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Direct simulation of plastocyanin and cytochrome f interactions in solution

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Abstract

Most biological functions, including photosynthetic activity, are mediated by protein interactions. The proteins plastocyanin and cytochrome f are reaction partners in a photosynthetic electron transport chain. We designed a 3D computer simulation model of diffusion and interaction of spinach plastocyanin and turnip cytochrome f in solution. It is the first step in simulating the electron transfer from cytochrome f to photosystem 1 in the lumen of thylakoid. The model is multiparticle and it can describe the interaction of several hundreds of proteins. In our model the interacting proteins are represented as rigid bodies with spatial fixed charges. Translational and rotational motion of proteins is the result of the effect of stochastic Brownian force and electrostatic force. The Poisson–Boltzmann formalism is used to determine the electrostatic potential field generated around the proteins. Using this model we studied the kinetic characteristics of plastocyanin–cytochrome f complex formation for plastocyanin mutants at pH 7 and a variety of ionic strength values.

1. Introduction

In green plants and algae light absorption and charge separation followed by photosynthetic electron and proton transfer take place in the photosynthetic membrane of chloroplasts. Protein complexes photosystem 1, photosystem 2, cytochrome bf complex and ATP-synthase are embedded in a photosynthetic membrane (figure 1). A mobile carrier plastocyanin, a small copper protein, transfers electrons from cytochrome f, a subunit of the cytochrome bf complex, to P700, the reaction center of photosystem 1 (Hope 2000, Gross 1996). Plastocyanin molecules are assumed to diffuse for long distances in the luminal space of chloroplasts, shuttling electrons between granal and stromal areas of the thylakoid membrane (Hope 2000, Albertsson 2001). A shift in the plastocyanin concentration between granal and stromal areas of the membrane during light–dark transition was interpreted as favoring the long distance transport between these compartments (Haehnel *et al* 1989). In native chloroplasts the luminal space is narrow (40–100 Å) and contains proteins protruding through the thylakoid membrane. Since the size of plastocyanin (40 × 28 × 30 Å) is about the thickness of the lumen, the diffusion of plastocyanin in the lumen is probably hindered by the membrane protein complexes.

There is a conceptual difficulty in explaining the mechanism of plastocyanin diffusion in the lumen. It is not clear whether plastocyanin can rapidly diffuse over a distance of hundreds of nanometers, between grana stacks and stroma lamellae. The 30–50 μs lag phase of cytochrome f oxidation, after a saturating pulse of light, shows that it takes some time for the oxidized plastocyanin to diffuse from P700 to the nearest cytochrome bf complex (Haehnel *et al* 1980). This lag is followed by multicomponent relaxation kinetics that may correspond to different plastocyanin diffusion distances.

It is widely recognized that electrostatic interactions play a crucial role in the binding of plastocyanin to its reaction partners, photosystem 1 and cytochrome f (Durell *et al* 1990, Gross 1996). Long-range electrostatic interactions govern the diffusion of plastocyanin to its docking sites at protein complexes and thus influence the rate of association, whereas short-range electrostatic interactions make plastocyanin molecules orient in the electrostatic field of the protein complex (Gross 1996).

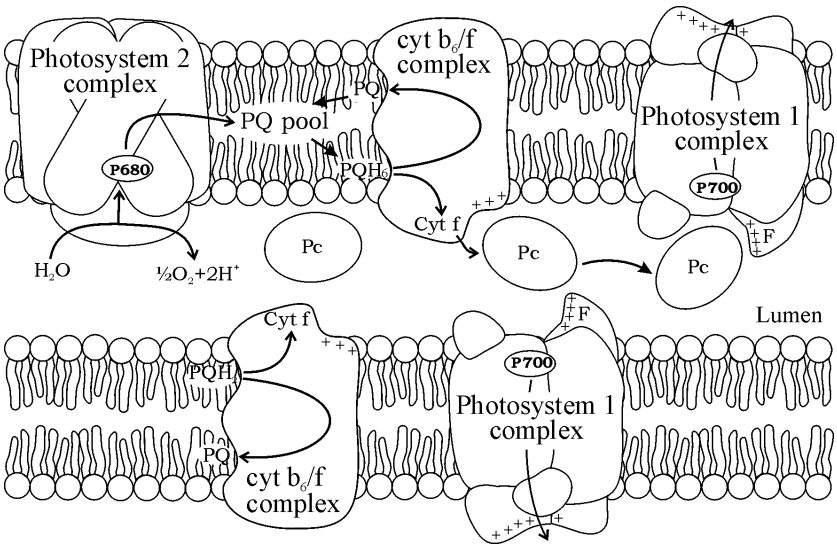

Figure 1. Schematic view of photosynthetic electron transport. Two thylakoid membranes and the luminal space between them are shown. Multiprotein complexes photosystem 1, photosystem 2, and cytochrome bf are embedded in the membrane. Mobile electron carrier protein plastocyanin (Pc) diffuses in the lumen. The arrows denote electron transport.

The limited diffusion of plastocyanin and its interaction with photosystem 1 and cytochrome bf complexes in the narrow thylakoid lumen are difficult to study by experimental techniques. A computer simulation model can give some information on plastocyanin diffusion and interaction in lumen. On one hand, this simulation should take into account the geometry of the luminal space packed with many protein molecules. On the other hand, it is necessary to consider electrostatic interactions of plastocyanin with its reaction partners in a thylakoid membrane.

