Vol. 90, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 12, 1979 Pages 110-116

LOCALIZATION OF POLYLYSINE INHIBITION IN A PHOTOSYSTEM I SUBCHLOROPLAST PARTICLE

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Received July 25,1979

Summary: Polylysine was found to increase the efficiency of electron donation from plastocyanin to $P700^+$ in highly resolved Photosystem I subchloroplast particles. This increase in efficiency is due to a decrease in the Km for plastocyanin in the presence of polylysine and is similar to results obtained with divalent cations. Cytochrome f photooxidation is observed in the presence of plastocyanin and divalent cations but not in the presence of plastocyanin and polylysine. The results indicate that the binding of polylysine to plastocyanin prevents the reduction of plastocyanin by cytochrome f.

Polycations such as histones and poly-L-lysine exhibit two contrasting effects in photosynthetic systems. With chloroplast membranes, polycations inhibit electron transport in the region of Photosystem I (1-3); the suggested site of inhibition is at the copper-containing protein, plastocyanin. Additional evidence in support of plastocyanin as the site of inhibition is the observation that polylysine inhibited the photooxidation of cytochrome f while P700 photooxidation was only slightly affected (4). Since plastocyanin is an electron carrier between cytochrome f and P700 (5) and the inhibition of Photosystem I activity was partially reversed by addition of plastocyanin (2), the suggested polycation inhibition of plastocyanin activity has become widely accepted.

A second effect of polylysine was demonstrated with chloroplast membranes from spinach and *Chlamydomonas reinhardii* from which

0006-291X/79/170110-07$01.00/0

plastocyanin was removed (6). With such membranes, low concentrations of polylysine increased the effectiveness of added plastocyanin as an electron carrier (6).

We have investigated the effect of polylysine on electron transfer reactions involving plastocyanin, cytochrome f and P700 in a highly resolved Photosystem I subchloroplast particle. Our results show that polylysine stimulates electron transfer from plastocyanin to P700 and inhibits electron transfer from cytochrome f to plastocyanin.

Materials and Methods. Photosystem I particles highly enriched in P700 were prepared according to the method of Lien and San Pietro (7). Spinach plastocyanin was prepared as described by Davis and San Pietro (8). Plastocyanin from *Anabaena variabilis* was prepared by the procedure of Lightbody and Krogmann (9). A partially purified preparation of spinach cytochrome f was prepared by a variation of the method of Nelson and Racker (12) as described by Davis and San Pietro (8). Polylysine (degree of polymerization = 15, M.W. = 3000) was obtained from Sigma Chemical Company.

P700 photooxidation and reduction were measured as described by Lien and San Pietro (7) using a Kok design dual-wavelength spectrophotometer. Cytochrome f photooxidation was measured as described by Davis and San Pietro (8) using the same instrument.

RESULTS

Lien and San Pietro (7) described recently the preparation of a highly resolved Photosystem I particle in which plastocyanin is an efficient electron donor to photooxidized P700 in the presence of divalent cations but not in their absence. The data in Figure 1 show that a similar increase in the efficiency of electron transfer from plastocyanin to photooxidized P700 is observed when divalent cations are replaced by polylysine. The polylysine concentration dependence for stimulation of electron transfer from plastocyanin to photooxidized P700 is shown in Figure 2. The effect saturates at about 4 μg/ml with half-maximal stimulation occurring near 1.5 μg/ml.

We have recently shown that the increase in the efficiency of plastocyanin as an electron donor to photooxidized P700 in the

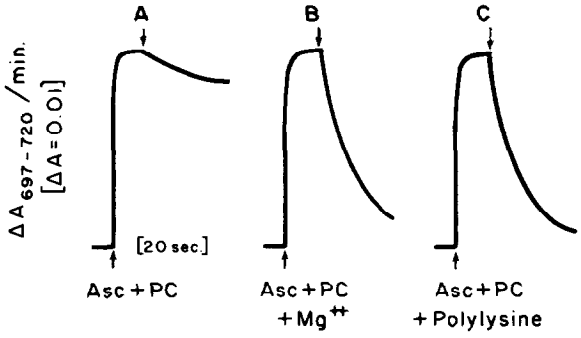

Figure 1. Effect of Polylysine and Divalent Cations on Electron Donation to P700. The basic reaction medium (A) consisted of 40 mM HEPES, pH 7.0, 2.5 mM Ascorbate, 0.5 μM plastocyanin, and Photosystem I particles added to a concentration of 0.26 μM P700. The reaction media for (B) and (C) were identical except for the addition of either 40 μg/ml polylysine or 20 mM $mgCl_2$. The upward turned arrows indicate the point at which the actinic light was turned on and the downward arrows the point at which illumination was ended.

