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STRATEGIES FOR MIMICKING THE PRIMARY EVENTS OF BACTERIAL PHOTOSYNTHESIS: STRUCTURE, FUNCTION, AND MECHANISM

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ABSTRACT. Some of the possible implications of the photochemistry of photosynthesis for artificial systems are presented. A major aspect of this paper involves the special conditions required to prevent the undesirable charge recombination via the excited triplet state of the primary donor. If a multi-jump model is operative in the primary events of photosynthesis, then artificial model systems are relatively easy to synthesize. Such systems prevent back reactions via a series of downhill chemical reactions. Each downhill step in energy results in an increase in charge separation distance. The annihilation reactions, even to the less energetic triplet state, involve intermediate states uphill in energy and consequently are greatly diminished. However, if superexchange is a correct explanation of photosynthesis, then model systems have not been developed that properly mimic the natural process. In particular, the triplet back reaction can occur due to the lack of any thermal activation barrier. In nature this downhill back reaction appears to be prevented by carefully balancing the energetics using four molecules in the electron transport system. If this is the case, artificial photosynthesis would also require fine tuning of the coupling and energetics with three or four molecules making duplication more difficult to achieve.

Introduction

Photosynthesis is the natural process of light induced charge separation which occurs in a reaction center (RC) protein within about three picoseconds of singlet state excitation. The resulting electron-hole pair lasts for at least ten nanoseconds in the form of a donor-

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acceptor radical pair separated edge-to-edge by about 11 Å. As a result of this long-lived chemistry, nature has a means of producing efficient charge separation with negligible back reactions. This paper in general describes some of the possible implications of this RC chemistry for artificial photosynthesis with emphasis on special conditions required to prevent charge recombination annihilation via the indirect back reaction to the excited triplet state of the primary donor. The basis for such discussions has evolved from a knowledge of the purple photosynthetic bacteria.

The photosynthetic process is so efficient that every photon absorbed in a RC produces a chemical reaction, and therefore, the initial energy conversion act of photosynthesis proceeds with a quantum yield of nearly unity (>0.95) [1-3]. Consequently, an artificial chemical system utilizing the fundamental energy conversion processes of photosynthesis should be able to duplicate the biological process of energy conversion and storage. Not surprisingly, one goal of RC research is to optimize the use of solar energy in chemical systems for the production of useful compounds and fuels.

The bacterial RC can be described in terms of two main systems, the protein matrix and the donor-acceptor complex. The protein part of the *Rb. sphaeroides* RC is further comprised of three separate protein subunits labeled L-, M- and H-. The L- and M-parts contain the actual donor-acceptor pigments and UQs which perform the electron transfer chemistry. The arrangement of the various pigments in the complex without the protein matrix is shown in the X-ray structure of *Rb. sphaeroides* R26 [4], Figure 1. There are four bacteriochlorophylls (Bchls), two bacteriopheophytins (Bpheos), two ubiquinones (UQs), and a high spin ferrous(II) ion (Fe). Two of the four Bchls form the primary donor known as the special pair with one half of the special pair associated with the L-subunit (P_L) and the other half associated with the M-subunit (P_M). The remaining two Bchls will be referred to as the bridging Bchls (B_L), (B_M) and the two Bpheos as (H_L), (H_M) where the subscripts L and M refer to the corresponding protein subunits. The UQs are represented as Q_A and Q_B .

The pigments and the UQs occur in pairs as shown in Figure 1. One half (the right half of Figure 1) of the pigment-UQ complex is primarily associated with the L-subunit and the other half is primarily associated with the M-subunit. Only the Fe does not have a corresponding pair. Moreover, the L-subunit, the M-subunit, and the donor-acceptor complex including the Fe, exhibits an approximate two-fold axis of symmetry, C_2 . As a consequence of this two fold symmetry of the pigments and UQ-Fe complex, potentially there exists two pathways for the electron transfer chemistry to occur. If rigorous C_2 symmetry were in operation for the components of electron transfer, both pathways would necessarily be functioning. Interestingly, only the L-branch seems to be photochemically active, implying that the C_2 symmetry must be broken. Despite the knowledge of many symmetry breaking residues of the protein, no mechanism has yet been established for this preferred pathway. The origin of the L-side activity will not be discussed further and any reference to the electron transfer process can be assumed to be

associated with the L-branch. The L-bridging Bchl is generally believed necessary for electron transfer between the primary donor special pair and the primary acceptor Bpheo. We will now focus on the chemistry involved with the first step of charge separation; this restricts the discussion primarily to the Bchls and the Bpheos.