Brownian dynamics (BD) models are usually used to simulate the interaction of two individual protein molecules to investigate the process of complex formation, to predict the structure of the complex and to estimate the rate constant of protein association. In BD simulations the interaction of two proteins is investigated in solution, the proteins are treated as rigid or semi-rigid bodies, their geometric shape is considered at atomic resolution and electrostatic interactions of the proteins are presented in great detail. Simulation of plastocyanin and cytochrome f association by the Brownian dynamics method was done by Pearson and Gross (1998), Gross and Pearson (2003), Rienzo *et al* (2001) and Ullmann *et al* (1997). In Ullmann *et al* (1997), BD simulations were followed by molecular dynamics and quantum chemistry studies. These BD simulations cannot provide the concentration time courses for different biochemical species which are registered experimentally and which are usually used as a source of information about rate constants of biochemical reactions.

In our studies we develop models of the limited diffusion of plastocyanin and its interaction with photosystem 1 and cytochrome bf complex in a narrow thylakoid lumen. In this paper we present the results on the simulation of spinach plastocyanin and turnip cytochrome f diffusion and interaction in solution, but not in the lumen. This is the first step in the development of the complete luminal model, where we consider many plastocyanin molecules that compete to form complexes with PS1 and cytochrome bf. This study develops the method of the direct 3D simulation of electron transport processes in a thylakoid, which was described previously (Kovalenko *et al* 2003). In contrast to models described in Pearson and Gross (1998), Gross and Pearson (2003), Rienzo *et al* (2001) and Ullmann *et al* (1997), our model is multiparticle and includes many plastocyanin and cytochrome f molecules. We do not describe complex formation in detail (such as hydrophobic interactions and conformational changes of proteins which are important for the final complex formation). Our model uses a continuum electrostatic approach that describes molecules at the atomic level using a macroscopic description. From crystallographic studies, we know the structure of plastocyanin–cytochrome f encounter complex (Ubbink *et al* 1998, PDB ID is 2PCF). We use this structure in our simulation.

2. Description of the model

2.1. The model scene

The model scene is a cubic 3D reaction volume (figure 2). Initially, the protein molecules are distributed randomly in the reaction volume. In our simulation, protein molecules are considered as Brownian particles. These particles move stochastically in a viscous medium guided by random Brownian force and electrostatic force. As shown in Doi and Edwards (1986), one can use the Langevin equation for the mathematical description of such a process.

The Langevin equation for translational motion has the following form:

$$\xi_{\mathrm{tr}}^{x}\frac{\mathrm{d}x}{\mathrm{d}t} = f_x(t) + F_x.$$

where x is the coordinate, ξ_{tr}^{x} is the viscous friction coefficient at x, $f_x(t)$ and F_x are the projections of the random Brownian force and electrostatic force onto the x axis, respectively.

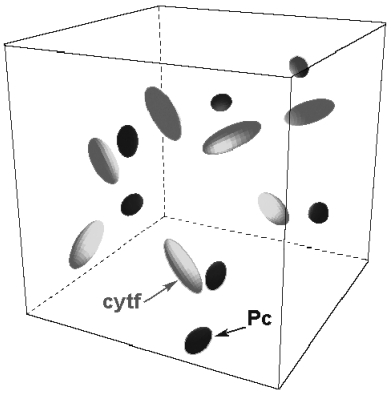

Figure 2. Visualization of the model scene of the computer simulation of protein plastocyanin and cytochrome f in solution. Protein molecules are visualized by ellipsoids of revolution.

The Langevin equation for rotational motion is

$$\xi^x_{\text{rot}} \frac{\mathrm{d}\varphi}{\mathrm{d}t} = m_x(t) + M_x,$$

where ξ^x_{rot} is the viscous friction coefficient for rotational motion around axis x, $m_x(t)$ and M_x are the moments of the random Brownian force and electrostatic force relative to the x axis, respectively. The random Brownian force has a normal distribution with zero mean value and dispersion $\frac{2kT\xi^x_{\text{tr}}}{\Delta t}$. Here k is the Boltzmann constant and T is the temperature.

To simplify the calculation of viscous friction coefficients, the molecules of plastocyanin and cytochrome f were represented as ellipsoids of revolution with axes a, b and c (a is the axis of revolution, $b = c$). The axes and sizes of ellipsoids were chosen in such a way that the moment of inertia relative to the axis of revolution (axis a) was minimal and equal to the moment of inertia of the protein molecule. The obtained ellipsoids of revolution are apt representations of the shape of plastocyanin and cytochrome f (figure 3). Translational and rotational viscous friction coefficients of an ellipsoid are calculated as described in Bloomfield (2000).

For the description of the position of each object we chose the Cartesian coordinate system with mirror boundary conditions at the edges (the scene coordinate system). Every object is assigned its local Cartesian coordinate system, which coincides with the axes of the respective ellipsoid. Such a choice of local coordinate system is very convenient because the viscous friction coefficients for the ellipsoid in this system are well known (Bloomfield 2000).

The Langevin equation is solved numerically in the local coordinate system: on each time step, the displacements of the ellipsoid along its axes and rotations around its axes are calculated. The displacements and rotations found in the local coordinate system for each object are then recalculated in the scene coordinate system. The position of every object in the model is specified by the displacement and rotation of the local coordinate system of the object with respect to the scene coordinate system.

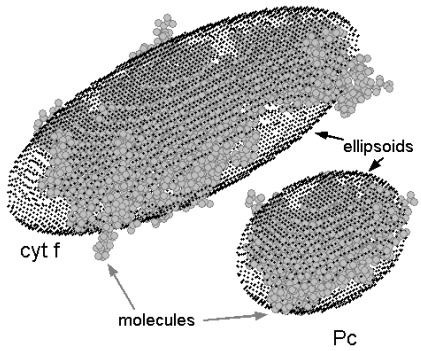

Figure 3. Description of plastocyanin and cytochrome f by ellipsoids of revolution for calculation of the viscous friction coefficients of the molecules.

