Effects of Poly (amino acid)s on Photophosphorylation in a Cyanobacterial Thylakoid

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Poly- L -glutamic acid accelerated by about 25% the cyclic photophosphorylation in the thylakoid of thermophilic cyanobacterium. A mixture of poly- L -glutamic acid and poly- L -ornithine of a ratio 2/1 (w/w) also enhanced by 20~30% the photophosphorylation. The acceleration behaviors by poly- L -glutamic acid alone are different from those by the mixture of acidic and basic poly (amino acid) s.

Key word : Cyanobacterium, Photophosphorylation, Poly (amino acid), Thermophile, Thylakoid membrane.

Basic poly (amino acid)s such as poly-L-lysine and poly-L-ornithine inhibited the cyclic photophosphorylation in the thylakoid of the thermophilic cyanobacterium, *Synechococcus* sp. by uncoupling the thylakoid membrane¹⁾. It was also suggested that these poly (amino acid)s block the function of cytochrome c-553, a soluble electron carrier between photosystem 2 and photosystem 1¹⁾. The degree of these effects was related to molecular weights of the polymers¹⁾. On the other hand, poly-L-histidine did not affect the activities of the thylakoid¹⁾. These results suggest that the charge and structure of poly (amino acid)s have a significant effect on the thylakoid activities. Recently, we have studied on effects of poly-L-glutamic acid and a mixture of poly-L-glutamic acid and poly-L-ornithine on the thylakoid activities. Some of the results will be shown below.

The cells of the thermophilic cyanobacterium, *Synechococcus* sp., were cultivated at 50°C as reported previously²⁾. The spheroplasts were prepared from the cells by the lysozyme-osmotic shock treatment and used for thylakoid preparation³⁾. Cyclic photophosphorylation mediated by phenazine methosulfate was measured for 1 min

Fig. 1 Effects of poly-L-glutamic acid on the thylakoid activities.

○ : photophosphorylation, □ : Δ pH, Δ : electron flow. Without added poly-L-glutamic acid, the activity of the photophosphorylation and electron flow were 580 μ mol ATP/mg Chl h and 304 μ eq/mg Chl h, respectively.

at 40°C and pH 7.0 as previously reported³⁾. Formation of H^+ -gradient (Δ pH) across the thylakoid membrane coupled with light-induced cyclic electron flow was measured as fluorescence decrease

Table 1 Effects of poly (amino acid)s on the thylakoid activities

Poly (amino acid) ($\mu\text{g/ml}$)		Activity (%)		
Poly-L-glutamic acid	Poly-L-ornithine	Photophosphorylation	ΔpH	Electron flow
0	20	23	76	112
40	20	123	119	107
0	50	6	53	129
100	50	130	128	101

*Activities are expressed as percent of the activity in the absence of poly (amino acid).

of 9-aminoacridine for 10 sec at 40°C and pH 7.5^{3, 4)}. Electron flow mediated by photosystem 1 was measured as oxygen uptake with a Clark-type electrode for 10 sec at 40°C , pH 7.5³⁾. In all the experiments, poly (amino acid) was added to the reaction mixture before the addition of spheroplasts. Poly-L-glutamic acid (Mw. 60,000) and poly-L-ornithine (Mw. 200,000) were obtained from Sigma.

Poly-L-glutamic acid accelerated by 26% at maximum the photophosphorylation in the thylakoids, but scarcely affected ΔpH formation and electron transport (Fig. 1). These results indicate that poly-L-glutamic acid does not affect ability of the thylakoid membranes to retain ΔpH in contrast to other poly (amino acid)s such as poly-L-ornithine and poly-L-lysine. There might be a possibility that poly-L-glutamic acid increases catalytic activity of coupling factors of the cyanobacterium. By the addition of Na-glutamate at concentrations of 50 and 100 $\mu\text{g/ml}$, the photophosphorylation rates became 100 and 86% of the control value of activity, respectively. So, the effect of poly-L-glutamic acid might not be ascribed only to its ionic property. Even if poly-L-glutamic acid may have some ionic effect on the substrates (ATP and inorganic phosphorus), 50 $\mu\text{g/ml}$ of the polymer is equivalent to only 0.35 mM as glutamic acid residue, which is lower than the substrate concentration (1 mM).

Poly-L-ornithine inhibited markedly the photophosphorylation (Table 1) by uncoupling the

thylakoid membranes¹⁾, while mixtures of poly-L-glutamic acid and poly-L-ornithine of a ratio of 2/1 (w/w) enhanced by 23~30% the photophosphorylation (Table 1). ΔpH was also increased a little by the poly (amino acid)s mixtures, though the electron transport was nearly affected. These results suggest that the photophosphorylation was accelerated by the increase of ΔpH which is a main driving force of the reaction. The reason of the ΔpH increase is not yet clear. As a possibility, the acidic and basic poly (amino acid)s form poly-ionic complexes, which suppress leak of H^+ through the thylakoid membrane by stabilizing the membrane. It might be probable that the thylakoid membrane became unstable and partially uncoupled during the preparation process, and then polyionic complexes repaired the membrane stability.

In summary, though the degree of acceleration is not so large in any cases, poly-L-glutamic acid alone and the mixture of acidic and basic poly (amino acid)s accelerate the photophosphorylation probably in different ways.

References

- 1) Ichimura T., Miyairi S., Matsui I. and K. Honda : *Membrane*, **13**, 117 (1988).
- 2) Yamaoka T., Satoh K. and S. Katoh : *Plant & Cell Physiol.*, **19**, 943 (1978).
- 3) Miyairi S. : *Membrane*, **9**, 37 (1984).
- 4) Pick U. and M. E. McCarty : *Methods in Enzymology* Vol. 69, ed. by Pietro A. S., Academic Press. New York pp. 538-546 (1980).

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