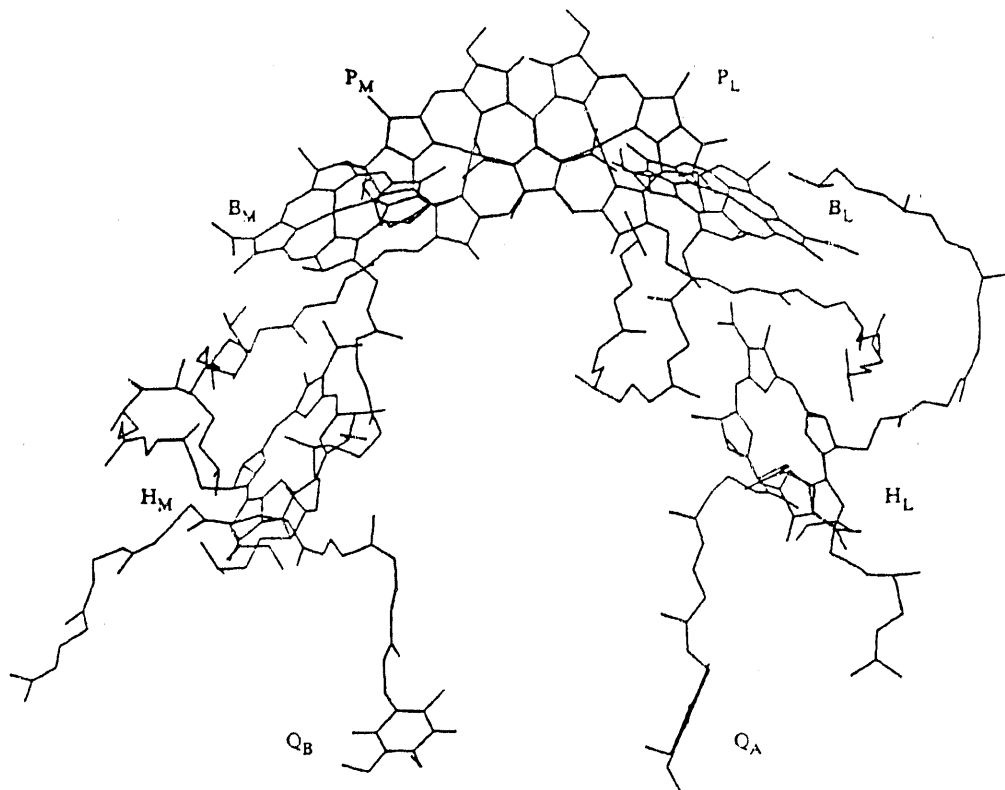


Figure 1. Structure of chromophores in *Rb. sphaeroides* R26 reaction center complex. P_L and P_M are the two Bchls constituting the special pair (P). B_L, H_L and Q_A are the Bchl, Bpheo and the ubiquinone in the photoactive branch L. B_M, H_M and Q_B are the same on the inactive branch M.

Chemical Principles of Photoinduced Charge Separation

Within approximately three picoseconds of optical excitation an electron is transferred from the primary donor to the primary acceptor forming an anion on the Bpheo and a cation on the special pair. The existence and speed of this initial electron transfer reaction is established through transient optical absorption spectroscopy with sub-

picosecond time resolution. Whether or not electron transfer involves the bridging Bchl molecule remains to be proven. Moreover, because of the difficulty in experimentally establishing a clear participation of the bridging molecule in the electron transfer act, two basic mechanisms of electron transfer have been predominant. One mechanism involves a short lived, discreet intermediate state where the transferred electron resides for a brief time on the bridging Bchl. The lifetime of the electron on the bridging Bchl is too small for easy experimental verification. The other mechanism invokes superexchange, where participation of the bridging molecule is quantum mechanical. In this latter case, experimental observation of a discrete oxidized or reduced state of the intermediate bridging molecule is intrinsically impossible.

In any event, as soon as the electron has been pumped from the special pair to the L-side Bpheo, within about 200 picoseconds the electron moves from the L-side Bpheo to the UQ of the L-side. Shortly thereafter it moves on to the UQ of the M-side (Figure 1). Eventually, the entire process is repeated so that the UQ on the M-side is ultimately reduced twice. Since the reaction center spans a membrane, this initial process of photosynthesis pumps electrons across the membrane via a single-sided pathway even though the RC structurally exhibits two potential pathways.

