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Electron transfer between cytochrome c_2 and the isolated reaction center of the purple bacterium *Rhodobacter sphaeroides* ☆

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Abstract

A short review is presented to account for the functional aspects of electron transfer from cytochrome c_2 to isolated *Rhodobacter* (*Rb.*) *sphaeroides* reaction centers. It incorporates new developments from several laboratories and from my own.

Key words: Electron transfer; Cytochrome c_2; Reaction center; (*Rb. sphaeroides*)

1. Introduction

Electron transfer is an essential function of most bioenergetic systems. It takes place either within large protein complexes containing several redox centers held in a well-defined geometry (this is the case for photosynthetic reaction centers, cytochrome b/c complexes, cytochrome oxidases, nitrate reductase, etc.), or between partners which are not always associated: they form transient complexes which are the seat of electron transfer. There are many instances of this latter situation, which usually involves a small soluble protein (such as cytochrome c, ferredoxin, plastocyanin, ...) which shuttles electrons from one large complex to another. These systems face interesting problems of protein-protein recognition, because the reactions have to be specific (i.e., take place between the right donor-acceptor couple) and of positioning, because the electron transfer reaction rate is highly sensitive to the distance between redox centers. Many systems have been actively studied, such as cytochrome c/cytochrome oxidase, cytochrome f/plastocyanin, etc. with the objectives of answering the following questions:

- the mechanism of complex formation;
- the structure of the complex and the nature of the interactions which determine that structure (is there a unique structure, or several well-defined structures, or a large number of slightly different structures in equilibrium?);
- the factors which determine the rate of electron transfer in the complex (driving energy, reorganization energy, effects of the protein medium, ...).

It is important, however, to focus on good models which could allow a deeper insight in the structure/function relationships. The cytochrome c/cytochrome c peroxidase system has been much studied in this respect because the partners have a known structure, mutants have been obtained and recently the atomic structure of the complex has been solved by X-ray crystallography [1]. The cytochrome c_2/reaction center complex, however, may prove to be a much better model, essentially because: (i) the kinetics of electron transfer can be studied very precisely by flash absorption spectroscopy; (ii) photoinduced electron transfer takes place at low temperature, a property of importance when nuclear factors of the reaction are investigated; (iii) the atomic structure of the complex is on the way to being determined [2].

In purple photosynthetic bacteria, cytochrome c_2

Abbreviations: RC, reaction center; P, primary electron donor; cyt c_2,cytochrome c_2.

☆ Dedicated to Professor Achim Trebst in honor of his 65th birthday.

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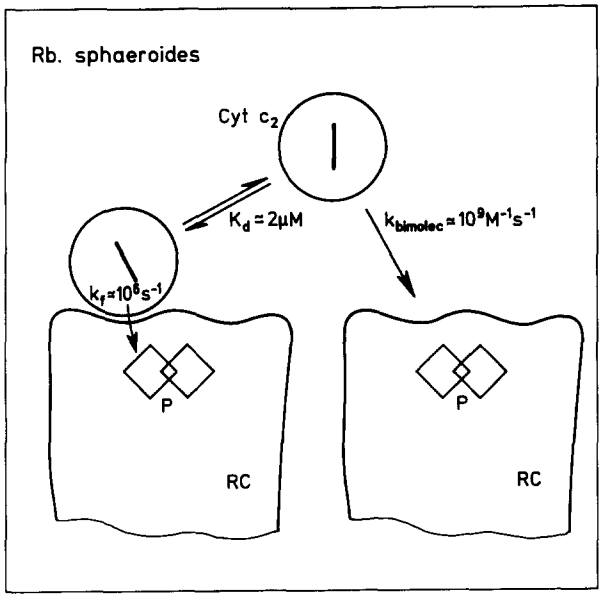

Fig. 1. Scheme for the functional interaction of cytochrome c_2 with isolated *Rb. sphaeroides* reaction centers. k_f: rate of fast electron transfer for docked cytochrome c_2. Free cytochrome c_2 is in equilibrium with the bound state and can also react bimolecularly with free reaction centers (presumably via a unique bound state).

picks an electron from the b/c_1 complex and then reacts with the reaction center to reduce P^+, the primary electron donor (a dimer of bacteriochlorophyll) which has been oxidized by light. The literature on this system is discussed in detail in Refs. [3] and [4]. I will summarize some of our recent results on the in vitro behavior of the cytochrome c_2/reaction center (*Rb. sphaeroides*) system and discuss a few of their implications.

