTID METABOLISM BY THIOREDOXIN IN THE MARINE DIATOM *PHAEODACTYLUM TRICORNUTUM*

Yusuke Matsuda¹, Sae Kikutani¹, Satoshi Hara², Toru Hisabori² & Peter G. Kroth³

¹Research Center for Environmental Bioscience, Department of Bioscience, Kwansai Gakuin University

²Chemical Resources Laboratory, Tokyo Institute of Technology

³Fachbereich Biologie, Universität Konstanz, Konstanz

Thioredoxin (Trx), a major regulator of photosynthetic fixations of inorganic carbon and nitrogen in plant chloroplasts, was thought to play little role in controlling the Calvin cycle in marine diatoms. Nevertheless diatoms possess a set of subtypes of chloroplastic Trxs. In the present study we examined influences of redox state and the involvement of Trxs on activities of two pyrenoidal carbonic anhydrases, PtCA1 and PtCA2, in the marine diatom *Phaeodactylum tricornutum* which are presumably a critical part of CO₂-concentrating mechanism (CCM). The recombinant mature PtCA1 and 2 (mPtCA1 and 2) completely lost their enzymatic activity when oxidized by 50 μM CuCl₂ whereas DTT activated CAs in a concentration-dependent manner. The maximum activity of mPtCAs in the presence of 6 mM reduced DTT were significantly stimulated by the addition of 10 μM Trxs from *Arabidopsis thaliana* (AtTrx-f2 and -m2) and 5 μM of Trxs from *P. tricornutum* (PtTrxF and M). Kinetic analyses of mPtCA activation by Trxs in the presence of 6 mM DTT revealed that the maximum mPtCA1 activity was enhanced about 3 fold in the presence of Trx whereas mPtCA2 was only weakly activated by Trxs, and that half saturation concentration of Trxs were significantly lower in PtTrxs compared to AtTrxs. Site-directed mutagenesis of potential disulfide-forming cysteines in mPtCA1 and mPtCA2 resulted in a lack of oxidative inactivation of both mPtCAs. These results reveal the first direct evidence of a target of plastidic Trxs in diatoms, indicating that Trxs may participate in the redox control of inorganic-carbon flow in the pyrenoid, a focal point of the CO₂-concentrating mechanism.