However, if the ubiquinone Q_A has been chemically removed or prereduced (e.g., by dithionite treatment in the dark), then the normal forward electron transfer chemistry is blocked in these RCs and charge recombination begins. This annihilation process requires about 10 ns [5]. Two distinct charge recombination routes exist simultaneously. One pathway returns the electron to the primary donor ground state via the singlet manifold. If this pathway alone were active then charge recombination would require about 20 ns [5]. The other pathway returns the electron to the singlet ground state via the first excited triplet state of the special pair. Actually when the triplet state of the primary donor special pair is formed in this process, charge recombination is complete since the hole and the electron have annihilated each other. This triplet annihilation process takes only about 2 ns [5]. Eventually, the excited triplet state of the special pair relaxes to the original ground state. We focus attention on this triplet annihilation process in this paper.

The chemistry that takes place in a RC is rather amazing. We shall proceed by describing such chemistry based on the RC structure and function in terms of artificial photosynthesis. In all our examples, neither the donor nor the acceptor is reactive if both are in their neutral ground state. The electron transfer requires at least one of the participants to be in an excited electronic state that can be generated by the absorption of light. For convenience, throughout this discussion we assume that the light energy is absorbed by the donor and that the donor is promoted into its first excited singlet state.

First we illustrate a hypothetical donor-acceptor pair where the distance of separation between donor and acceptor is relatively large, 25 Å (e.g., the distance between the special pair and Q_A). In this example the average lifetime of the lowest excited singlet state of the typical donor is so short (10 to 100 ns) that electron transfer cannot proceed

from the donor to the acceptor in this time and no radical pair formation occurs. In general, each 1.5 Å of separation decreases the electron transfer rate by roughly ten [6]. As a point of reference electron transfer over 10 Å (edge-to-edge) should occur in about 10 ns for molecules like the Bchls. Consequently, the long-distance 25 Å model system is nonfunctional in the excited singlet state since completion of electron transfer would require that the lifetime of the excited state be longer than a second.

In contrast, the longer lived but less energetic triplet state of the donor may survive long enough for the electron transfer event to occur. For a number of years chemists have been able to generate charge separation in model systems based on excited triplet states. In general, the triplet state is formed by loss of energy from an excited singlet state. Thus, for maximum conversion the energy available in the singlet state should be used directly, not indirectly via a triplet state. As a consequence, the energy of the charge separated radical pair state should be below the energy of the excited singlet state but well above the energy of the triplet state. In any event, the goal of RC research is to learn how to use the more energetic singlet state.

We therefore proceed to the next example with a hypothetical donor-acceptor pair separated by a very short distance of less than ~4 Å (e.g., the special pair to bridging Bchl distance). In this example, electron transfer occurs during the lifetime of the singlet state of the donor. However, a new problem arises; any charge separation is quickly destroyed by a very efficient and rapid back reaction. The back reaction is the self-annihilation of charge separation and concomitant collapse of the donor and acceptor radical pair. In this case the self-annihilation proceeds so rapidly that the lifetime of the charge separation exists on the order of a few hundred picoseconds. Since the goal of artificial photosynthesis is the production of millisecond charge separation, such a short lifetime is an unsatisfactory solution to the problem. As we move the donor-acceptor further apart (for example to the 11 Å distance of separation between the special pair and Bpheo), then both the reaction rate and the reaction yield decreases precipitously and very quickly the photochemistry does not proceed during the lifetime of the excited singlet state.

One conceivable route to longer-lived charge separation in the next example is via the well known three-site arrangement, where electron transfer proceeds from the initial donor to the terminal acceptor by some mechanism involving the intermediate bridging molecule. This example is similar to the special pair donor, the bridging Bchl molecule and the primary acceptor, Bpheo, of the RC as shown in Figure 2. The technical difference between our hypothetical three site model and the RC is that nature uses four molecules for three sites. The arrangement of molecules is such that electron transfer proceeds within a few picoseconds after light excitation resulting in a donor cation and the third molecule as an anion. In this three molecule system the charge separation can remain stable for longer than 10 nanoseconds. As before, one of the reaction requirements is that the distant, terminal molecule be a good acceptor relative to the excited state of the initial donor molecule and the bridging molecule. In general, the

pheophytins are better acceptors than the corresponding chlorophylls, or in other words, bacteriopheophytin is easier to reduce than the corresponding bacteriochlorophyll; thus, the electron would rather be on the Bphea than on the Bchl.