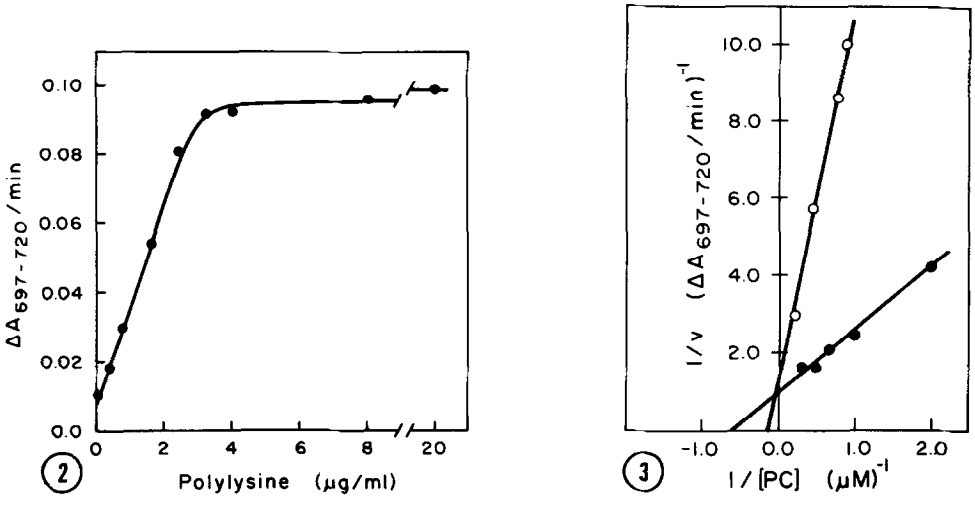

Figure 2. Concentration Dependence for Polylysine Stimulation of Electron Donation from Plastocyanin to P700. The reaction mixture was as in Figure 1A with the indicated concentration of polylysine added.

Figure 3. Double Reciprocal Plots for Electron Transfer from Plastocyanin to P700 in the Presence and Absence of Polylysine. Conditions were as in Figure 1A and 1C except that the plastocyanin concentration was varied. (Open circles - no polylysine, closed circles - 40 μg/ml polylysine present).

presence of divalent cations is due to a decrease in the apparent Km for plastocyanin (10). Divalent cations were found to decrease the apparent Km for spinach plastocyanin from 10 μM to 1.6 μM. It

was thus of interest to determine whether the increase in efficiency observed in the presence of polylysine was due also to a change in the Km for plastocyanin. Figure 3 shows double reciprocal plots of the rate of $P700^+$ reduction by spinach plastocyanin as a function of plastocyanin concentration in the presence and absence of polylysine. The Km's for plastocyanin were 10 μM and 1.7 μM in the absence and presence of polylysine respectively. Thus, polylysine appears to act in essentially the same manner as do divalent cations; i.e., by increasing the affinity of the Photosystem I particle for plastocyanin.

In contrast to electron transfer from plastocyanin to $P700^+$, which was stimulated by both divalent cations and polylysine, electron transfer from cytochrome f to plastocyanin occurs in the presence of divalent cations but not in the presence of polylysine (Figure 4). The site of inhibition by polylysine thus appears to

