S14-011

Fast and slow recovery processes of photosynthetic systems during rewetting in a terrestrial cyanobacterium, *Nostoc Commune*

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Keywords: Drought stress, Energy transfer, Nostoc commune, Terrestrial cyanobacterium, Water stress.

Introduction

Nostoc commune is a well-known, terrestrial, and highly drought-tolerant cyanobacterium. It covers and binds soil, and under wet conditions, it can perform both photosynthetic CO_2 fixation and nitrogen fixation. Therefore, *N. commune* is thought to participate in protection against soil erosion and in enrichment of soil by producing organic matter in dry areas. Although *N. commune* occupies an important ecological position in some areas on earth, as mentioned above, little is known on physiological changes during rewetting and drying processes of this cyanobacterium. During rewetting of the colony of *N. commune*, respiration recovered quickly (within less than 30 min), but the recovery of photosynthesis was slower, and nitrogen fixation started much later (Scherer et al. 1984). However, more detailed information on the recovery processes of photosynthesis was missing.

In this work, we measured recovery processes of induction of millisecond delayed light emission (DLE) and light state1-state 2 transition during rewetting of *N. commune* colonies. Relationship among the amount of water absorbed, time after initiation of rewetting, and the recovery of various partial reactions of photosynthesis was summarized. In cyanobacteria, the maximum fluorescence level obtained with the first saturating pulse of light after dark incubation of the cells (Fm) is usually smaller than the maximum fluorescence levels recorded with the saturating pulses applied during illumination of the cells with photosynthetically active radiation (Fm'). The increase in the maximum level of fluorescence during illumination of the actinic light (Fm'-Fm) can be attributed to state 2 to state 1 transition (Fork and Satoh 1983, Satoh and Fork 1983a, b).

Materials and Methods

Colonies of *N. commune* were collected in and around the Harima Science Garden City Campus of Himeji Inst. Tech., Hyogo Prefecture, Japan. If not otherwise mentioned, the colonies were dried for more than one week at 25°C under room light. Dry colonies were cut into small pieces using a blender with little damage to the individual cells, and the fragmented colonies were used in some experiments.

Chlorophyll (Chl) fluorescence was measured using a PAM 101-3 Chl fluorometer (Walz, Germany) as mentioned by Yamane et al. (1997). Fluorescence spectra at 77 K were obtained using a laboratory-constructed spectrophotometer as reported by Yamane et al. (1997). The excitation light from a 12 V, 100 W halogen lamp was passed through a Corning 4-96 filter. Time courses of DLE were measured using a laboratory-constructed instrument as reported by Satoh and Katoh (1983).

Enough amounts of water were added to the dry colonies, and Chl fluorescence and DLE were measured after incubation for various periods shown in the figures.

Results and Discussion

Our recent results (Nishio et al. 2001) and those presented in this report were summarized in Table 1. It shows that 1) with absorption of water, weights of colonies of *N. commune* increased in three phases; half-increase times of them were about 1 min, 2 h, and 9h. 2) Fluorescence intensities of phycobiliproteins and Photosystem (PS) I complexes recovered largely in 1 min, suggesting that their structure restored to functional forms within this period of time. 3) Recovery of energy transfer from allophycocyanin to anchor protein occurred within 1 min, but that from anchor protein to PSII took place quite slowly. 4) The PSI activity and cyclic electron flow around PSI simultaneously recovered within 2 min, while the recovery of the PSII activity had a time lag of about 5 min. After this time lag, the activity recovered in two phases, half times of which were about 20 min and 2 h. 5) Photosynthetic CO_2 fixation was restored almost in parallel with the first phase of the recovery of the PSII activity. 6) When there was enough water, the amount of absorbed water reached more than 20 times of the initial dry weight of the cyanobacterial colony. However, it was found that the amount of water, which is about twice of the initial dry weight of *N. commune* colonies, was enough for the recovery and maintenance of the PSII activity.

Recovery of delayed light emission --Time courses of DLE were measured in *N. commune* colonies after rewetting for various periods of time (Fig. 1). Up to 10 min, no remarkable change in the intensity of DLE was observed. The extent of the slow increase reached maximum after 1 h, but full recovery of the induction required more than 24 h of rewetting. The initial instant rise of DLE was attributed to the charge separation reaction of PSI and PSII, and the following fast and slow increases were to electric field and ΔpH formation across the thylakoid membranes (Satoh and Katoh 1983), although signals due to scattered light contribute to the instant rise as well. In cyanobacteria, state 2 to state 1 transition also contributes to the slow increase in DLE (Satoh and Fork 1983b). The dip or inflection point between the first and second peak is due to regulation of electron flow at a site on the reducing side of PSI (Satoh and Katoh 1983). That is, the electron flow on this site is restricted in a dark-adapted state, but after illumination, electrons started to flow through this site fluently.