2.2. Interactions of proteins: simulation of the geometric shape of the proteins and protein collisions

The interaction of two protein–electron carriers can be divided into four stages (Gross 1996): (1) convergence due to diffusion and electrostatic attraction; (2) interaction of separate parts of the molecules determined by their charge configuration, mutual orientation of the molecules, with the possible formation of a predocking complex; (3) final binding at the site where electron transfer can occur; and (4) electron transfer. In our simulation we consider the diffusion of molecules, electrostatic interactions and formation of a predocking complex.

In our model proteins are considered as rigid bodies. They diffuse in the simulated reaction volume and can come into close proximity to each other but without overlapping. The 3D geometric surfaces of plastocyanin and cytochrome f are known at atomic resolution based on crystal structures of these proteins. We approximated the surfaces of the molecules by a set of spheres. With this approximation, two molecules overlap if the respective sets of spheres overlap. Geometric interpretation of the surface of the molecule by a small number of spheres (10–50 spheres), on one hand, ensures a sufficiently realistic representation of the molecular surface. On the other hand, it is less time consuming than calculation of each atom overlap, because the number of spheres is by two orders of magnitude less than the number of atoms. To verify the overlapping of two spheres is very easy—it is enough to know the radii and coordinates of their centers.

2.3. Simulation of the electrostatic interactions of cytochrome f and plastocyanin

Electrostatic interactions play a key role in the binding of plastocyanin to its reaction partners, photosystem 1 and cytochrome f (Durell *et al* 1990, Hope 2000, Rienzo *et al* 2001, Gross 1996). Charged amino acid residues and partial charges of the proteins produce a heterogeneous electrostatic field. This field is screened by the polar water molecules and thus the electrostatic potential rapidly decreases with distance from the protein surface. If a protein is far away from other proteins, it moves under the effect of Brownian force but not electrostatic force, and hence its motion is determined only

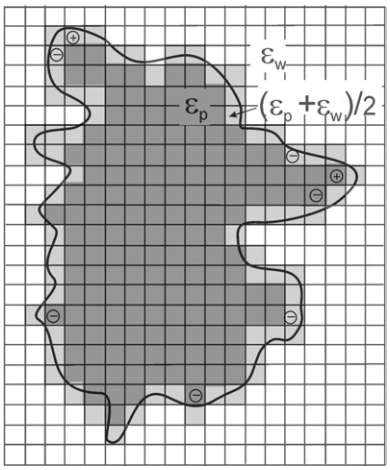

Figure 4. The grid for calculation of the electrostatic potential field around a protein. Dark gray represents the cells occupied with the protein, light gray represents the cells of separation, white cells represent water.

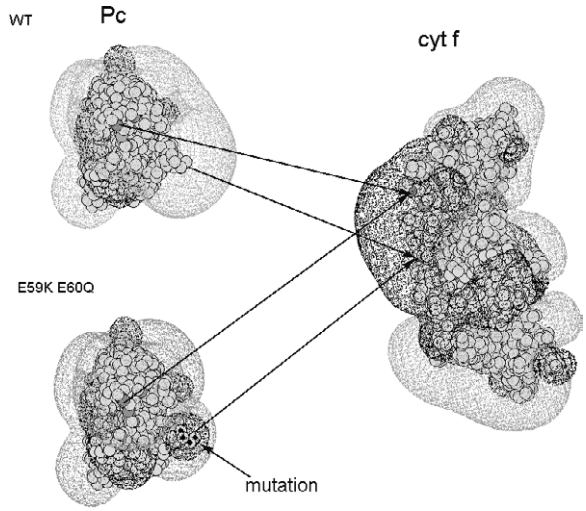

Figure 5. Equipotential surfaces –6.5 mV (light gray) and + 6.5 mV (dark gray) for reduced cytochrome f and oxidized plastocyanin (wild-type and mutant E59K/E60Q), calculated by the Poisson–Boltzmann equation; ionic strength is 100 mM, pH = 7, $\varepsilon_{sol} = 80$, $\varepsilon_{prot} = 2$. The lines connect the amino acid residues on plastocyanin and cytochrome f that were used in the simulation for calculation of the distance between the proteins. For mutant plastocyanin the black points represent atoms with changed charges relative to the wild type.

by Brownian diffusion. As the protein comes close to other proteins, its behavior is directed by the electrostatic field of the latter so that a favorable position may be reached for the formation of a predocking complex.

In our simulation a protein is presented as a low dielectric area ($\varepsilon = 2$) with spatially fixed partial charges. The solution is presented as a high dielectric area ($\varepsilon = 80$) with mobile charges (ions).

When the proteins are separated from each other by more than 100 Å, the electrostatic interactions are very weak because of the dielectric medium (water with ions) screening. In our simulation electrostatic interactions are considered only when proteins converge to some distance which we call the radius of the electrostatic interaction.

Since a single protein is influenced by all other proteins inside the radius of electrostatic interaction, it is necessary to calculate the electrostatic force value and its moment. For this reason we chose a 3D rectangular region around each protein, set up a rectangular grid in the region and mapped the charges, dielectric constants and ionic strength to the grid. Then we calculate the electrostatic potential value for a given ionic strength and pH in every cell of the grid. We calculate this electrostatic potential map around all types of the objects in our model scene (both for plastocyanin and cytochrome f in the oxidized and reduced forms). The step of the grid is 2 Å. The formal charges on the proteins were calculated as described in Durell *et al* (1990). The dielectric constant of the cells inside the protein was assigned 2; inside the solution—80; at the boundary protein solution—40; and the value of the ionic strength inside the protein was assigned 0 (figure 4).

