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Research Article

Properties of The Docking Site of Photosystem 1 From Spinach

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Abstract: The bimolecular interaction between Plastocyanin and Photosystem I under steady-state illumination was studied. The dependence of the O_2 consumption rate on Plastocyanin concentration, electrostratic effect in the presence of mono- and divalent cations and pH-dependence were investigated. A mechanism of activating complexes formation between Plastocyanin and Photosystem I in frame of trimeric structure of Photosystem I is proposed.

Key Words: Photosystem I, Plastocyanin, electron transport, subunit II, surface charge density.

Ispanak Bitkisinden Fotosistem I'de Doking Yerinin Özellikleri

Özet: Sürekli aydınlatma durumunda fotosistem I ve plastosiyanin arasındaki bimoleküler karşılıklı etkileşme incelendi. O_2-nin tüketilme hızının plastosiyanin konsantrasyonuna, mono ve divalent katyonların elektrostatik etkilerine ve sistemin pH-sına bağımlılığı araştırıldı. Fotositem I'in üçlü yapı organizasyonu çerçevesinde, plastosiyanın ile fotosistem I arasında aktif kompleks oluşumunun tasarlanan düzeni sunuldu.

Anahtar Sözcükler: Fotosistem I, Plastosiyanin, altbiri 3, yüzey yük yoğunluğu.

Indroduction

Photosynthetic as well as respiratory electron transport chains involve two processes: (i) the intraprotein electron transfer between redox cofactors and; (ii) interprotein electron transfer involving water soluble proteins which can diffuse and bind to the reaction partner. The understanding of intraprotein electron transfer has been remarkably improved by knowledge of structure after the work of Deisenhofer and Michel (1) on purple bacteria and H. Witt et al. (2) on Photosystem I (PS I) from cyanobacteria. The interprotein interactions are now under active investigation.

The physiological partner in higher plants on the donor side of PSI is a water soluble copper protein-Plastocyanin (Pc). The structure and physico-chemical properties of Pc have also been studied in detail by Heahnel (3) and Sykes (4). However, the mechanism of activating complex formation between Pc and PS I this still quite controversial. Under the steady state (5, 6) and fast kinetic (7) conditions, the specific role of divalent cations as well as the pH effect have been shown on the cyanobacteria and green algae. The model of Pc's interaction with PS I proposed by H. Bottin and P. Mathis (8) is based on the assumption that there are two kinds of plastocyanin: one tightly bound to PS I and one free in lumenal space. In contrast, Hippler et al. (9) by cross-linking experiments, proposed that there is no evidence for electron trasnfer via the bound plastocyanin to PS I.

Materials and Method

Spinach leaves (*Spinacia oleracea* L.) were obtained from a local market. Plastocyanin was isolated by acetone treatment by the method described by A.A. Mutuskin (10) with purification on DEAE cellulose (DE-52, Whatman) using gradient of 50-400 mM KCl. Further hydrophobic chromatography on Toyoperl SW-65 allowed us to obtain purity near 1.2-1.3. Purity of plastocyanin was determined from absorption ratio at 597 nm and 278 nm. The concentration of protein was determined after oxidation with ferricyanide with an extinction coefficient of 4.9 mM at 597 mm (11).

Digitonin solubilised Photosystem I particles (D-144) were obtained from spinach chloroplasts as described by Boardman (12). Chlorophyll concentration was estimated by the method described by MacKinney (13). The ratio of chlorophyll a/b= 5.7. The content of P700 in the samples was calculated from the oxidized minus reduced difference spectra using an absorption coefficient of 64

