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Partial equilibration of photosynthetic electron carriers under weak illumination: a theoretical and experimental study

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When submitting photosynthetic material to weak illumination, in uncoupled conditions, quasi-equilibrium states of the redox carriers are attained that should presumably reflect the equilibrium constants deduced from the mid-point potentials. However, the apparent constants experimentally obtained are, in several instances, much lower than expected. In order to account for such discrepancies, we examine the consequences of 'clustering' of neighboring carriers, assuming rapid equilibration within a cluster, but much slower relaxation towards the intercluster equilibrium. A general treatment is developed and its predictive content is discussed. Its application to two experimental systems is described, namely the donor chain of isolated reaction centers of *Rhodopseudomonas viridis*, and the donor chain of *Rhodobacter sphaeroides* in vivo, investigated in the accompanying paper (Joliot, P., Verméglio, A. and A. Joliot) (1989) Biochim. Biophys. Acta 975, 336–346). Possible extension of this class of hypothesis to the donor chain of Photosystem I and to the acceptor chain of Photosystem II in chloroplasts is suggested.

Introduction

The thermodynamic properties of the electron-transfer chains involved in the various photosynthetic apparatus (oxygenic or bacterial) can be characterized by measuring the mid-point redox potentials of the carriers. Such redox titration experiments are usually run using mediators at low concentration, with the purpose of establishing thermodynamic equilibrium between the measuring electrode, the solution, and the carriers of the photosynthetic chain. A basic technical problem arising when titrating components of a membrane system is the equilibration rate between the mediators and the carriers embedded within very hydrophobic proteins. This equilibration process is often rather slow (several minutes), and it may sometimes be difficult to ascertain that a true equilibrium state has been reached. Nevertheless, provided sufficient caution is given to this difficulty, this technique gives reliable results, although, as discussed by Walz [1], it may not be straightforward to derive true thermodynamic parameters therefrom. With the exception of a few particular cases, a consensus has now been reached as to the midpoint potentials of most carriers in the oxygenic or bacterial photosynthetic chains. Indeterminacies seldom exceed a few tens of millivolts. From these values for the mid-point potentials, equilibrium constants are readily obtained. On the other hand, equilibrium constants can also be estimated through another methodology, where mediators are not required. This is done by measuring the redox state of the carriers (using some appropriate technique, e.g., absorption spectroscopy, chlorophyll fluorescence, oxygen evolution rate) under equilibrium or close to equilibrium conditions. Such is expected to be the case in the dark-adapted state, or under a sufficiently weak illumination so that the rate of electron flow through the chain is strictly limited by the photochemical turnover. Many experiments of this type have been run, using either the unicellular alga Chlorella, or isolated spinach chloroplasts. Joliot, Joliot and Kok [2] measured, in chloroplasts, the redox state of the primary quinonic acceptor of PS II and of the primary donor of PS I at steady state under limiting intensity illumination. The Q_A-P-700 equilibrium constant thus obtained ranged between 5 and 10. On the other hand, the difference in the mid-point potentials of both carriers is on the order of 450 mV, which corresponds to an equilibrium constant greater than 10^7, in dramatic disagreement with the foregoing measurements. It should be stressed that these experiments were run in totally

Abbreviations: PS I (II), Photosystem I (II).

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uncoupled chloroplasts, excluding energy conservation under the $\Delta\tilde{\mu}_{H^+}$ form. Besides, varying the illumination intensity by a factor of 10 (within the light-limited range) did not significantly alter the value of the apparent equilibrium constant. In the case of living algal cells, submitted again to weak illuminations at various wavelengths, an apparent equilibrium constant of 1 was obtained between Q_A and the plastoquinone pool [3], whereas a value close to 50 would be expected from the mid-point potential values. Forbush and Kok [4] also obtained values close to 1 for this constant, using isolated chloroplasts in the absence of electron acceptor. Similar discrepancies arise when measuring apparent equilibrium constants under weak illumination between the plastoquinone pool, the secondary donors of PS I and the primary donor P-700 [5]. The values obtained are all smaller than 10, whereas the true equilibrium constants are expected to range between 10^2 and 10^5. The investigation of the donor chain of a photosynthetic bacterium reported in the previous paper [6] reveals disagreements of the same order between the equilibrium constants measured under weak illumination or computed from redox titrations.