A Poisson–Boltzmann calculation (Ullmann and Knapp 1999) is used to determine the electrostatic potential grid around each type of the object. In the finite difference representation, the electrostatic potential of a given cell on the grid in the *n*th iteration is calculated by an iteration formula (1) depending on the potential values in the neighboring cells in the previous time step and the entire charge of the cell. In the *n*th iteration the electrostatic potential value in the cell is

$$\varphi = \frac{\left(\sum_{i=1}^{6} h\varepsilon_i\varphi_i\right) + 4\pi q}{\left(\sum_{i=1}^{6} h\varepsilon_i\right) + h^3\kappa^2} \qquad (1)$$

$$\kappa^2 = \frac{8\pi N_A e^2 I}{k_B T}, \qquad I = \frac{1}{2}\sum_{i=1}^{K} c_i^{bulk} Z_i^2.$$

Here φ is the electrostatic potential, ε is the dielectric constant, ρ is the charge density, c_i^{bulk} is the concentration of the *i*th ion in the solution, Z_i is the charge of the *i*th ion, e is the electron charge, T is the temperature (K), N_A is the Avogadro number, I is the solution ionic strength and h is the step of the grid. Figure 5 demonstrates equipotential surfaces of the proteins cytochrome f and plastocyanin.

After the calculation of the potential grid of each type of object in the model scene, we ran a Brownian dynamics simulation. In calculating the electrostatic force and its moment applied to a charge in a protein, it is necessary to calculate the potential gradient created by other proteins at the point where the charge is situated. To calculate the electrostatic force and its moment applied to the entire protein molecule, we geometrically sum the forces and their moments applied to every charge of the molecule.

2.4. Simulation of the plastocyanin–cytochrome f complex formation

In the model, plastocyanin and cytochrome f molecules move randomly, guided by Brownian and electrostatic forces. As two molecules approach each other they are directed by the electrostatic field and may occupy a favorable position for

docking. In the model it means that the distances between certain amino acid residues of the molecules become less than certain parameters of the model, which we call the docking distances. The corresponding amino acid residues are in a close contact in the crystal structure of the plastocyanin–cytochrome f complex. From crystallographic studies we know the structure of the plastocyanin–cytochrome f encounter complex (we use the structure of the complex described in Ubbink *et al* (1998), PDB ID is 2PCF). In the model the distances between these amino acid residues determine the position and orientation of one molecule with respect to another.

The program monitors the distances between chosen amino acid residues at every time step in the process of diffusion. If at some time step the distances are less than the docking distances, then the molecules can form a complex with probability p, which is a model parameter. We do not explicitly consider the process of complex formation. Instead, we simulate this event implicitly by introducing the probability, p, of the accidental complex formation. The accidental nature of a complex formation in every particular case is represented by a random choice of a number from the segment [0; 1]. At each time step, the algorithm generates a random number, uniformly distributed from 0 to 1. If the generated number is less than the given docking probability p, it means that the probability of the choice of this random number is the same as the probability of the complex formation. In other words, it means that the complex formation occurs. If the generated number is greater than the given docking probability p, the program flows to the next step. Provided the proteins still satisfy the docking conditions, the described procedure is repeated. As two molecules form a complex, it is considered that electron transfer occurs immediately and the molecules change their redox states.

In our model we consider only electrostatic interactions and do not simulate explicitly the final complex formation and electron transfer. We do not consider hydrophobic interactions, possible complex rearrangements and any conformational changes of proteins which may be important for the final complex formation. We think that probability p is a convenient parameter that can take into account all these processes implicitly. Introducing probability p, we are able to simulate not only diffusion-controlled reactions but also the reactions in which the reaction rate is influenced by diffusion and some other processes.

A recent paper concerning a cyanobacterial plastocyanin (Schlarb-Ridley *et al* 2005) suggests that the reaction between plastocyanin and cytochrome f is activation controlled, because the increase in the solution viscosity by addition of high molecular mass viscogens (Ficoll 70 or Dextran 70) had little or no effect on the second-order rate constant of the protein interaction. On the other hand, when the viscosity of the solution was raised by addition of low molecular mass viscogens, a marked inhibition of second-order rate constant was observed, as would be expected for a reaction in which diffusion plays an important part (a diffusion-controlled reaction). We suppose that the reaction is diffusion controlled because the true behavior of plastocyanin in a solution of high molecular mass viscogens such as Ficoll or Dextran is not known in detail.

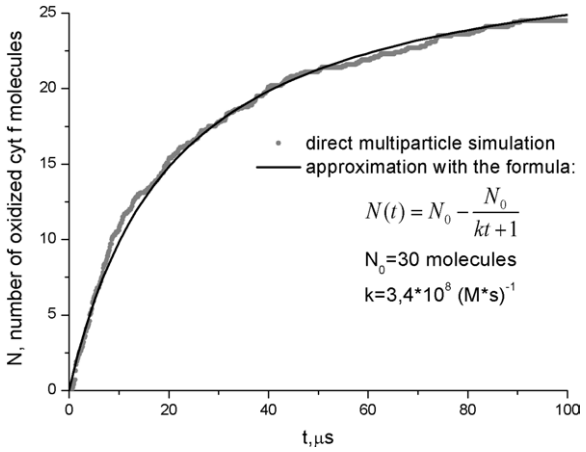

Figure 6. Kinetic curve of cytochrome f oxidation, obtained from the simulation, and its approximation by the mass action law (see the formula in the figure). N_0 is the total number of cytochrome f molecules, k is a second-order rate constant.

3. Simulation algorithm parameters

Calculating the number of oxidized (Pc^{II}) and reduced (Pc^{I}) plastocyanin and cytochrome f (cyt f) molecules at each time step, we obtain the kinetic curves of oxidation of cytochrome f and reduction of plastocyanin (figure 6). We can use the kinetic curves to compare the simulation results with experimental data and identify the model parameters. The second-order reaction rate constant can be determined by fitting kinetic curves by the mass action law. For this purpose we consider a simple kinetic scheme of a second-order reaction:

$$Pc^{\mathrm{II}} + \mathrm{cyt\ f} \xrightarrow{k} Pc^{\mathrm{I}} + \mathrm{cyt\ f}^{-},$$

where k is a second-order rate constant.