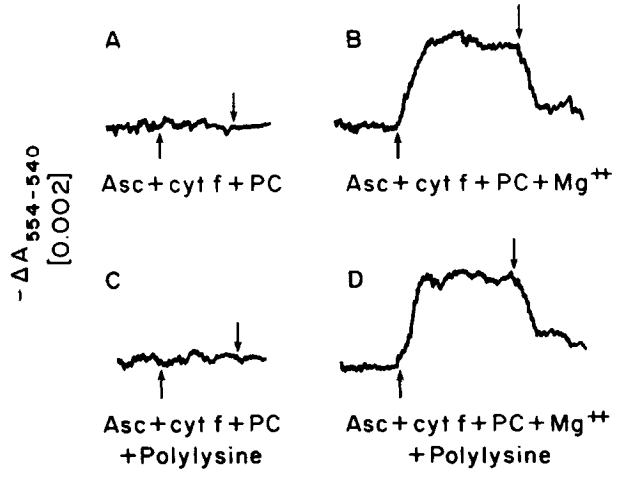

Figure 4. Effect of Polylysine and Divalent Cations on Cytochrome f Photooxidation. The basic reaction medium (A) consisted of 30 mM HEPES, pH 7.0, 5 mM ascorbate, 0.5 μM plastocyanin, 2 μM cytochrome f, and Photosystem I particles added to a concentration of 0.26 μM P700. Additions were as follows: (B) 20 mM $MgCl_2$, (C) 40 μg/ml polylysine, (D) 20 mM $MgCl_2$ + 40 μg/ml polylysine. The upward turned arrows indicate the point at which the actinic light was turned on and the downward arrows the point at which illumination was ended.

prevent the oxidation of cytochrome f by plastocyanin even though electron transfer from plastocyanin to $P700^+$ is stimulated (Fig. 1 and 2). In the presence of both polylysine and divalent cations, cytochrome f photooxidation was observable suggesting that divalent cations can displace polylysine from the site of inhibition.

Berg et al (11) observed that polylysine had no effect on photosynthetic membranes from the blue-green alga, *Anabaena variabilis*. In contrast to plastocyanin from spinach which is an acidic protein, plastocyanin from *Anabaena variabilis* is a basic protein (9). When tested against the Photosystem I particle used in these studies, the blue-green algal plastocyanin was found to be a more efficient electron donor to photooxidized P700 than was spinach plastocyanin (10). While polylysine increased the rate of reduction of photooxidized P700 by spinach plastocyanin, no stimulation was observed with the plastocyanin from *Anabaena variabilis* (Table I). Poly-

TABLE I

Comparison of Effect of Polylysine on Spinach and *Anabaena variabilis* Plastocyanin

	Rate of P700+ Reduction[1]	Extent of Cyt. f Photooxidation[2]
Spinach plastocyanin (0.5 μM)	0.004	0.000
+ 4 μg/ml polylysine	0.039	0.000
+ 20 mM $MgCl_2$	0.050	0.015
Anabaena variabilis plastocyanin (0.02 M)	0.016	0.012
+ 4 μg/ml polylysine	0.016	0.011
+ 20 mM $MgCl_2$	0.014	0.011

[1] $A_{797-720}$/min

[2] $A_{554-540}$

Conditions were as in Fig. 1A and 4A with additions as indicated.

lysine also had no effect on the extent of cytochrome f photooxidation in the presence of *Anabaena variabilis* plastocyanin.

DISCUSSION

Earlier studies of the effects of polycations on photosynthetic membranes led to the conclusion that the site of action was at plastocyanin (1-4, 6). Such studies did not, however, define whether the inhibition by polycations was on the reduction of plastocyanin by cytochrome f or on the oxidation of plastocyanin by $P700^+$. Using an *in vitro* system, we have shown that polylysine stimulates electron transfer from plastocyanin to $P700^+$ in a manner similar to the stimulation observed in the presence of divalent cations. The observation that cytochrome f photooxidation could be observed in the presence of divalent cations but not in the presence of polylysine strongly suggests that the inhibition by polylysine is due to prevention of electron transfer from cytochrome f to plastocyanin.

The effects of polylysine appear to depend on the charge characteristics of the plastocyanin used. When the acidic plastocyanin from spinach was replaced with the basic plastocyanin from *Anabaena variabilis*, no effect of polylysine was observed. Since all other components of the assay medium were the same, the polylysine binding site is most likely associated with spinach plastocyanin rather than the Photosystem I particle or cytochrome f. Since no polylysine effects were observed with the basic algal plastocyanin, negative charges on the spinach plastocyanin are implicated in the binding of polylysine.

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