

Determination of NAD(H) and NADP(H)

- 1) The frozen samples (0.4 g) were extracted with ethanol-water (1:1, v:v) containing either 0.1 M KOH (for NADH and NADPH) or 0.1 M HCl (for NAD^+ and NADP^+).
- 2) Centrifugation (20,000 g) for 20 min. at 4°C twice. The obtained supernatants were used for the enzymatic cycling method according to Tamoi et al. (2005).
- 3) The NADP^+ and NADPH contents were determined in a 990 μl of reaction medium containing 100 mM HEPES-KOH (pH 8.0), 0.5 mM EDTA, 0.42 mM 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT), 2.5 mM glucose-6-phosphate, 1.66 mM phenazine ethosulphate (PES), and 10 μl of an extract or a standard solution (25, 50, and 100 μM NADP^+ or NADPH) in a final volume of 1 ml. For the determination of NAD^+ or NADH, the reaction medium was the same except that 96 % ethanol (0.1 ml) was substituted for glucose-6-phosphate. Standard solutions for the determination of NAD^+ or NADH were prepared as follows: 25, 50, and 100 μM NAD^+ , and 12.5, 25, and 50 μM NADH.
- 4) The reaction was initiated by adding 0.7 units of glucose-6-phosphate dehydrogenase for the NADP^+ and NADPH assays or 0.7 units of alcohol dehydrogenase for the NAD^+ and NADH assays at 37°C.
- 5) The rate of thiazolyl blue reduction was recorded by measuring the change of absorbance at 570 nm.
- 6) The amounts of NADP^+ , NADPH, NAD^+ and NADH in the extracts were calculated from the standard curve using the absorbances of respective standard solutions.

Tamoi, M., Miyazaki, T., Fukamizo, T., and Shigeoka, S. (2005) The Calvin cycle in cyanobacteria is regulated by CP12 via the NAD(H)/NADP(H) ratio under light/dark conditions. *Plant J.* **42**, 504-513.

Determination of ATP content

- 1) The frozen leaf samples (200 mg) were homogenized with 1 ml of 5 % trichloroacetic acid and neutralized with 5N KOH (approx. 37.5 μl).
- 2) After centrifugation (20,000 g) for 20 min. at 4°C (x2) the supernatants were used for the luciferin-luciferase reaction.
- 3) ATP was quantified using the LL-100-1 ATP assay system (TOYO INK, Tokyo, Japan). One hundred microliters of the extract was mixed with 100 μl of reaction mixture.
- 4) Chemiluminescence was detected using a luminometer, AB-2200-R (ATTO, Tokyo, Japan).

- 5) The amounts of ATP in the extracts were calculated from the standard curve using the chemiluminescences of respective standard solutions (12.5, 25, 50, and 100 nM ATP).