All the foregoing equilibrium constant determinations, that give blatant disagreement with the titration data, were obtained under weak illumination conditions. On the other hand, the measurements carried out in the dark-adapted state, even in the absence of exogenous redox mediators, yielded results that are consistent with the titration data. Two main lines of interpretation may be considered. First, one may assume that thermodynamic quasi-equilibrium is achieved under weak light illumination, but the thermodynamic constants would be, somehow, changed by the illumination. Such an interpretation was more or less hinted in the early papers mentioned above. However, the idea of a dramatic switch of the thermodynamic properties of the system under weak illumination – and in uncoupled conditions – has become still more difficult to substantiate, both on theoretical and experimental grounds. Another class of interpretation consists in assuming that true equilibration is not achieved under weak illumination (ranging from 5 to 50 absorbed photons per center and per second) in spite of this illumination being intensity-limiting. This apparent contradiction may be solved in the following way. First, there must be rapid partial equilibration processes, accounting both for the known kinetic constants and for the fact that, under the weak illumination conditions described above, the evolution of the system is completely controlled by the amount of absorbed photons (halving the intensity results in doubling the time scale with no other change in the kinetics). On the other hand, one must assume that the pathways leading to total equilibration are much slower. Depending on the process by which electrons are removed or added to the system, states of partial equilibration are attained, which should account for the anomalous apparent equilibrium constant found under continuous illumination. We investigate in this paper a particular hypothesis of this type, in which fast electron transfer and equilibration occur within limited clusters of neighboring carriers. On the other hand, equilibration between clusters is assumed to be a very slow process, requiring, say, several tens of seconds. As shown below, this hypothesis provides a simple interpretation, at least in some instances, for the discrepancy between apparent and true equilibrium constants. Indeed, a very good quantitative agreement with the experimental data is obtained in the case of bacterial donor chains. The Q_A-P-700 apparent equilibrium is a more difficult problem which may require additional – or different – assumptions, as will be discussed later. The basic content of the cluster hypothesis is a large kinetic gap between the fast equilibration occurring within a cluster, and the slow equilibration between clusters. Thus, the structural entities involved may range from the membrane protein complex (for instance, a reaction center complex) to more extended domains involving soluble components (plastoquinone or plastocyanin), provided diffusion between these domains occurs only on a markedly slower time-scale than it does within one domain. A theoretical investigation of this hypothesis is reported in the next sections. A quantitative treatment is then given for two examples involving the donor chains of *Rps. viridis* and *Rb. sphaeroides*.

Theoretical section

We consider a complex – or 'cluster' in the meaning discussed above – involving several rapidly interacting redox carriers, one of them being a primary photochemical reactant, for instance a donor chain with a primary photochemical donor and some secondary donors. We shall focus on the redox states of the carriers resulting from photochemical turnover (either saturating flashes or weak continuous light), assuming a time-range in which equilibration is achieved within a complex, but not between complexes. A convenient index to characterize these states is the apparent equilibrium constant K_{app} between two carriers. This is the ratio of the oxidized over reduced fractions for both components, which equals the true equilibrium constant when equilibrium is achieved. It thus indicates whether the distortions from equilibrium in a given situation tend, e.g., to over-oxidize a low potential carrier and over-reduce its higher potential partner. These metastable states will eventually relax slowly towards equilibrium (through intercomplex interactions) where K_{app} and the true constant K_{eq} coincide.

Before treating a more general case, the main points we wish to make may be exemplified in the simple case of a binary complex.

The two-partner complex

Let AB denote the complex, where A and B are one-electron redox centers with mid-point potentials E_A and E_B. It is assumed that the redox state of one of the partners does not influence the mid-point potential of the other. The oxidation degree of the complex can be 0 (state AB), 1 (states A^+B and AB^+), or 2 (state A^+B^+). In the absence of inter-complex equilibration, there is not a single equilibrium state for a given content of plus charges in the overall system: it is also necessary to specify the distribution of the complex among the three sub-classes (oxidation degree 0–2). From an experimental standpoint, accessible quantities are generally the oxidized fractions of A and B, from which one may compute an apparent equilibrium constant:

$$K_{app} = \frac{[A]}{[A^+]} \Big/ \frac{[B]}{[B^+]} \tag{1}$$

When inter-complex equilibration is allowed to occur, K_{app} is equal to the true equilibrium constant K_{eq}, related to the difference of the midpoint potentials of the two couples. If the complexes are isolated, however, K_{app} will differ from K_{eq}, in a manner that depends on the history of the system, i.e., the statistical factors which have determined its particular distribution among the three sub-classes.

Within the sub-population of complexes with oxidation degree 1, one has:

$$\frac{[AB^+]}{[A^+B]} = \exp\frac{E_A - E_B}{RT/F} = K_{eq} \tag{2}$$

where R is the gas constant, T the temperature, and F the Faraday constant. Here, and throughout the paper, we assume $K_{eq} \geqslant 1$ (thus, $E_A \geqslant E_B$). Eqn. 1 expresses intra-complex equilibration, and remains true all the more when inter-complex equilibrium is achieved. We now assume that only the former type of equilibrium is achieved, and that all the complexes have been prepared with oxidation degree 1, for instance, through a flash-induced photochemical reaction. The apparent equilibrium constant is easily obtained from Eqn. 1 by noticing that, since the only available states are A^+B and AB^+, $[A]/[A^+] = [B^+]/[B] = [AB^+]/[A^+B]$. Therefore,

$$K_{app} = \frac{[AB^+]^2}{[A^+B]^2} = K_{eq}^2 \tag{3}$$

Thus, the apparent equilibrium constant is equal under such conditions to the square of the true constant, meaning that the low-potential partner is over-oxidized in comparison with the inter-complex equilibrium situation. During the relaxation towards inter-complex equilibrium, a net electron flow from the high- to the low-potential partner will take place. This may be easily rationalized by considering that the newly accessible states, AB and A^+B^+ can only be formed in equal amounts, according to the dismutation reaction:

$$2(AB)^+ \rightarrow AB + A^+B^+$$

Therefore, if $K_{eq} > 1$, most of the $(AB)^+$ complexes are in the AB^+ form, whereas the dismutation reaction results in the formation of equal amounts of A^+B^+ and AB, thus a net increase of A^+ and decrease of B^+. If $K_{eq} = 1$, $K_{app} = K_{eq}$, and no net transfer occurs during the relaxation. However, this does not mean the intra-complex and inter-complex equilibrium situations are thermodynamically equivalent. Actually, the relaxation corresponds to an entropy increase $\Delta S = R \ln 2$, since in the relaxed state, each complex has equal probabilities to be found in one of four states, instead of two in the initial situation. The free-energy decrease is also $\Delta G = -RT \ln 2$, since the process is purely entropic in this case.

The situation we have considered, namely, all the complexes put in the singly oxidized state, is not uncommon in experimental practice. For instance, the Q_AQ_B acceptor system of PS II or bacterial centers can easily be set in the singly reduced state through a saturating short flash illumination of the oxidized system:

$$Q_AQ_B \xrightarrow{h\nu} Q_A^- Q_B \rightarrow Q_AQ_B^-$$

The experimental estimate of K_{eq} in this case should make use of Eqn. 3 rather than Eqn. 1.

We shall now focus on another distribution of the complexes among the three subclasses with oxidation degree 0, 1 and 2, resulting from the use of continuous illumination instead of one single turnover flash. It turns out that the deviation of K_{app} with respect to K_{eq} is now in the opposite direction. This may be easily seen by considering the case when $K_{eq} \gg 1$, so that most of the singly oxidized complexes are in the AB^+ state (AB is here assumed to be a donor chain, in which the high-potential partner A is a photochemical donor). The efficiency of photochemical conversion is then about the same whether a photon hits a totally reduced or singly oxidized complex. Assuming the complex is initially totally reduced, the population of the various states may thus be obtained from a Poisson distribution $P_m(n) = m^n e^{-m}/n!$, where n is the number of photochemical hits on a complex, m the mean of this quantity:

$$[AB] = P_m(0) \tag{4a}$$

$$[AB^+] + [A^+B] \approx [AB^+] = P_m(1) \tag{4b}$$

$$[A^+B^+] \approx 1 - (P_m(0) + P_m(1)) \tag{4c}$$

where Eqns. 4b and 4c make use of the fact that $K_{eq} \gg 1$. Thus:

$$K_{app}(m) = \frac{([AB]+[AB^+])([AB^+]+[A^+B^+])}{[A^+B^+][AB]}$$

$$= \frac{(1+m)(1-e^{-m})}{1-e^{-m}(1+m)} \quad (5)$$

Taking, e.g., $m = 1$, one gets: $K_{app} \approx 4.8$. Thus, when K_{eq} is large enough, K_{app} is not controlled by thermodynamic factors, but only by the statistics of photochemical conversion on the complex. Fig. 1 shows simulations obtained from the general equations derived in the next section, for the AB complex under weak continuous illumination at various values of K_{eq}. For $K_{eq} = 2$, the $[A] = f([B])$ curve is still close to the equilibrium curve. For $K_{eq} > 10$, a limiting curve is obtained, close but significantly different from the equilibrium curve $K_{eq} = 4.8$ (see also Fig. 2).

The general case

We now consider a N-partner complex, with carriers at m different mid-point potentials. One assumes n_1 redox centers have mid-point potential E_1, n_2 have E_2, and so forth, so that $n_1 + n_2 + \ldots n_m = N$. For definiteness, we treat the case of a donor chain, assuming $E_1 > E_2 > \ldots > E_m$, and where the species 1 is the photochemical donor. We want to compute the redox state of the various carriers when the complexes are submitted to either flashing or weak continuous illumination. In the absence of inter-complex equilibration. This amounts to a stochastic Markov process in which the states of the system are defined by the number of oxidized equivalents present on a complex. The transition probabilities between these states are calculated from the distribution of photochemically open reaction centers (reduced primary donors). Therefrom, the master equation describing the evolution of the probabilities of each state can be derived. The initial dark-adapted condition may be an inter-complex equilibrium (usually with all carriers in their reduced state).