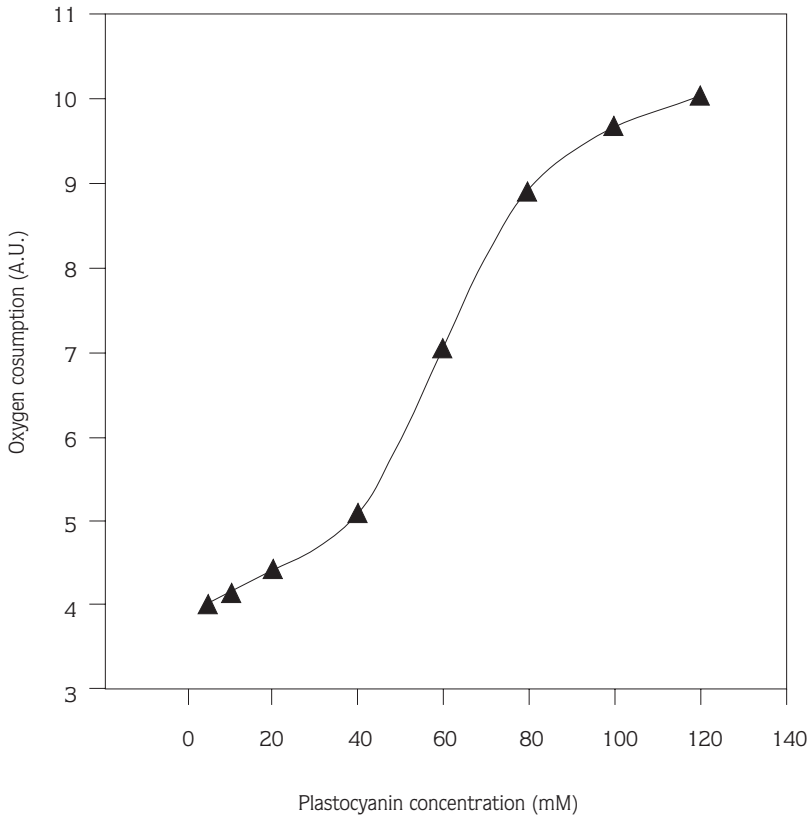

Figure 1. Dependence of oxygen consumption by methyl viologen from plastocyanin concentration. Incubation medium: 20 mM HEPES (pH 7.5), 0.1 mM methyl viologen, 1 mM sodium ascorbate, 0.1 mM diaminoduorene, 5 mM $MgCl_2$

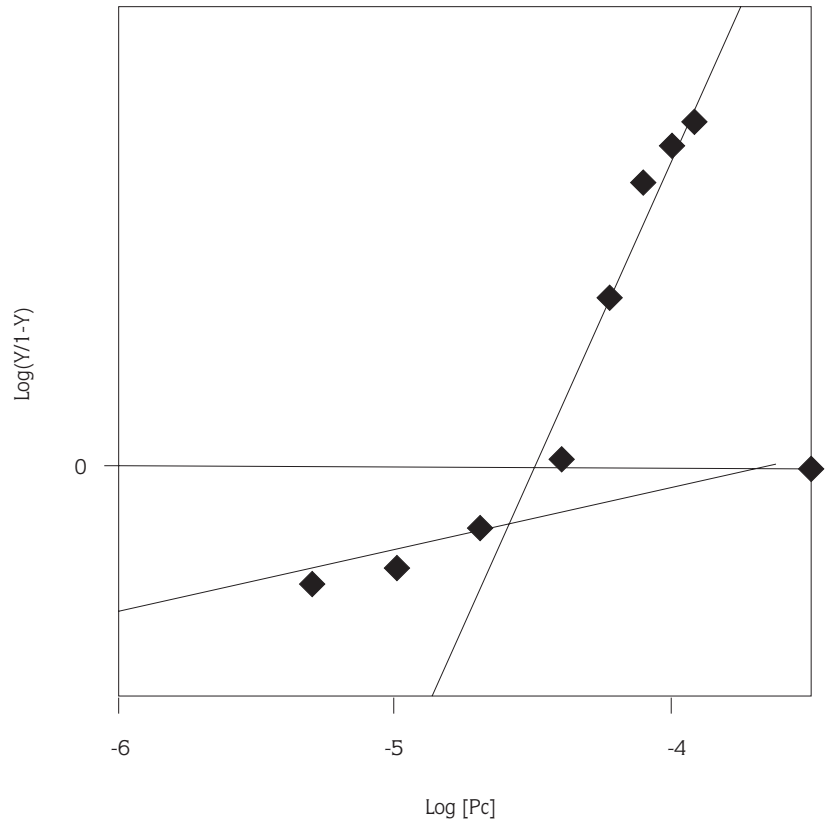

Figure 2. Hill plot for data in fig.1: the y/1-y values correspond to the ratios between the rate at given concentration and the rate at half-saturating concentration. The upper and lower parrallel lines correspond to strong and weak binding sites respectively.

