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A brownian dynamics study of evolutionary changes in electrostatic interactions between plastocyanin and cytochrome f in cyanobacteria and green plants.

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Introduction

In higher plants and green algae, positive charges on cytochorme f (cyt f) intereact with negative charges on plastocyanin (PC) to form an electrostatic complex in which electrons are transferred from the Fe of cyt f to the copper of PC (Anderson et al., 1987; Gross, 1996; Pearson et al., 1996; Kannt et al., 1986; Pearson and Gross, 1998; Nelson et al., 1998). However, in cyano-bacteria such as *Phormidium* (Carrell et al., 1999), K66 is the only one of the interacting positively-charged residues on cyt f which is conserved and *Phormidium* cyt f has many negatively-charged residues giving it a very large negative electrostatic field (Fig..1a) instead of a positive electrostatic field observed for turnip cyt f (Pearson et al., 1996). All of the acidic residues that contribute to the negative electrostatic field in higher plant and algal PC's are absent except for D44 (and D45 in *Phormidium)* resulting in a large positive electrostatic filed (Figs. 1b and 1d) replacing the negatively-charged electrostatic field (Durell et al., 1998) observed in higher plant and algal PC's. The question arises as to whether electrostatic fields play apart in the binding of cyanobacterial PC's to the corresponding cyt f's, and if so, what is the nature of the interaction.

Brownian Dynamics (BD simulations (Northrup, 1995) were used to investigate this question. Previously, BD simulations were used to study the interactions of poplar (Pearson and Gross, 1998) and spinach (Nelson et al., 1998) with turnip cyt f.

Methods

The atomic coordinates for all molecules were obtained from the protein data bank (Berman et al., 2000), Molecular representations were made using the software package GRASP (Nicholls and Honig, 1991) BD, simulations were carried out using the program Macrodox (Northrup, 1995) running on a Silicon Graphics O2 workstation For each experiment, five runs of 1000 trajectories each were run at an ionic strength of 10mM at pH 7.0 to maximize electrostatic interactions. For other conditions see Pearson and Gross (1998) and Nelson et al. (1988).

Results

The BD study of the interaction of *Prochlorothrix* PC (Babu et al., 1999) with *Phormidium* cyt f (Carrell et al. (1999) is compared with the interaction of spinach PC (Ubbink et al., 1998) with turnip cyt f (Martinez et al., 1994) (Fig. 2a). As many complexes are formed for the *Prochlorthrix-Phormidium* system as for the spinach-turnip system proving that that electrostatic forces are involved in both cases. But the copper-Fe distance for maximal complex formation is 22 Å compared to 15 Å found for spinach PC-turnip cyt f system. One reason is that *Prochlorthrix* PC has a tyrosine at position 10 instead of a glycine found in all other plastocyanins. Replacing the tyrosine in *Prochlorothrix* PC with a glycine decreases the Cu-Fe distance and slightly increases the number of complexes formed. Furthermore, the spinach PC used in the BD simulations was taken from the energy-minimized NMR complex of Ubbink et al. (1998) which forms tighter complexes than the spinach PC structure determined by