Charge distribution

Our first step is to calculate the charge distribution on the various carriers among the sub-population of complexes with 1,2,..., N oxidation degree (for brevity, (+) charges). Consider the set of complexes with ν charges. This corresponds to various sub-states with e.g. $\nu_1 \leqslant n_1$ charges on the carriers 1, $\nu_2 \leqslant n_2$ on 2, and so forth, so that $\nu_1 + \nu_2 + \ldots + \nu_m = \nu$. The weighting factor for this particular configuration is:

$$z(\nu_1 \ldots \nu_m) = \binom{n_1}{\nu_1} e^{-\nu_1 E_1/(RT/F)} \ldots \binom{n_m}{\nu_m} e^{-\nu_m E_m/(RT/F)} \quad (6)$$

where $\binom{n}{\nu}$ is the combination number $\binom{n}{\nu} = \frac{n!}{(n-\nu)!\nu!}$. The appropriate normalization factor for the z's is the partition function:

$$Z(\nu) = \sum_{\nu} z(\nu_1 \ldots \nu_m) \quad (7)$$

where Σ_ν denotes summation over all configurations of $\nu_1 \ldots \nu_m$ such as $\nu_1 + \ldots + \nu_m = \nu$. The probability of a given configuration is thus:

$$\bar{z}(\nu_1 \ldots \nu_m) = \frac{z(\nu_1 \ldots \nu_m)}{Z(\nu)} \quad (8)$$

The fraction $\varphi(i,\nu)$ of the oxidized form of carrier i, within the complexes possessing ν charges is therefore:

$$\varphi(i,\nu) = \sum_{\nu} \frac{\nu_i}{n_i} \bar{z}(\nu_1 \ldots \nu_m) \quad (9)$$

Equilibrium distribution

The initial distribution (dark-adapted state) of the sub-populations of 0 to N-oxidized complexes may be assumed to be an inter-complex equilibrium. We calculate here this particular type of distribution, at some ambient redox potential E.

Let ρ_i be the oxidized fraction of carrier i equilibrated at potential E. It is easily found that:

$$\rho_i = \frac{1}{1+\exp\frac{E_i - E}{RT/F}} \quad (10)$$

The probability of the ith carrier having ν_i charges is:

$$\pi_i(\nu_i) = \binom{n_i}{\nu_i} \rho_i^{\nu_i} (1-\rho_i)^{n_i - \nu_i} \quad (11)$$

Therefore, the probability of ν charges on the complex is:

$$\pi(\nu) = \sum_{\nu} \pi_1(\nu_1) \ldots \pi_m(\nu_m) \quad (12)$$

Evolution equation

We now derive the evolution (master-) equation for $\pi(\nu)$ under flash illumination. Carrier 1 is assumed to be the primary photochemical donor, active only in its reduced state. Considering again the complexes posessing ν charges, the probability of $\mu_1 = n_1 - \nu_1$ of the primary donors being reduced (thus, ν_1 oxidized) is given by:

$$r(\mu_1,\nu) = \sum_{\nu,\nu_1} \bar{z}(\nu_1 \ldots \nu_m) \quad (13)$$

where Σ_{ν,ν_1} means summation over $\nu_2 \ldots \nu_m$, keeping ν_1

constant, and also the sum $\nu_2 + \nu_3 + \ldots + \nu_m = \nu - \nu_1$ constant. The change in $\pi(\nu)$ upon a flash is made up of two contributions. First, the increment $\Delta^+\pi(\nu)$ arising from complexes with less than ν charges that join the ν-charged state upon photo-oxidation. The other contribution is the decrease $\Delta^-\pi(\nu)$ due to all oxidations occurring on the complexes with ν charges.

The general term in the increasing contribution to $\pi(\nu)$ corresponds to complexes with $(\nu - l)$ charges, losing l electrons, thus having $k \geqslant l$ reduced primary donors. One takes into account the photochemical efficiency of the flash by defining the miss parameter α as the probability of a reduced primary donor failing to achieve charge separation. Hence:

$$\Delta^+\pi(\nu) = \sum_{l=1}^{n_1} \sum_{k=l}^{n_1} \pi(\nu - l) r(k, \nu - l) \binom{k}{l} (1-\alpha)^l \alpha^{k-l} \tag{14}$$

The last factors account for the number of ways of selecting k reduced donors among l, and the probability of having l photochemical hits and $k - l$ misses.

The general term for the decreasing contribution corresponds to ν-charged complexes with k reduced primary donors that lose one or more electrons. The probability to do so is $(1 - \alpha^k)$, i.e., one minus the probability of all k reduced donors to undergo a photochemical miss. Accordingly:

$$\Delta^-\pi(\nu) = \pi(\nu) \sum_{k=1}^{n_1} r(k, \nu)(1 - \alpha^k) \tag{15}$$

From Eqns. 14 and 15 we get the evolution equation that gives the net change in the $\pi(\nu)$ distribution caused by a flash:

$$\Delta\pi(\nu) = \left(\sum_{l=1}^{n_1} \sum_{k=l}^{n_1} \pi(\nu - l) r(k, \nu - l) \binom{k}{l} (1-\alpha)^l \alpha^{k-l} \right) - \pi(\nu) \sum_{k=1}^{n_1} r(k, \nu)(1 - \alpha^k) \tag{16}$$

Starting from a given $\pi(\nu)$ distribution, such as the equilibrium one given by Eqn. 12, its changes during a series of flashes is obtained by iterating Eqn. 16. At each step, the fractions of the oxidized form of each carrier may be obtained using Eqn. 9:

$$\frac{[\text{carrier } i \text{ oxidized}]}{n_i} = \sum_{\nu=0}^{N} \pi(\nu) \varphi(i, \nu) \tag{17}$$

The case of a weak continuous illumination is easily dealt with as a limiting case of flash illumination when $\alpha \to 1$, that is, approximating continuous light as a series of largely non-saturating flashes. Clearly, this procedure may fail to simulate the real time-course of reduction whenever the photochemical efficiency depends cooperatively on the primary reactant state, e.g., if energy transfer from closed traps, within a complex or between complexes, takes place. Nevertheless, the distribution of charges among the various carriers should be reliably obtained, for instance in a plot [reduced carrier i] versus [reduced carrier j] with the number of flashes as a parameter.