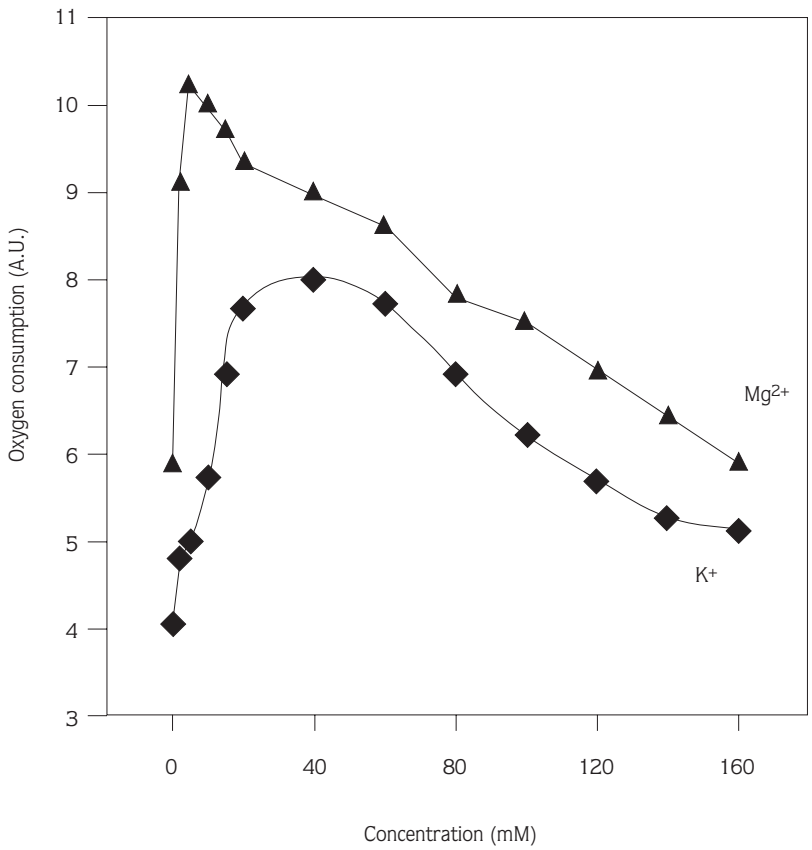

Figure 3. Dependence of oxygen consumption by methyl viologen from $MgCl_2$ and KCl concentration. Incubation medium: 20 mM HEPES (pH 7.5), 0.1 mM methyl viologen, 1 mM sodium ascorbate, 0.1 mM diaminoduorene, 10 μM Pc.

mM^{-1} * cm^{-1} determined by Hiayma and Ke (14). The assay was carried out by measuring the rate of O_2 consumption polarographically and 25°C in a Clark type oxygen electrode with detection using an OH-102 (Radelskis, Hungary) polarographic unit at saturating (800 $\mu E/m^2$ * s) white light intensity. Unless otherwise noted, the reaction mixture contained in final volume of 3 ml 20 mM HEPES buffer, pH=7.5, an amount of D-144 particles adjusted to the final Chl. concentration 10 μg/ml, 0.1 mM methyl viologen, 0.1 mM DAD, 1 mM Na-ascorbate, 5 mM $MgCl_2$ and 10 μM plastocyanin. In all experiments the minimal ratio of Pc/P700 was 100.

Results and Discussion

The dependence of oxygen consumption by methyl viologen is coupled with electron acceptance in PS I from Pc, which can be detected polorographically. When the observed rate constants were plotted as a function of protein concentration, a sigmoidal curve was obtained (Fig. 1). The data can be represented in the form of the Hill equation, as shown in Fig. 2. The value for the Hill coefficient calculated from this plot was 1.87, clearly indicating positive cooperativity. It is also possible to calculate values for the low-affinity and high-affinity binding constants from the cross-over points on the zero axis of lines with the slope drawn through the low and high protein concentration data. The values obtained were 120 μM and 19 μM, respectively.

We also analysed the electrostatic interaction between the Pc and PS I particles. The effect of varying concentration of $MgCl_2$ and KCl at neutral pH is presented in Fig. 3. As can be seen from this dependence, the effect of $MgCl_2$ exhibits a profile similar to KCl but the maximum rate was obtained at 10 mM for $MgCl_2$. The KCl-induced activity was 75% of the $MgCl_2$-induced activity, and the optimum KCl concentration shifted to 40 mM. These differences cannot be explained simply as arising from an ionic strength effect, but rather suggest a specific role of Mg^{+2} ions. Such a role could be extended to divalent cations basically, since Tamure et al. (7) detected the same effects with Ca^{+2}.

Fig. 4 shows the pH-dependence of electron transport to PS I. It is shown that the electron transport rate increased with decreasing pH from 7.5 to 5.5 (maximum value) and a second maximum was observed in alkaline region at pH=8.5.