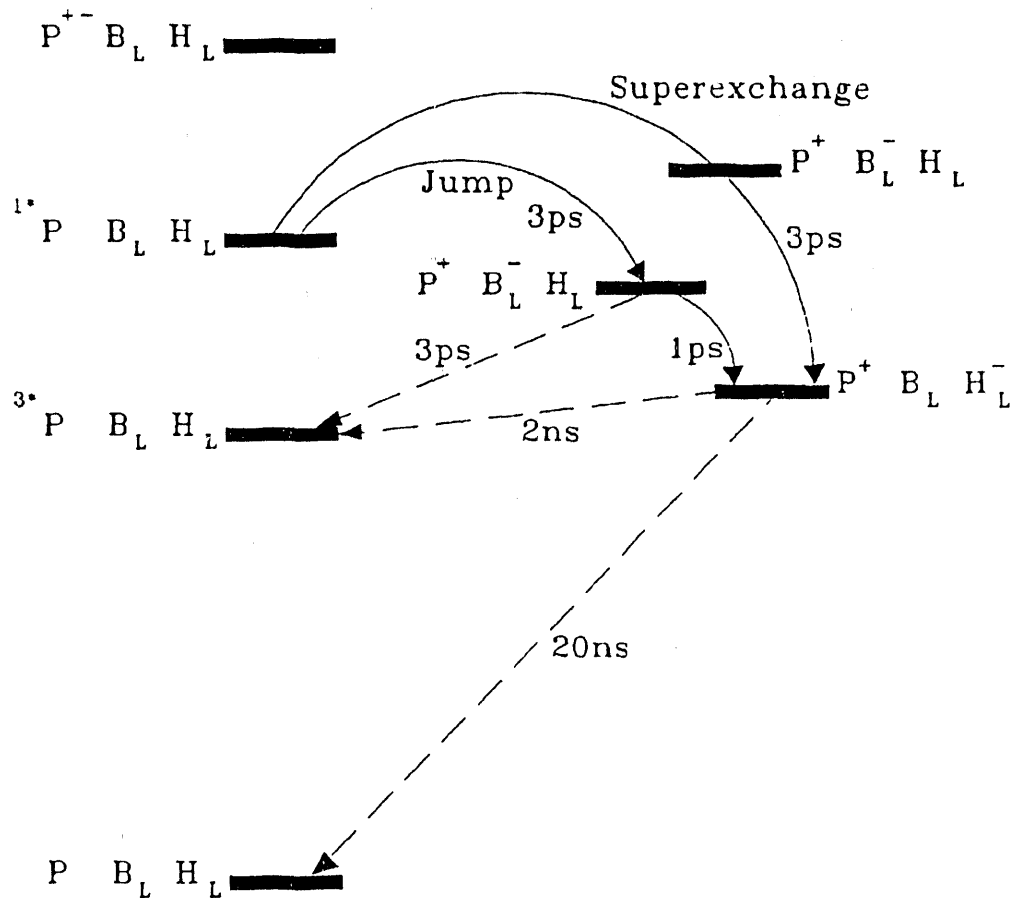


Figure 2. Schematic representation of the primary charge transfer process in the reaction center. P, B_L and H_L are the three different sites involved in electron transfer reaction. The different states are shown as the thick solid lines. The energies of the states are scaled with reference to the ground state energy of the primary donor (P). $P^+ B_L^- H_L^-$ is the charge resonant state of 1^*P believed to participate in the super exchange mechanism.

The speed at which the undesirable back reaction occurs depends critically on the distance of charge separation. By having a three-site reaction, the distance of charge separation can be much larger than in a two-site reaction, thus minimizing the direct back reaction rates to the ground state. Suppose that the three-site reaction occurs as it might in photosynthetic bacterial RCs as illustrated in Figure 2. In this mechanism the first

electron transfer step, from the donor of site 1, (P), to the intermediate acceptor of site 2, (B_L), may take less than a few picoseconds. If the second forward reaction, i.e., from B_L to H_L proceeds in a time much less than one picosecond, then the overall forward reaction leading to the distant charge separated state $P^+B_LH_L^-$ occurs in about three picoseconds. Because of the large distance between site 1 and site 3, the direct back reaction (i.e., the annihilation of charge separation without participation of site 2) is slow. If the reaction scheme were stopped at site 2, then the time for back electron transfer to the ground state or an excited triplet state located in the donor of site 1 would be significantly short, typically less than one nanosecond. This faster back reaction directly results from the shorter distance between site 1 and site 2.