Simulations

We consider here a complex with carriers of two distinct mid-point potentials ($m = 2$). Let us again call A the high-potential species (photochemical donor), and B the low potential one. Figs. 1–4 show plots of (reduced) [A] versus (reduced) [B]. The initial state was taken at total reduction of both A and B. The evolution under weak light was simulated as explained above by a series of nonsaturating flashes, using Eqns. 16–17. The miss parameter α was given a value of 0.98. Increasing α further ($\alpha < 1$) caused negligible change in the output (the [A] vs. [B] plot). Fig. 1 shows the effect of varying the relative mid-point potentials (i.e., the K_{eq}) of A and B in a two-partner complex ($n_1 = n_2 = 1$), and was already discussed in the subsection dealing with the two-partner complex. The major conclusion is that when $K_{eq} > 10$, the [A] vs. [B] plot becomes independent of K_{eq} and approaches a low equilibrium constant ($K_{app} \simeq 5$) relation.

Fig. 2 shows the effect of increasing N (the number of partners within a complex) at constant stoichiometry ($n_1 = n_2$) and constant K_{eq} ($= 100$). Also shown are the equilibrium plots for $K_{eq} = 100$ and $K_{eq} = 4.8$. As discussed earlier the curve for the 1–1 complex is close to the $K_{eq} = 4.8$ equilibrium curve, although significantly different, especially when B is highly reduced. Increasing N gradually shifts the curves towards the $K_{eq} = 100$ equilibrium curve. However, the 'cluster' effect is still quite noticeable for the 11–11 complex.

Fig. 3 shows the effect of varying the stoichiometry, keeping $n_1 = 1$ and $K_{eq} = 100$. Again, increasing n_2 shifts the curves towards the equilibrium plot. However, it is noteworthy that large differences still persist for the 1–100 complex, now especially about the end of the kinetics (B mostly oxidized).

An example of the flash-induced evolution is presented in Fig. 4. A complex AB_3 was used with $K = 20$. The points indicate the successive states of the system submitted to a series of flashes with various miss coefficients. As explained when discussing the two-partner complex, when the flashes are saturating, and 100% efficient ($\alpha = 0$), the apparent equilibrium constant is higher than the true one. On the other hand a low apparent constant is obtained for $\alpha = 0.5$, not very far from the weak continuous light curve (simulated with $\alpha = 0.98$). With $\alpha = 0.15$, i.e., close to the experimental situation when using saturating flashes, an intermediate

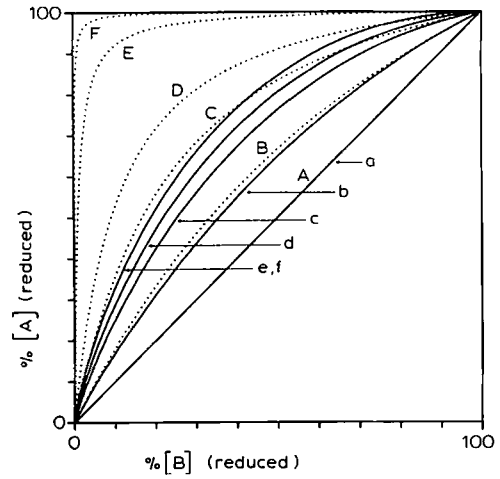

Fig. 1. A plot of [A] vs. [B] for a two-partner complex with various K_{eq} values. The solid lines (a–f) show the simulated evolution under weak light illumination as described in the text, starting from A and B totally reduced. The dotted lines (A–F) are the corresponding (inter-complex) equilibrium curves, a and A, $K_{eq} = 1$; b and B, $K_{eq} = 2$; c and C, $k_{eq} = 5$; d and D, $k_{eq} = 10$; e and E, $K_{eq} = 100$; f and F, $K_{eq} = 1000$. Curves a and A on the one hand, e and f on the other hand, are superimposed.

pattern is obtained which happens to approach roughly the true equilibrium curve. The continuous light regime is, generally speaking, a more discriminating tool for detecting cluster structuration.