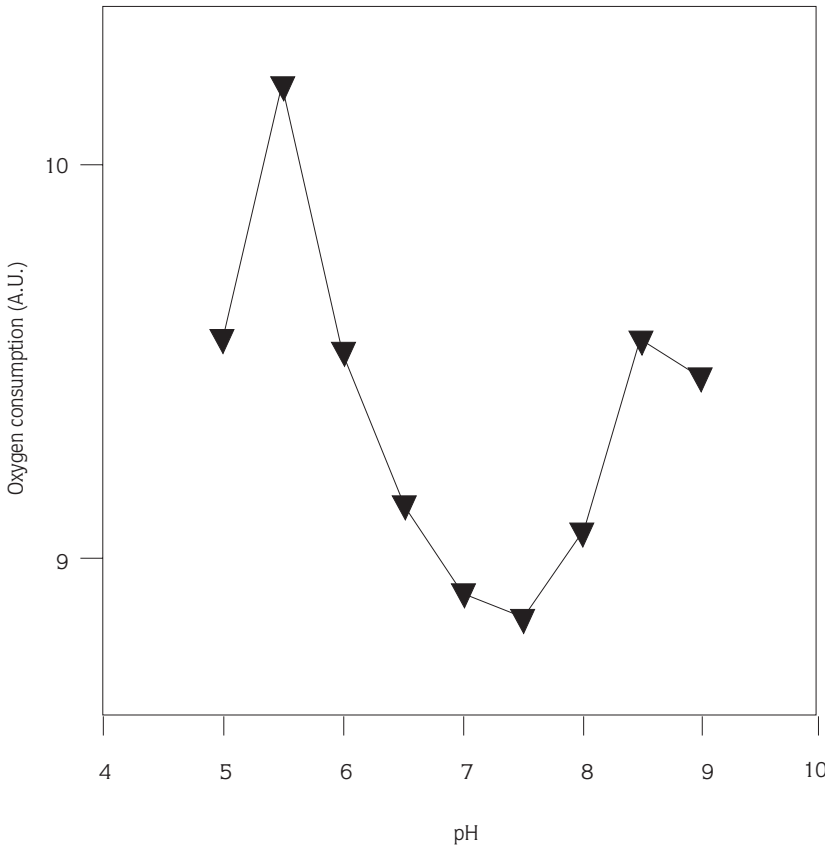

Figure 4. Dependence of oxygen consumption by methyl viologen from pH. Incubation medium: 20 mM MES (pH= 5 - 6.5), 20 mM HEPES (pH= 7-8), 20 mM Tris (pH= 8-9), 0.1 mM Methyl viologen, 1 mM sodium ascorbate, 0.1 mM diaminoduorene, 10 µM Pc.

The sigmoidal kinetics cannot be explain in simple models of bimolecular reactions between Pc and PS I. The Hill coefficient n=1.87 means that there is a positive cooperativity in Pc-PS1 complex formation and that more than one Pc binding site in PS I take part in the process. An earlier model (8) explained multiphasic kinetics of P700 reduction by proposal of one binding site on the donor side of PS I and the existence of two kinds of Pc: one of them tightly bound to docking site and second free in lumenal space. In this model electron transfer occurs through the bound Pc.

Drepper et al. (15) proposed a model where the sigmoidal behaviour is explained by the decreasing binding affinity of oxidised Pc and its removal from the docking site. Also this research group have shown by cross-linking of Pc to PS I the impossibility of electron transfer through the bound Pc. But there is a contradiction between the data mentioned above and the data reported by Wynn and Malkin (16) where the electron trasnfer from cross-linking Pc to PS I was inhibited. Probably during cross-linking Pc "locked" into a less productive orientation in its binding site. In the attempt to explain our data we considered the process of formation and dissociation of activating complexes Pc-PS I through the model based on trimeric structure of PS I. In fact, the existence of trimeric complexes of PS I in vitro as well as in vivo stated previously (17, 18). The trimeric complexes of PS I (3-PS I) have a mass near 1 MDa (3x340 kDa) and it possesses three binding sites for Pc. Principally, for explanation of sigmodial kinetics, we assumed that there is an interaction between three subunits III which led to formation of activating complexes. Hence, we could assume an interaction between the three docking sites. In the case of protein-protein incteraction the process of formation of activating complexes might involve cooperativity. This mechanism can be explained in frame of electrostatic interaction of negatively charged subunits III in 3-PS I and 3 molecules of Pc. Each binding to subunit III molecule of Pc facilitates binding of the following molecule of Pc. The sequential neutralisation of the negative charge on the surface of subunits III finds a good agreement with a shielding effect of Mg^{+2} and K^{+}.