2. Kinetic model

We have studied the rate of P^+ re-reduction with reaction centers without cyt c_2 (P^+ is reduced in hundreds of milliseconds by back-reaction with a bound quinone, Q_A^- or Q_B^-) and following addition of a variable concentration of reduced cyt c_2. The results can be described by the model of Fig. 1, where the dissociated and bound partners are in equilibrium with a dissociation constant $K_d \approx 2\ \mu M$; electron transfer in the complex is practically monophasic with $k_f \approx 10^6\ s^{-1}$; and unbound partners react following a bimolecular reaction with $k_{bimolec} \approx 1 \cdot 10^9 \cdot M^{-1}s^{-1}$. This behavior has been observed with cyt c_2 prepared in several laboratories and several kinds of reaction centers were utilized: the wild-type strain named 2.4.1, the R-26 (carotenoidless) strain from two laboratories; two strains reconstituted from reaction center deletion strains (Refs. [5–7] and P.M., unpublished data). The R-26 reaction center has been studied either solubilized in the detergent LDAO or incorporated in phospholipids liposomes, and nearly identical results were obtained [6].

The model to which we arrive is the simplest possible for electron transfer from a small water-soluble protein to a large membrane complex. It was proposed by Rosen et al. [8] who, however, did not present detailed data. Several groups [9,10] proposed a more complex model with two possible binding sites, named proximal and distal, in a 1:1 equilibrium, with half-times $t_{\frac{1}{2}}$ for electron transfer of 1 μs and 100 μs. Tiede et al. recently suggested that the distal site may be due to the presence of reaction centers dimers with only one cyt c_2 bound [4]. There is, however, no firm basis for that proposal. In our work we found no evidence for a distal site. In addition to aggregation, several experimental problems may have been at the origin of the kinetic behavior attributed to a distal site: insufficient time resolution, presence of salts brought together with cyt c_2, non-specific binding of cyt c_2, excessive size of the measured aborbance change. The only clear deviation we have found in R-26 reaction centers could be attributed to the triplet state of P, formed in reaction centers where Q_A is reduced and having a $t_{\frac{1}{2}}$ in the 10 μs range. This phase was not present in carotenoid-containing reaction centers where ^3P is deactivated very quickly by energy transfer to a carotenoid molecule. We recently used the proximal/distal model to discuss the results obtained with L162 mutants (tyrosine replaced by other amino acids) [5]. The reasons for this choice were twofold: (i) the proximal/distal model constituted a well-accepted frame for an interpretation of our data; (ii) an effect of glycerol was better understood within that model. This aspect deserves some discussion. Glycerol is often added to increase the viscosity of the solution and (in situations equivalent to that shown in Fig. 1) an effect of glycerol is usually intepreted as a slowing down of a bimolecular reaction. This type of reasoning was employed by Moser and Dutton [10], as well as by us [5], to conclude that 'reorientation' toward the proximal site of cytochrome c_2 bound at a distal site involves molecular migration. According to a few recent results, however, glycerol may have other effects and change the dissociation constant. Local effects have been reported on the structure of proteins (e.g., Ref. [11]) and glycerol is well known to stabilize the structure of proteins [12]. More directly relevant to our subject, glycerol (like other solutes) increases the potential of water in a solution and thus influences protein structure when different conformations are differently solvated [13,14]. Protein–protein interactions are accompanied by a change in protein solvation and are thus susceptible to modification by addition of glycerol. So it should not be taken for granted that glycerol does not change the K_d of cyt c_2-RC complexes: as a matter

of fact, we observed a substantial increase of K_d with 60% glycerol [6]. In our study of the kinetics of P^+ re-reduction by cyt c_2 in mutated reaction centers where Y L162 is replaced by other amino acids, we found that in the two strains with aromatic residues (Y, F) the kinetics were slowed down by glycerol much more than in strains with aliphatic residues (L, G) [5]. It is quite possible that these differences reflect a differential effect of glycerol on affinity and docking rather than a simple effect of viscosity. To conclude on this question, I think that it is not possible to decide between the one-site and the two-site model in the case of the L162 mutants of *Rb. sphaeroides*.

Our kinetic experiments [5–7] can be interpreted by a single rather well-defined binding site for cyt c_2 on isolated *Rb. sphaeroides* wild-type reaction centers. They do not exclude other binding sites provided they do not inhibit binding at the site responsible for the ≈ 1 μs electron transfer. The most detailed biochemical experiments on binding were reported by Rosen et al. [15,16]. From equilibrium dialysis, these authors concluded to a 1:1 stoichiometry between cyt c_2 and the reaction center in the complex. They found no evidence for a structural heterogeneity in binding.