A conclusion from these simulations is that the apparent equilibrium constant under weak light conditions is a very sensitive indicator of 'clustering', provided the true constant is not close to 1. Even in cases where the statistical effects might have been expected, at first thought, to be very small, such as the 11–11 complex in Fig. 2 or the 1–100 complex in Fig. 3, rather large discrepancies between K_{app} and K_{eq} are still found. On

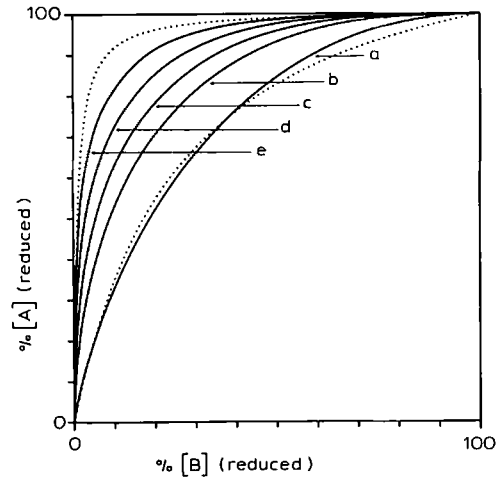

Fig. 2. Effect of varying the cluster size, keeping constant the stoichiometric ratio (1/1) and $K_{eq} = 100$, a, (AB) complex; b, $(AB)_2$; c, $(AB)_3$; d, $(AB)_5$; e, $(AB)_{11}$. The dotted lines are the equilibrium curves for $K_{eq} = 4.8$ (close to a) and 100 (close to e). Other conditions as in Fig. 1.

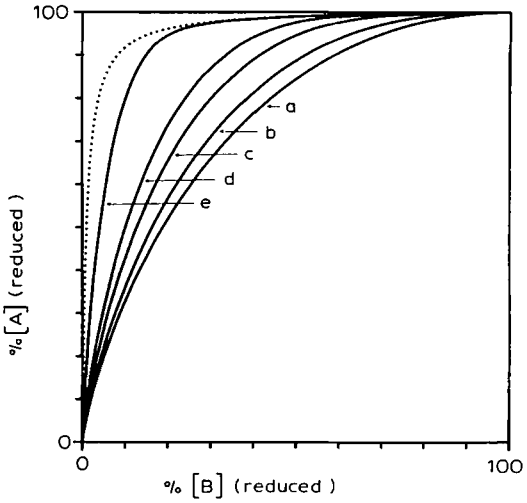

Fig. 3. Effect of varying the stoichiometry of the AB complex, at constant $K_{eq} = 100$. a, (AB); b, (AB_2); c, (AB_5); d, (AB_{10}); e, (AB_{100}). Dotted line, equilibrium curve for $K_{eq} = 100$.

the other hand, as soon as the equilibrium constant is larger than 10, very little information on its precise value can be drawn from the light-induced evolution.

Experimental example

The Rhodopseudomonas viridis reaction center

To give a straightforward illustration of the type of effects we have been discussing, we present here experiments with isolated reaction centers from *Rps. viridis* – a paradigm for membrane electron-transfer complexes. The donor chain includes five electron carriers: the

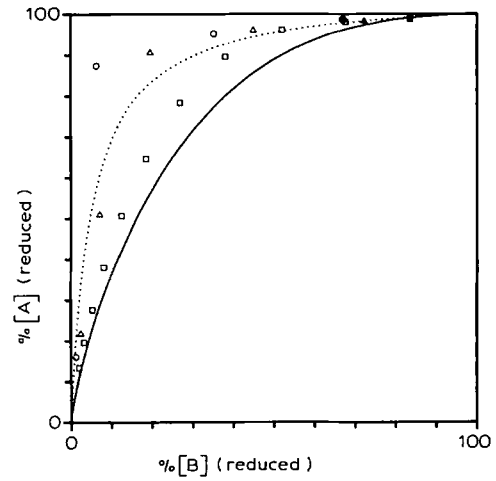

Fig. 4. A plot of [A] vs. [B] simulating a series of flashes, varying the miss parameter α. The complex has stoichiometry (AB_3), with $K_{eq} = 20$. Circles, $\alpha = 0$; triangles, $\alpha = 0.15$; sqares, $\alpha = 0.50$. The symbols show the successive values reached during the flash sequence, starting from [A] = [B] = 100%. Solid symbols were used for the first flash (notice that the points for the first flash with $\alpha = 0$ and second flash with $\alpha = 0.50$ are almost superimposed). The solid line is the continuous light curve ($\alpha \to 1$). The dotted line is the equilibrium curve for $K_{eq} = 20$.

primary donor (the bacteriochlorophyll-*b* special pair, P-960), and four hemes in the cytochrome subunit. However, under moderate redox-potential conditions, such as used here, only the two high-potential hemes are reduced.