In all computer experiments the number of plastocyanin and cytochrome molecules was $N_0 = 30$, the reaction volume was cubic with 70 nm on a side and the time step was 100 ps. Every simulation was repeated ten times and the obtained kinetic curves were averaged.

3.1. Rate constant dependence on the model parameters

The protein association rate constant depends on numerous factors: the type of the proteins, diffusion coefficients, temperature, pH, ionic strength, etc. In our model, the second-order rate constant k is a function of the parameters of the model such that the probability of final complex formation, docking distances, time step, ionic strength, pH and temperature is $k = F(p, r_i, \mathrm{d}t, I, \mathrm{pH}, T)$. In this section we study the rate constant dependence on the docking distance and docking probability. In section 4 we provide the dependence of k on I.

We studied the dependence of the rate of the plastocyanin and cytochrome f reaction on the docking distance. The docking distance is the model parameter that determines

Table 1. Distances between contacting atoms in the complex plastocyanin–cytochrome f, obtained by the NMR method in Ubbink *et al* (1998) and distances chosen in our simulation as the condition of predocking complex formation.

Amino acid residues of plastocyanin and cytochrome f, which are in close contact in plastocyanin–cytochrome f complex	Numbers and names of the atoms to calculate the distances between		*R*, A distance in a complex (experiment)	*R*, A distance in a complex (simulation)
	Plastocyanin	Cytochrome f		
Asp42–Arg209 (D42-R209)	591, OD2	3278, HH2	4.1	18
Glu43–Lys187 (E43-K187)	607, HB	2930, HE	1.34	18
Asp44–Lys185 (D44-K185)	618, OD2	2895, HZ	3.56	18
Glu60–Lys58 (E60-K58)	842, HA	912, HZ	4.35	25
Glu59–Lys65 (E59-K65)	825, OE2	1014, HE	3.4	–
His87–Tyr1 (H87-Y1)	1270, HE2	21, HE2	2.45	–
His87–Phe4 (H87-F4)	1270, HE2	65, CE2	3.67	–
–	1435, Cu	3881, Fe	10.7–11.3	40

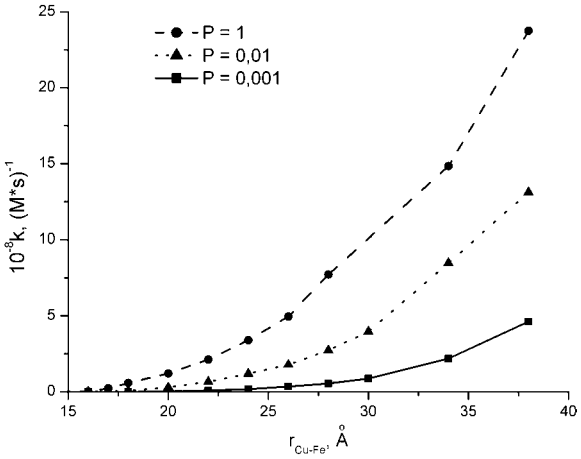

Figure 7. Dependence of the rate constant of the reaction plastocyanin + cytochrome f on the docking distance (the distance between metal centers) at $I = 100$ mM for different docking probabilities p.

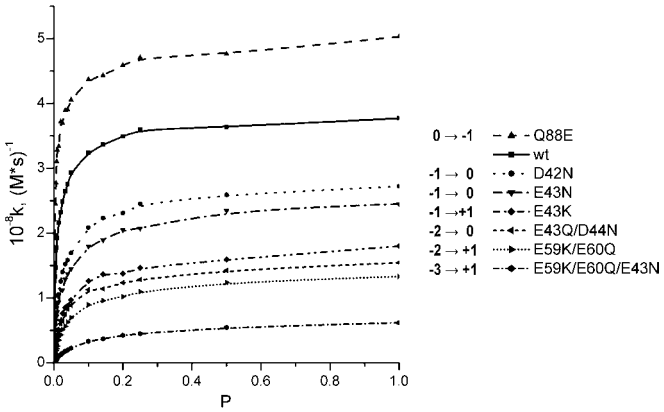

Figure 8. Dependence of the reaction rate on the docking probability p for the wild-type and mutant plastocyanins at $I = 100$ mM, pH = 7.0. The changes of charge on amino acid residues for different mutations and docking distances are presented. The values of distances between contacting amino acid residues are given in table 1.

the distance between the proteins when they are ready to form a complex capable for electron transfer. As the docking distance we chose the distance between metal centers (Cu in plastocyanin and Fe in cytochrome f). Figure 7 demonstrates the obtained dependences for three values of docking probabilities p (1, 0.01 and 0.001) and 100 mM ionic strength.

As seen in figure 7, the rate of the plastocyanin and cytochrome f reaction increases with an increase in the docking distance value. The dependence is nonlinear: at large docking distances the rate of the reaction increases faster than at small docking distances. In fact, due to the electrostatic interactions and steric factors, diffusing protein molecules find themselves much more frequently at large distances from each other than at short distances.

We also studied the dependence of the reaction rate on docking probability. In the simulation we changed the docking probability, while docking distances were fixed (see table 1). Figure 8 presents the dependence of the reaction rate on the docking probability for a wild type and some mutants of plastocyanin. The mutations modify the charge distribution on the surface of plastocyanin and thus significantly influence the process of protein docking, which is mainly determined by electrostatic interactions. Some mutations lead to an increase in the rate constant, while some mutations actually decrease it.

As seen in figure 8, there is a saturation dependence of the reaction rate on the docking probability. At high docking probabilities the rate of the reaction is almost constant. Proteins stay close to each other in the position ready to form a complex for a time period that depends on the diffusion coefficients and the time step value. At low docking probabilities they leave this position before the complex is formed. However, at high docking probabilities they have enough time to form a complex, which is observed as shown in figure 8, at $p > 0.1$.