Furthermore, it has previously been shown that subunit III is necessary for effective electron transfer from Pc to PS I and optimal orientation of Pc in the docking site (19). The Pc removal from the binding site occurs after its oxidation and, as a consequence,

decreases the electrostatic field at the negative patch near Tyrosine-83 (20). An analogous effect is exhibited under increasing $MgCl_2$ and KCl concentration which act as an ionic screen between Pc and subunit III. In support of the electrostatic mechanism of formation of Pc-subunit - III complexes we note that in green algae and cyanobacteria, where Pc is substituted by cytochrome C_6 (it has no negative patches like Pc), only a weak effect of cations was found (21).

pH dependence could be explained in terms of co-operative electrostatic interaction. Lowering the pH also increased the reaction rate by decreasing the net negative charges on both Pc and subunit III (6, 22). The maximum rate of electron transport activity at pH=5.5 could be explained by neutralisation of negatively charged groups under protonation. The maximal activity in alkaline region (pH=8.5) is probably related to deprotonation of Hystidine-87 at the hydrophobic patch of Pc.

In conclusion, we would like to note that three-dimensional structuref PS I and Pc was resolved, but attempts to obtain the Pc-PS I complex in crystal state was unsuccessful. Thus, the native structure of the docking site for Pc and the real electron pathway from Pc to P700 remain the tasks of future research.

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References

1. J. Deisenhofer, O. Epp, K. Miki, R. Huber, H. Michel (1985), Nature, (London), 318, 618-624.
2. P. Fromme, W.-D., Schubert, N. Krauss, W. Hinnrichs, I. Witt, P. Fromme, W. Pritzkov, Z. Dauter, C. Betzel, K.S. Wilson, H. Witt, W. Saenger. (1993) Nature (London), 361, 326-331.
3. W. Haehnel (1986) in Photosynthesis III (L.A. Staehelin & C.J. Arntzen eds.) pp.547-559, Springer-Verlag, Berlin.
4. A.G. Sykes (1985) Chem. Soc. Rev. 14., 283-315.
5. S. Lien, A. San-Pietro (1979) Arch. Biochem. & biophys., 194, 128-137.
6. T. Takabe, H. Ishikava, S. Niwa, S. Itoh (1983) J. Biochem. (Tokyo) 94, 1901-1911.
7. N. Tamura, S. Itoh, Y. Yamomoto, M. Nishimura (1981) Plant Cell Physiology, 22, 603-612.
8. H. Bottin, P. Mathis (1987) Biochim. Biophys. Acta, 892, 91-98.
9. M. Hippler, R. Ratajczaak, W. Haehnel (1989), FEBS letters, 250, Vol.2, 280-284.
10. A.A. Mutuskin, K.V. Pshenova (1975), In "The methods of modern biochemistry" Mosc ow, "Science" publ. (Russia), pp. 12-14.
11. S. Katoh, I. Shiratori, A. Takamiya (1962), J. Biochem., 51, 32-40.
12. N. Boardman (1971) In "Methods of Enzymology", (A. San-Pietro eds.), vol.VXXIV, 268-276.
13. G. MacKinney (1941), J. Biol. Chem., 140, 315-322.
14. T. Hiyama, B. Ke (1972), Biochim. Biophys. Acta, 267, 595-599.
15. F. Drepper, M. Hippler, W. Nitschke, W. Haehnel (1996), Biochemistry, 35, 1282-1295.
16. R. Wynn, R. Malkin (1988) Biochemistry, 27, 5863-5869.
17. W.D. Schubert, O. Klukas, N. Krauss, W. Saenger, P. Fromme, H. Witt (1995). In: Photosynthesis: From light to Biosphere (P. Mathis eds.) Kluwer Acad. Publish., pp. 3-10.
18. N.V. Karapetyan, V.V. Shubin, I.N. Bessmertnaya, R. Strasser (1995). In: Photosynthesis: From light to Biosphere (P. Mathis eds.) Kluwer Acad. Publish., pp.55-58.
19. M. Hipler, F. Drepper, J. Farah, J-D. Rochaix (1997), Biochemistry, 36, 6343-6349.
20. S.R. Durrel, J. Lobanovski, E. Gross (1990), Arch. Biochem. Biophys., 277, 241-254.
21. M. Hervas, M. De La Rosa, G. Tollin (1992) Eur. J. Biochem., 205. 113-120.
22. T. Takabe, H. Ishikava, S. Niwa, Y. Tanaka (1984) J. Biochem. (Tokyo), 96, 385-393.