Materials and Methods

Reaction centers were isolated and purified from *Rps. viridis* cells grown for 24 h in degassed Hutner medium, according to the procedure of Clayton and Clayton [7]. The reaction centers were suspended in Tris (pH 7), 10 mM, 0.1% LDAO. Ubiquinone-6 was added at a final concentration of 50 μM. Reduction of the high-potential cytochrome hemes was achieved by addition of 200 μM sodium ascorbate. Light-induced absorption changes were measured with an apparatus similar to that described in Ref. 6. Continuous illumination was provided by a 100 W quartz-iodine lamp filtered through two RG 695 Schott filters. Under such illumination, the half-time for cytochrome photo-oxidation was about 50 ms, that is 4 orders of magnitude larger than the half-time for electron transfer from secondary to primary donor. The amount of photo-oxidized primary donor, P-960, was estimated from the difference of the absorption changes at 612 nm and 595 nm. The kinetics of photo-oxidation of cytochromes *c*-559 and *c*-556 were measured separately as the differences $(\Delta A_{560\ nm} - \Delta A_{550\ nm})$ and $(\Delta A_{554\ nm} - \Delta A_{562\ nm})$, respectively, according to the difference spectra reported in Refs. 8–9. This procedure does not eliminate completely the contribution from photo-oxidized P-960, for which an additional minor correction was applied. Alternatively, the total amount of photo-oxidized cytochromes (*c*-559 + *c*-556), denoted c_t, was estimated as the difference $\Delta A_{557\ nm} - \Delta A_{535\ nm}$. 557 nm is a wavelength where both cytochromes have the same oxidized-reduced absorption change.

Using the spectra and extinction coefficients reported in Refs. 7–9, it was checked that the absorption changes measured when photo-oxidation was completed corresponded to a stoichiometry of two c_t's per one P-960. This confirms that dark-adaptation in the presence of ascorbate results in a fully reduced chain. The absorption changes at various times during the photo-oxidation kinetics were measured at the appropriate wavelengths for deconvoluting the amounts of oxidized P-960 and cytochromes as indicated above. These quantities, normalized to their full photo-oxidation extent were plotted in Fig. 5.

Results

Fig. 5 is a plot of the relative amount of photo-oxidized *c*-559, *c*-556, and c_t against the relative amount of photo-oxidized primary donor P-960. The theoretical curves were computed assuming the following values for the mid-point potentials: 500 mV, 380 mV and 310 mV,

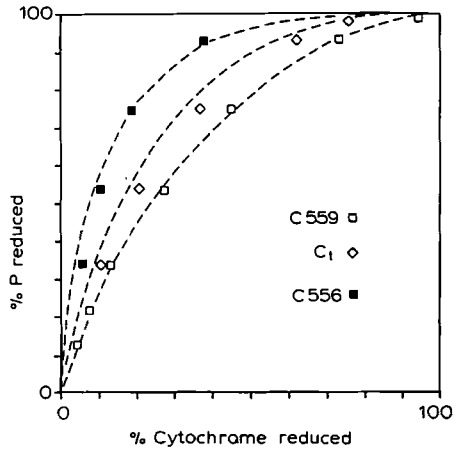

Fig. 5. Experimental results with isolated reaction centers from *Rps. viridis* submitted to weak continuous illumination. The relative amount of reduced P-960 was plotted against that of cytochrome *c*-559 (open squares), cytochrome *c*-556 (closed squares), or c_t, a collective indicator of both cytochromes (open diamonds). The dashed lines are the corresponding simulated curves as explained in the text.

for P-960, *c*-559, and *c*-556, respectively (see Refs. 8–9). A satisfactory fit is obtained between theoretical and experimental curves. Slight deviation in the c_t curve may be ascribed to inaccuracy in its deconvolution. As expected, the apparent equilibrium constant in the light is considerably lower than the constant deduced from redox-titration data. The agreement with the theoretical prediction confirms that no significant inter-complex equilibration takes place during the photo-oxidation kinetics in the absence of added redox mediators. In other experiments (not shown), the ambient potential was poised so as to obtain partial oxidation of the donor chain in the dark-adapted state. The starting point of the cytochrome vs. P curves was then close to the equilibrium curve with high K_{eq}.

By varying the parameters used for simulation, we could investigate several points pertaining to the information that can be gained from such experimental data. As discussed in the subsection dealing with simulations, the behavior of such systems becomes quite insensitive to the value of K_{eq} whenever it exceeds about 10. Such is the case with the viridis donor chain, with a 120 mV jump ($K_{eq} = 100$) from P-960 to *c*-559, and 70mV ($K_{eq} = 15$) between the 559 and 556 hemes. Thus the photo-oxidation data bear very little information on the mid-point potential differences – except that they must be larger than, say, 50 mV. Indeed, varying the mid-point potentials in a reasonable range about the values given above caused negligible change in the simulated curves, except when the gap between the two hemes was taken below 50 mV. On the other hand, the simulation is quite discriminative on the size of the functional 'cluster'. For example, one can definitely exclude (data not shown) a dimeric functional association, where rapid equilibration would occur between a pair of reaction centers.

We also investigated the dark re-reduction of the system, following complete photo-oxidation (not shown). In the presence of ascorbate, total re-reduction is achieved in about 90 s, following multiphasic kinetics. It is noteworthy that the reductive pathway (in a $[c_1]$ vs. [P-960] plot) is again markedly distinct from the equilibrium curve, with K_{app} values lower than K_{eq} (but greater than observed during photo-oxidation). This is to be expected if the reduction process is faster than inter-complex equilibration.