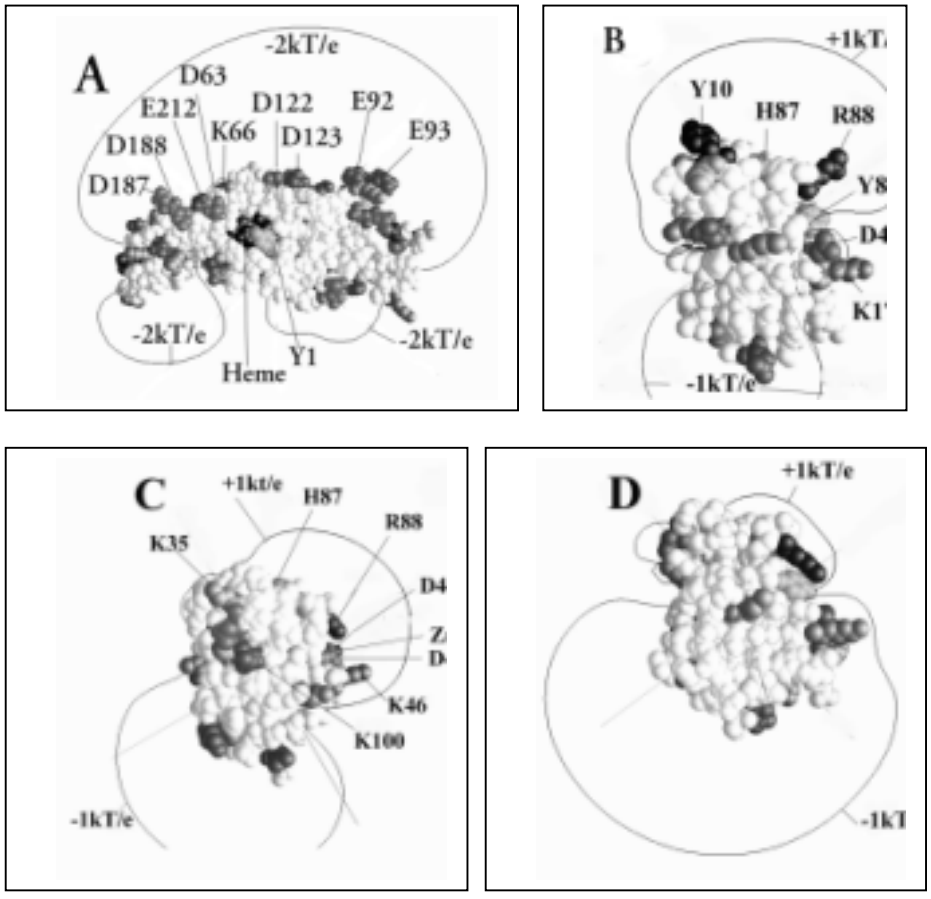

Fig. 1. A. *Phormidium* cyt f. Electrostatic contours at ± 2kT/e are shown. **B.** *Prochlorothrix* PC. Electostatic contours at ± 1 kT/e are shown. **C.** *Phormidium* PC + Zn^{2+} **D.** *Phormidium* PC – Zn^{2+}. Higher plant number systems are used for the residues

crystallography (Xue ea al. (1998) (Gross, unpublished observations) indicating that the PC structure is changed slightly upon complex formation to maximize interactions. Similar results have been observed for the barnase-barstar system (Gabdoulline et al., 1998) showing that Macrodox simulations are sensitive to protein conformational changes. Also, *Phormidium* cyt f did not interact with spinach PC nor did turnip cyt f interact with *Prochlorothrix* PC confirming

that negatively-charged residues on *Prochlorothrix* PC interact with positively-charged residues on *Phormidium* cyt f.

A comparison of five different cyantobacterial PC's with *Phormidium* cyt f is shown in Fig. 2b. *Prochlorothrix* PC with a net charge of 1.0 shows of the greatest number of complexes formed followed by *Anabaena* PC (net charge = +1.1) (Badsburg et al., 1996) and *Phormidium* (net charge = -0.3) (Bond et al., 1996) whereas *Sumechocystis* (net charge = -1.8) and *Synechococcus* PC (net charge = -5.1) (Inoue et al., 1999) showed no reaction at all. Thus, a PC must be positively-charged or near neutrality in order to interact with Phormidium cyt f.

The *Phormidium* PC-*Phormidum* cyt f system was studied in more detail (Fig 2c). All three PC molecules in the unit cell gives identical results (not shown). All three PC structures contains one Zn^{2+} adjacent to acidic residues #44 and 45. When the Zn^{2+} is removed, almost no complexes are formed suggesting that the Zn^{2+} ion may also be present in solution. Q88 found in higher plant and algal PC's (Gross, 1996) is replaced by an arginine in all cyanobacterial PC's. Substution of R88 with an alanine severely inhibits complex formations showing the importance of R88 in the binding of the two proteins.