Figure 8 shows that for the plastocyanin mutant Q88E with high negative potential, the rate constant is higher than for the wild-type plastocyanin. This demonstrates that high electrostatic attraction between the negative patch on plastocyanin and the positive patch on cytochrome f leads to the increase in the rate constant. This is in accordance with experimental results (Kaant *et al* 1996) and other simulations (Rienzo *et al* 2001). All other plastocyanin mutations that we simulated have a lower negative potential at the binding

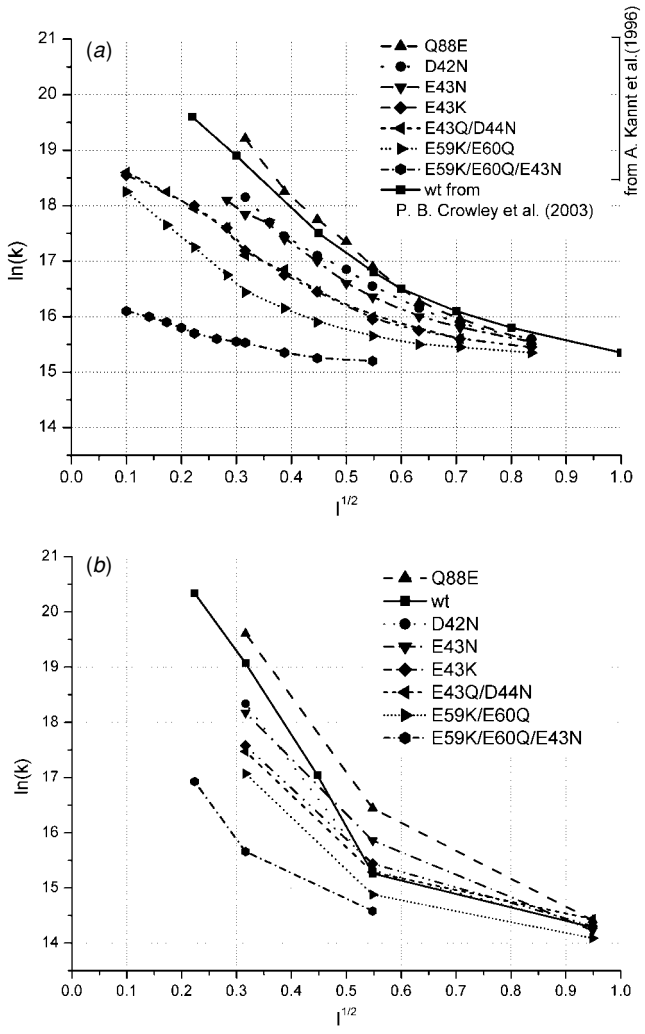

Figure 9. The logarithm of the second-order rate constant k dependence on the root square of the ionic strength I for the wild-type and mutant plastocyanin and cytochrome f: (*a*) experimental data reprinted from Kaant *et al* 1996 The role of acidic residues of plastocyanin in its interaction with cytochrome f *Biochim. Biophys. Acta* **1277** 115–26, Copyright (1996), with permission from Elsevier and from Crowley *et al* 2003 The parsley plastocyanin-turnip cytochrome f: a structurally distorted but kinetically functional acidic patch *Biochem. J.* **378(1)** 45–57, reproduced with permission from the Biochemical Society; (*b*) simulation results (the parameter values are given in table 1, docking probability is 0.01). k is in $(\mathrm{M\ s})^{-1}$, the ionic strength I is in M.

site than the wild-type plastocyanin, and thus the rates of the reactions are lower.

3.2. Estimation of the model parameters

In Ubbink *et al* (1998), the amino acid residues that take part in plastocyanin–cytochrome f complex were found using the NMR method. We have suggested that the favorable docking position when the reaction occurs is determined by the distances between these residues. On the basis of the crystal structure of the plastocyanin–cytochrome f complex, we calculated the distances between these residues and between Cu and Fe atoms in plastocyanin and cytochrome f. The values of estimated docking distances for the reaction in solution are given in table 1.

Table 2. The experimental (Kaant *et al* 1996) and calculated rates of reaction plastocyanin and cytochrome f at $I = 100$ mM, pH = 6, for different mutants of plastocyanin. Model parameters are given in table 1; docking probability is 0.01.

Mutation	Charge change of the respective amino acid residue	k, 10^6 $(\mathrm{M\ s})^{-1}$ experiment	k, 10^6 $(\mathrm{M\ c})^{-1}$ simulation
Wt	–	185 (±20)	198 (±4)
Q88E	$0 \rightarrow -1$	220 (±30)	345 (±7)
D42N	$-1 \rightarrow 0$	76.5 (±1.5)	94 (±3)
E43N	$-1 \rightarrow 0$	56.1 (±1)	80 (±3)
E43K	$-1 \rightarrow +1$	29.3 (±0.7)	44 (±2)
E43Q/D44N	$-2 \rightarrow 0$	26.9 (±0.6)	40 (±3)
E59K/E60Q	$-2 \rightarrow +1$	13.8 (±0.2)	27 (±2)
E59K/E60Q/E43N	$-3 \rightarrow +1$	5.56 (±0.01)	6.4 (±0.9)

On the other hand, we estimated the values of docking distances for which the calculated second-order rate constants for the wild-type and mutant plastocyanins at the ionic strength of 100 mM were close to the experimental values in Kaant *et al* (1996). As seen in table 1, the estimated docking distances are several times greater than the experimentally determined ones between plastocyanin and cytochrome f arranged in a unique complex. This is because in the model the representation of the surface of the protein molecule is quite rough (due to the approximation by a set of spheres) and it is impossible for the proteins in the model to come close at a distance of several angstroms. In the model the values of docking distances correspond to the position of separate molecules, cytochrome f and plastocyanin, in solution as they approach each other to form a complex.