The Rhodobacter sphaeroides donor chain

In the preceding paper [6] were reported the results of an experimental investigation of the donor chain in whole cells of *Rb. sphaeroides*. The data obtained upon flash photo-oxidation in various conditions of ambiant potential suggested that two kinds of structures were present, namely a three-partner complex P–P/c_2, and a five-partner complex, P–P/c_2/c_1–FeS. In both types, a pair of reaction centers P are associated with one cytochrome c_2. In the second type, the *b-c* complex (c_1–Fes) is also part of the 'super-complex', adding two more carriers, namely the *c*-type heme and the Rieske type iron-sulfur center. The relative amount of both types of structures was usually found to be about 30/70%, respectively. The experimental data also gave values for the relative mid-point potentials of the carriers. A miss coefficient $\alpha = 0.12$ for saturating flashes was estimated from the damping in the Q_B/Q_B^- binary oscillation under flash illumination. Using this set of parameters, we could simulate the relative evolution of the redox state of the carriers during a series of single turnover flashes or during weak light photo-oxidation, starting from various equilibrium initial conditions. This was done using Eqns. 12 and 16–17. The photochemical behavior of the complexes was assumed to be independent, so that simultaneous iteration of Eqn. 16 was run for both types of complexes, and the overall states of the carriers obtained from the 30/70 weighting. As may be seen in Fig. 4 of Ref. 6, a quite satisfactory fit with the data was obtained. This provides strong support to the proposed model, since all parameters were obtained independently, and the simulated relations involved no further adjustment.

Conclusion

It is a rather puzzling fact that, as a common rule in photosynthetic systems, a dramatic discrepancy is found when comparing the equilibrium relationship between carriers with that found in the course of even very weak illumination. We have proposed here a basic scheme to explain this effect in terms of 'clusters' of carriers in which rapid equilibration occurs, whereas inter-cluster relaxation takes place on a much slower time-scale. This hypothesis has structural bearings, although the precise meaning of the structures involved remains to be elucidated in most cases. In the case of *Rps. viridis* donor chain, the experimental agreement that was obtained is, in a way, trivial. For here the 'cluster' involved is a very well known – to a few Ångströms, actually – structural entity. The findings in the donor chain of *Rb. sphaeroides* in vivo may seem more surprising (although similar suggestions have previously been put forward, as mentioned in the accompanying paper). Here we have two types of super-complexes, with three and five partners, involving bulky integral membrane complexes (two reaction centers and the *b-c*, in the latter case), and a soluble component, the c_2-cytochrome. Visualizing the five-partner super-complex, at the present stage, may require some imagination: nevertheless, we believe the experimental support for this model to be rather cogent.

The weak light photo-oxidation pattern of the Photosystem I donor chain in chloroplasts (see Ref. 5) exhibits qualitative similarity with that of *Rb. sphaeroides*, displaying an anomalously low apparent equilibrium constant. Taken together with the overall similarity between both systems, this suggests that here also, rapid equilibration occurs only within limited clusters including P-700, plastocyanin, and the b_6-*f* complex, with precise stoichiometries that remain to be determined. Nevertheless, it is quite clear that two or more plastocyanin molecules are present per P-700. This makes the hypothesis of a super-complex implying physical association of all the carriers somewhat harder to visualize. On the other hand, the cluster hypothesis does not really require a super-complex structure. Domains wherein diffusion would be confined would also satisfy the basic condition of rapid partial equilibration.

The problem of the low apparent constant between Q_A and the plastoquinone pool, on the acceptor side of Photosystem II, cannot be solved by the sole cluster hypothesis, at least without further elaboration. The same is true, consequently, for the overall Q_A-P-700 equilibrium. PS II and PS I centers are known to be segregated in different regions of the membrane and connected through pools of mobile carriers (plastoquinone, plastocyanin) that must diffuse over rather large areas. Moreover, it was shown [10] that several (more than two) PS II centers share a common plastoquinone pool. Since the stoichiometry is 5–8 plastoquinone molecules per PS II, this implies a large minimum size for a hypothetical cluster. Simulation of the photo-reduction of clusters of such size does not account for as low a K_{app} as experimentally found. A modification of the cluster hypothesis that may account for the experimental findings is to accept clusters of large size, but with a heterogeneous distribution in their stoichiometries. This would mean in some regions an excess of PS I over PS II, the opposite in other regions. The first situation would account for the over-oxidation of P-700, the latter for overreduction of Q_A. Similarly, a

distribution in the Q_A-PQ stoichiometry may explain the anomalous apparent equilibrium between these carriers. Further study is obviously needed to test and elaborate this line of interpretation.

As a concluding remark, it may be worth noting that the occurrence of quasi-equilibrium states with low apparent equilibrium constants is not just an experimental creation, but should concern broadly the actual physiological functioning of the photosynthetic systems (with complications arising from coupled conditions, higher illumination intensities, and so forth). The redox balance of the carriers under some steady-state regime can be widely different from what could be foreseen from the knowledge of the mid-point potentials. Should the ideas proposed in this paper prove to be of broader applicability, the study of partially equilibrated states could give access to valuable structural information at an intermediate scale between the individual complex and the membrane as a whole.

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