3. Other problems studied

(1) Role of tyrosine L162. In all purple bacteria, the cofactors for the primary photoreactions are held by two polypeptides named L and M. The amino acid sequence of these polypeptides has been determined for several species, and a tyrosine residue has always been found at the position 162 of subunit L. This residue is located on the potential path of electrons from the cytochrome to P^+ in *Rps. viridis* and in *Rb. sphaeroides* [17,18]. Its conservation led to the hypothesis that it plays a specific role, perhaps by facilitating electron transfer from the cytochrome to P^+. This hypothesis was tested with *Rb. sphaeroides* mutants made in D. Oesterhelt's laboratory, with Tyr L162 replaced by several other amino acids [19]. From a detailed study it was concluded that electron transfer to P^+ is impaired in the mutants because docking of cytochrome c_2 is severely disturbed [5]. Recently, similar mutants were made with the bacterium *Rps. viridis* where the tetrahemic cytochrome is firmly bound to the LM complex; it was found that electron transfer from the cytochrome to P^+ is not greatly disturbed in the mutants (Dohse, B., Mathis, P. and Oesterhelt, D., unpublished data). It can thus be concluded that Tyr L162 does not play any specific role in facilitating electron transfer, but that it plays a key role for the binding of soluble cytochrome c_2 to the reaction center.

Two models have been proposed for the docking of cytochrome c_2: one in which it is nearly on top of P [18] and one in which it is located asymmetrically toward the M subunit [4]. This latter positioning is favored by the recent structural data on co-crystals of reaction centers and cytochrome c_2 [2]. The large effect of replacement of Tyr L162 is, however, difficult to understand with this geometry (conversely, it is easier to understand in the model of Allen et al., Ref. [18]).

(2) Lack of role of the C-terminus of subunit M. It is usually assumed that electrostatic interactions play a key role in the binding of cytochromes to their functional partners, as shown by the effects of ionic strength and of chemical modification of residues (see, for example, Ref. [20] for cytochrome *c*, Ref. [3] for cytochrome c_2). In the models for the docking of cyt c_2 to the reaction center, it appears that the C-terminus of subunit M is close to the binding site (Refs. [4,18]; see also Refs. [21,22]). In the laboratory of J. Allen, mutants of *Rb. sphaeroides* have been made in which the last 10 residues at the C-terminus of M have been either deleted, or replaced by their equivalent in Rb. capsulatus, or replaced by the long extension found in *Rps. viridis*. Electron transfer from cyt c_2 to P^+ has been studied in all four strains and analyzed in terms of K_d for dissociation of the complex, of intra-complex electron transfer rate k_f, and of rate of bimolecular reaction (see Fig. 1) [7]. Without going into a detailed description of the results, it appears essentially that these three parameters are nearly the same in all four strains. This result is fairly surprising (specially when one considers that substitution of Tyr L162 has dramatic effects). It has to be analyzed in detail with respect to docking models.

(3) Effect of temperature on electron transfer in the complex. The effect of low temperatures on the rate of electron transfer from cytochrome c_2 to P^+ in the bound complex has been studied in collaboration with G. Venturoli [6,23]. Two features were observed:
- between 300 and 240 K, the reaction rate decreases, following an Arrhenius behavior with an activation energy $E_a = 213$ meV.
- when the temperature is lowered below 260 K, the extent of fast electron transfer decreases; it is negligible below 240 K and the temperature of half decrease is 252 K. A similar behavior has been found in *Rps. viridis* where the *c* cytochrome is bound to the reaction center [24]. Among several possible interpretations, we proposed that electron transfer stops because structural rearrangements associated to cytochrome oxidation are blocked at low temperature, supposedly because of water freezing in the protein [6,23].

It should be pointed out that, at temperatures around 250 K where the fast phase has about 50% of its maximum amplitude, this phase remains monoexponential. These are conditions where eventual sub-states of the complex would not quickly equilibrate, leading to a multiphasic reaction.

4. Conclusions

The manner in which proteins interact for electron transfer has been interpreted according to several models. Some views place emphasis on local interactions (such as salt bridges between lysine residues and carboxylates), leading to a precise structure for the complex [1,18]. At the other extreme, some authors give more importance to surface diffusion of the partners or consider a large number of protein-protein complexes with a different geometry [25,26]. An intermediate view consists in giving importance to patches of amino acids of opposite charges leading to an intermediate accuracy in the geometry of the complex [4]. It seems quite probable that there is no unique situation and that different points of view reflect different properties of the proteins. In the case of the complex between cytochrome c_2 and the *Rb. sphaeroides* reaction center, I would favor the hypothesis of a unique well-defined complex, for the following reasons:

– the rate of electron transfer k_f is well-defined (exponential kinetics) and it is not influenced by addition of glycerol [6];

– a cross-linked complex displays electron transfer kinetics very similar to those in the normal complex [16];

– lowering of temperature induces a modest increase of the rate k_f, without appearance of a multiphasic behavior, except for the sudden block which happens around 250 K.

As a model for electron transfer, this system still has to be studied in detail, as mentioned in the Introduction. One of its most interesting aspects is the existence of a counterpart in *Rps. viridis*, which has many properties in common [23], and which has a well-defined and well-known structure.

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