The value of docking probability p was estimated by comparison of calculated and experimental reaction rates. The good agreement with experimental rates for a wide range of mutants was achieved for $p = 0.01$. The comparison of experimental and model data at the ionic strength of 100 mM is represented in figure 9 and in table 1.

4. Results and discussion

On the basis of model parameters (docking distances and probability, figure 8 and table 1) we calculated rate constant values which fit the experimental values in Kaant *et al* (1996) for plastocyanin–cytochrome f reaction at $I = 100$ mM and pH = 7.0 (table 2). For the mutant Q88E the rate constant k is higher than for the wild-type plastocyanin both by experimental and model estimation, though the model value is higher than experimental. Q88E mutation adds a negative charge to the binding site near tyrosine 83 where a large region of negative potential is found (Gross 1996; see table 2 for the change of charge on the respective amino acid residue). This leads to the stronger attraction of this negative patch on plastocyanin to the positive patch on cytochrome f. As these patches are close to reaction sites both on plastocyanin and cytochrome f, it leads to the increase in the rate constant. In

all other mutants the net charge of plastocyanin becomes more positive and this leads to the decrease in the reaction rate. If the plastocyanin mutants possess the same net charge and the mutations take place at approximately the same locations in the proteins, then the rate constants are more or less the same (table 2, figures 9(*a*) and (*b*)). Indeed, the mutations D42N and E43N decrease the net negative charge by 1 compared to the wild type and this leads to the decrease in the rate constant. Mutations E43K and E43Q/D44N decrease the net negative charge by 2, which leads to a greater decrease in the rate constant. The triple mutant E59K/E60Q/E43N, whose charge is decreased by 4 compared to the wild type, has the lowest value of the rate constant.

As seen from table 2, for all the mutants at the ionic strength $I = 100$ mM the model reaction rate constant values are higher than the experimentally estimated values. The probable explanation is that in our model we consider only electrostatic interactions and do not simulate explicitly the final complex formation capable of electron transfer. We do not consider hydrophobic and hydrodynamic interactions and conformational changes of proteins which may be important for the final complex formation. Instead, we introduce the same probability, p, of the final complex formation for all the mutants. Of course the suggestion may not be true. However, the values of the rate constant for different plastocyanin mutants match the experimental results qualitatively well, which indicates that the electrostatic interactions, especially long-range electrostatic interactions, play an important role in the formation of the plastocyanin–cytochrome f complex.

Rienzo *et al* (2001) have done Brownian dynamics simulation of the reaction of spinach plastocyanin and turnip cytochrome f and calculated the rates of the association. The calculated association rates were generally overestimates of the experimentally obtained rate constants, and the overestimation factor varied from 8 to 49 for different datasets and mutants. They suggested that overestimation might be due to the limits of the method used, in which hydrodynamic interactions, protein conformational flexibility and post-diffusional association rearrangement steps were not simulated. In fact, our model has the same drawbacks as the model of Rienzo and coworkers, and it is even more rough because we approximate the surface of the proteins with sets of spheres while in the BD models of Pearson and Gross (1998), Gross and Pearson (2003) and Rienzo *et al* (2001) the protein structure is considered at atomic resolution. Nevertheless, introducing the probability of the final complex formation, we can take into account all preceding processes. Estimating the value of probability p we obtain good agreement between experimental and model rate constants for the wild type as well as for all the mutants of plastocyanin.

Pearson and Gross (1998) studied electrostatic interactions between poplar plastocyanin and turnip cytochrome f using the software package MacroDox for Brownian dynamics simulations. They found a preferred orientation for plastocyanin that minimizes the distance between the metal centers of plastocyanin and cytochrome f and concluded that electrostatic forces alone are sufficient to guide plastocyanin into a single preferred conformation with cytochrome f suitable for the electron transfer. The distance of 1.6–1.7 nm between the metal centers (reaction distance) gave a good estimate of the experimentally observed rate constant for plastocyanin–cytochrome f interaction. They did not consider molecular flexibility, final complex rearrangement, and hydrophobic and hydrodynamic interactions explicitly, but they could take them into account implicitly by estimating the reaction distance between the metal centers. Although they approximated the protein surface at the atomic resolution, they got the reaction distance value greater than that estimated experimentally (1.09 nm (Ubbink *et al* 1998)). We think that for the description of the final complex formation it is useful to introduce the probability of this process.

The experimental dependences of the rate constant on the ionic strength for the cytochrome f and plastocyanin reaction are given in Crowley *et al* (2003) for the wild-type plastocyanin and in Kaant *et al* (1996) for the mutant plastocyanin (figure 9(*a*)). Using our model parameters we obtained the dependences of the rate of the plastocyanin and cytochrome f reaction on the ionic strength for the wild-type and mutant plastocyanin (figure 9(*b*)). We calculated the rate constant for three different values of ionic strength. The results of the model are in a good agreement with the experimental data. The reaction rates match experimental values better at the ionic strength of 100 mM, at which we estimated the model parameters. At low values of ionic strength ($I < 100$ mM), association rate constants differ much for various mutants, while at high values ($I > 1000$ mM) they are almost equal. This is because at high ionic strength values the electrostatic field around the proteins is screened and thus electrostatic interactions become less specific.

In our simulations, 30 plastocyanin and 30 cytochrome f molecules were distributed in a cubic reaction volume with 70 nm on a side, resulting in a comparatively high concentration of the molecules and thus reduction of the computational time. If we further increase the concentration, we expect that protein diffusion will be hindered and protein crowding will influence the reaction rate. In the narrow thylakoid lumen, where the concentration of macromolecules is very high, crowding effects are supposed to take place (Hope 2000, Schlarb-Ridley *et al* 2005). We plan to address this problem in the future.