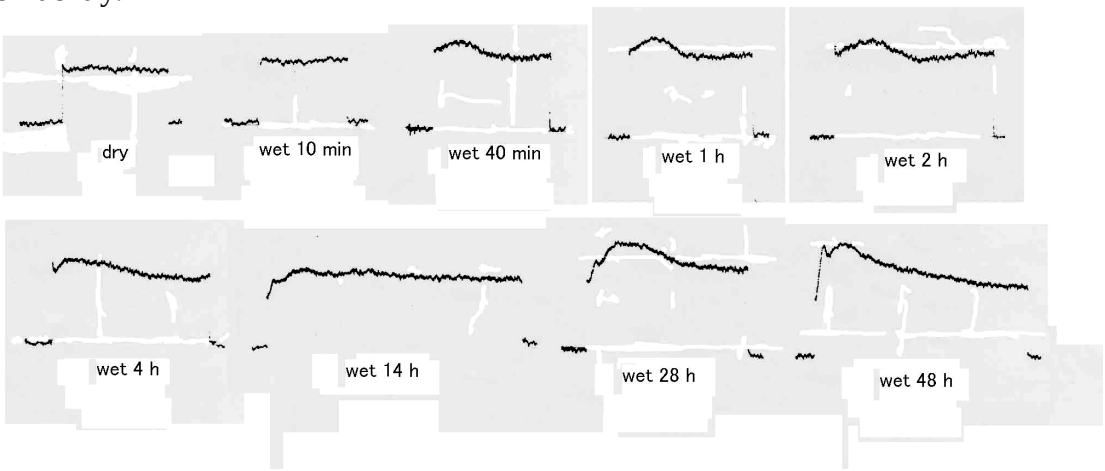

Fig. 1. Recovery of the time course of delayed light emission during rewetting of *N. commune* colonies.

Recovery of state 2 to state 1 transition—Fig. 2 shows increases in the (Fm'-Fm) value during rewetting of *N. commune* colonies. Although it changed from colony to colony, the

value kept increasing for 0-15 h of rewetting. Because these (Fm'-Fm) increases were observed even in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, data not shown), the values can be regarded as state 2 to state 1 transition as mentioned in "Introduction".

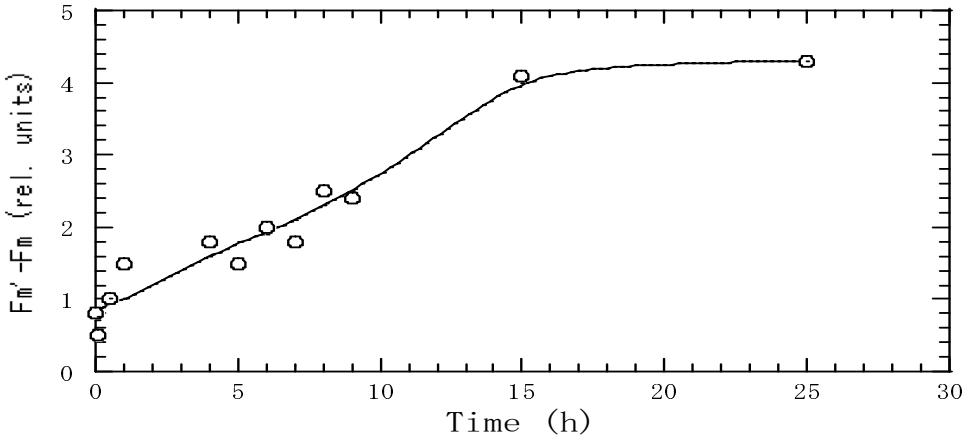

Fig. 2. Recovery of the Fm to Fm' increase during rewetting of *N. commune* colonies**.**

Relationship among recovery of fluorescence, restoration of activities, rewetting time, and amounts of water absorbed -- Table 1 summarizes the recovery of fluorescence and the photosynthetic activities during rewetting with enough amounts of water or by the incubation of *N. commune* colonies with various amounts of water for 24 h. The recovery of fluorescence from pigment-protein complexes can be regarded as the recovery of their structures, and water of more than one-fourth of the dry weight of the colony was required for restoration of phycobiliproteins and PSI complexes. Their fluorescence almost recovered within 1 min of rewetting and corresponded to the fast phase of water absorption. For PSII complexes, more than a half amount of water of the dry weight was needed. Fluorescence from PSII reaction center complexes increased in the time range of 1 min - 4 h, which corresponded only roughly to the middle phase of water absorption. Phycobiliproteins and PSI complexes fully recovered their fluorescence with a half amount of water of the dry weight of the colonies, but for PSII complexes, much larger amounts were required.

An interesting point is that the reaction center activity of PSII and photosynthesis recovered quite slowly as the recovery of PSII fluorescence, but for restoration and maintenance of the activities, relatively small amounts of water were needed. These characteristics seem to be quite important for *N. commune* because they might help the cyanobacterium ton perform photosynthesis for a long time during drying under sunlight.

Table 1. Recovery of fluorescence intensities and photosynthetic activities during rewetting with enough amounts of water or by incubation of dry *N. commune* colonies with various amounts of water for 24 h.

		Amounts of water required for recovery when incubated for 24 h (g/g dw)	Rewetting time required for recovery with enough amounts of water	Weight increase due to water absorption
Flu.	Phycobiliproteins	0.25→0.5	0→1 min	Fast phase
	PSI complex	0.25→0.5	0→1 min	
	PSII complex	0.5→10	1 min→4 h	Middle phase
Activity	PSI reaction center	0.25→1.0	0→2 min	Fast phase
	Cyclic electron flow	0.5→0.8	0→1 min	
	PSII reaction center	0.25→1.0	5 min→4 h	Middle phase
	Photosynthesis	0.25→10	5 min→1 h	
	Light state transition	0.82→10	20 min→15 h	Middle→slow phase
	Induction of DLE		2 h→48 h	

dw; dry weight. Flu; fluorescence.

Acknowledgements

This work was partly supported by grants to K.S. from the Ministry of Education, Science, Sports and Culture, Japan (No.11440238).

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