A typical *Phormidium* PC - *Phormidium* cyt f complex is shown in Fig. 3. Five out of ten random complexes studied show this type of structures. The other five were more varied. In this complex, H87 on PC is close to Y1 on cyt f and R88 is close to the interaction site.

In conclusion, cyanobacterial PC's interact electrostatically with cyanobactial cyt f's with positive charges on the PC molecule interacting with negative charges on the cyt f. This leaves the question as to why the charge on both types of molecules is reversed in algae and higher plants.

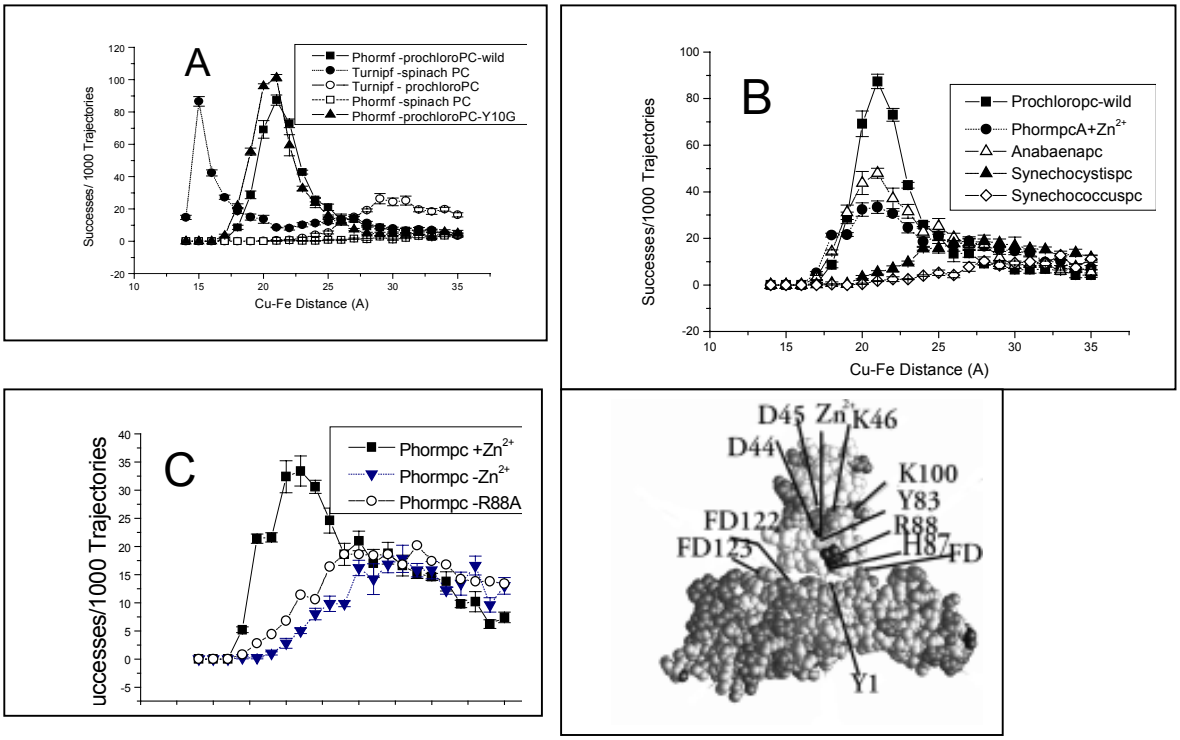

Fig. 2. Plots of number of complexes formed vs. Cu to Fe distance. **A.** Comparison of the *Phormidium* cyt f-*Prochlorothrix* PC system with the turnip cyt f-spinach PC system. **B.** The interaction of different PC's with *Phormidium* cyt f. *Synechocystis* PC was back-mutated to obtain the wild type form. **C.** The effect of Zn^{2+} and the mutation of R88 on the interaction of *Phormidium* PC with *Phormidium* cyt f.

Fig 3. A typical complex formed between *Phormidium* cyt f and *Phormidium* PC. White= PC; Gray= cyt f. Cyt f residues are labelled F

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