5. Conclusion and outlook

The model presented in this paper allows one to perform detailed simulation of diffusion and interactions of the proteins plastocyanin and cytochrome f in solution. Since electrostatic forces play a key role in the binding of these proteins, we consider electrostatic interactions as well as three-dimensional Brownian motion of the proteins. The model includes many molecules of plastocyanin and cytochrome f and thus allows investigation of the kinetics of the protein binding. From the simulation we obtain the time course and a rate constant of the plastocyanin–cytochrome f reaction directly, for various

electrical properties of the medium and various distributions of charges in the proteins.

For the plastocyanin–cytochrome f reaction in solution, the simulated dependences of the reaction rate constant on the ionic strength for different mutants of plastocyanin are in good agreement with the experiment. This indicates that for the simulation of plastocyanin–cytochrome f reaction as a first approximation it is enough to take into account only the electrostatic forces.

The model describes the behavior of numerous protein molecules simultaneously. In future we plan to simulate the diffusion and interaction of plastocyanin molecules in the lumen of thylakoid and to study the role of the geometrical shape of the lumen and the arrangement of protein complexes in the membrane in the redox reaction of cytochrome bf complex and plastocyanin.

The simulation method presented in this paper can be applied for description of diffusion and functioning of many macromolecules that interact in heterogeneous interior of subcellular systems. However, simulation of a large number of proteins in the compartment with complex geometry is computationally intensive. Parallel computing may be helpful in this situation.

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Glossary

Brownian dynamics. A simulation method for molecular diffusion in which each molecule takes a step chosen from a Gaussian distribution, at each time step.

Brownian motion. Diffusive motion of a molecule that obeys Fick's laws, with an infinitely detailed trajectory.

Plastocyanin. A small copper protein that diffuses in the luminal space of the chloroplast thylakoid and shuttles electrons from subunit f of a cytochrome bf complex to photosystem 1 reaction center.

Cytochrome f. A subunit of a chloroplast cytochrome bf complex that binds plastocyanin and transfers an electron to it.

Model scene. A set of objects in a three-dimensional space. The space may be divided into compartments. The objects are proteins, protein complexes, other molecules, ions and electrons.

References

Albertsson P-A 2001 A quantitative model of the domain structure of the photosynthetic membrane *Trends Plant Sci.* **6** 349–54

Bloomfield V A 2000 Survey of biomolecular hydrodynamics *On-Line Biophysics Textbook: Separations and Hydrodynamics* ed T M Schuster

Crowley P, Hunter D, Sato K, McFarlane W and Dennison C 2003 The parsley plastocyanin–turnip cytochrome f: a structurally distorted but kinetically functional acidic patch *Biochem. J.* **378(1)** 45–57

Doi M and Edwards S F 1986 *The Theory of Polymer Dynamics* (New York: Oxford University Press)

Durell S R, Labanowski J K and Gross E L 1990 Modeling of the electrostatic potential field of plastocyanin *Arch. Biochem. Biophys.* **277** 241–54

Gross E L 1996 *Oxygenic Photosynthesis: The Light Reactions* ed D R Ort and C F Yocum (Dordrecht: Kluwer) pp 413–29

Gross E L and Pearson D C Jr 2003 Brownian dynamics simulations of the interaction of Chlamydomonas cytochrome f with plastocyanin and cytochrome c6 *Biophys. J.* **85** 2055–68

Haehnel W, Propper A and Krause H 1980 Evidence for complexed plastocyanin as the immediate electron donor of P-700 *Biochim. Biophys. Acta* **593** 384–99

Haehnel W, Ratajczak R and Robenek H 1989 Lateral distribution and diffusion of plastocyanin in chloroplast thylakoids *J. Cell Biol.* **108** 1397–405

Hope A B 2000 Electron transfers amongst cytochrome f, plastocyanin and photosystem: I. Kinetics and mechanisms *Biochim. Biophys. Acta* **1456** 5–26

Kaant A, Young S and Bendall D S 1996 The role of acidic residues of plastocyanin in its interaction with cytochrome f *Biochim. Biophys. Acta* **1277** 115–26

Kovalenko I B, Ustinin D M, Grachev N E, Krendeleva T E, Kukarskih G P, Timofeev K N, Riznichenko G Y, Grachev E A and Rubin A B 2003 Cyclic electron transport around photosystem 1: an experimental and theoretical study *Biophysics* **48** 614–23

Pearson D C Jr and Gross E L 1998 Brownian dynamics study of the interaction between plastocyanin and cytochrome f *Biophys. J.* **75** 2698–711

Rienzo F, Gabdoulline R, Menziani M, Benedetti P and Wade R 2001 Electrostatic analysis and Brownian dynamics simulation of the association of plastocyanin and cytochrome f *Biophys. J.* **81** 3090–104

Schlarb-Ridley B G, Mi H, Teale W D, Meyer V S, Howea C J and Bendall D S 2005 Implications of the effects of viscosity, macromolecular crowding, and temperature for the transient interaction between cytochrome f and plastocyanin from the cyanobacterium *Phormidium laminosum Biochemistry* **44** 6232–8

Ubbink M, Ejdebeck M, Karlsson B G and Bendall D S 1998 The structure of the complex of plastocyanin and cytochrome f, determined by paramagnetic NMR and restrained rigid-body molecular dynamics *Structure* **6** 323–35

Ullmann G M and Knapp E-W 1999 Electrostatic models for computing protonation and redox equilibria in proteins *Eur. Biophys. J.* **28** 533–51

Ullmann G M, Knapp E-W and Kostic N M 1997 Computational simulation and analysis of dynamic association between plastocyanin and cytochrome f: consequences for the electron-transfer reaction *J. Am. Chem. Soc.* **119** 42–52