In addition to the distance, the size of the downhill energy gap also controls the reaction via the so called Frank Condon factor, where in general the larger the gap, the slower the reaction rate. Thus, a large energy gap between the charge separated state and the original ground state is desirable for maximum energy storage as well as for the prevention of the back reaction. However, this large energy gap between ground state and radical pair, charge separation state results in a small energy gap between the triplet state of site 1 and the same radical pair state. Thus, the triplet state back reaction has a favorable Frank Condon factor and thus is faster. Such an explanation agrees with the 2 ns for the triplet back reaction and the 20 ns singlet back reaction of RCs mentioned earlier.

How about the indirect back reaction mechanism? The electron of site 3, (H_L), can jump uphill, back to site 2, (B_L), and then directly return to the ground state of site 1. Note that the indirect mechanism involves one or more intermediate uphill states before a direct jump to the original ground state occurs with any efficiency as shown in Figure 2. Therefore, for efficient and long lived charge separation, the forward rate for the formation of $P^+B_LH_L^-$ must be larger than the direct recombination rate of $P^+B_LH_L^-$ to the ground state or the triplet state of site 1.

In general, to prevent the indirect back reaction mechanism from contributing, each forward reaction step should be slightly downhill in energy storage. In essence each forward reaction gives up some energy as "heat" but gains charge separation stability. Arguments based on microscopic reversibility and detailed balance show that, as long as sufficient energy is lost in the forward reactions (ultimately in the form of heat), the reverse uphill reaction rates are slower than the forward reaction rate constant. Thus, each forward step of the photosynthetic process wastes a little energy as heat in order to reduce the indirect back reaction rates via its preceding excited states. At the same time that the back reaction rates for the excited states are minimized by giving up a little energy in the forward reaction steps, the distance between cation and anion increases. As a result, the time for direct electron transfer back to the ground state and even more importantly to the excited triplet state of site 1, (3P), also increases.

Thus, in the photosynthetic RC both direct and indirect back reaction rates are minimized for both the singlet and the triplet manifold. In fact, in the RC all forward reaction steps increase the distance of charge separation except when the electron goes from Q_A to Q_B . By the time the electron is on the first UQ, the distance of charge separation is 25 Å. This distance between the special pair cation and the reduced ubiquinone, Q_A^- , is so large that the back reaction to the original ground state takes longer than the time required for cytochrome to reduce the special pair cation. After reduction of oxidized special pair by cytochrome, the 45 Å distance of charge separation (the distance between Q_B and a heme group of docked cytochrome) is so large that the back reaction is virtually eliminated.

The fact that photosynthesis uses at least five steps to generate stable charge separation is agreed upon, although, the mechanism of photosynthesis as detailed above has not yet been established. Instead, the above mechanism is just one of many that have been proposed. However, the general principles pertaining to kinetics, distance, and stable charge separation are the same for all models proposed to explain RC photochemistry.

Superexchange Mechanism

One of the other mechanisms for the initial charge separation of bacterial photosynthesis is based on the concept of superexchange. The relevance of this superexchange mechanism to artificial photosynthesis is the major purpose of this paper. In this model, regardless of experimental time resolution, detection of an electron transfer intermediate, $P^+B_L^-H_L$ (shown in Figure 2) prior to the reduction of the primary acceptor, bacteriopheophytin, is impossible. The justification of superexchange is to explain how an electron can be transferred at least 11 Å (nearest π edge-to-edge distance) in about three picoseconds without a measurable intermediate. In this view essentially a supermolecular complex exists such that the effective distance of electron transfer is less than 4 Å instead of 11 Å if the intermediate Bchl were not present. In other words, although at least three molecules must be involved in such a mechanism, this represents only a two site case where the edge-to-edge distance is quite short.

If this mechanism proves to be a correct explanation for photosynthesis, then its duplication in model systems appears to be rather difficult. The reason for the complexity is that some special considerations must be given to preventing the back reaction of charge recombination or self annihilation in such a two site case. We point out, that if a distance of less than 4 Å is involved in the forward reaction as a result of superexchange in the charge separation process, then the same distance and the same supermolecule complex may be expected to be involved in the back reaction. Thus, to be compatible with experimental observations on the RC, one would conclude that in nature the supermolecule complex can only be operative in the forward charge separation reaction and not in the back reaction. Especially if the effective distance between the donor supermolecule and the acceptor is 4 Å, then the back reaction to the triplet state of the

primary donor is slightly downhill in energy and thus in principle may be expected to occur very rapidly (in ps). Apparently, if this mechanism is operative in nature, this highly favorable downhill back reaction has been prevented. The next question to be addressed is how could nature diminish the back reaction for this "two site" case.

Friesner and Won [7] have expanded on a model proposed by Thurnauer et.al., [8] based on charge separation within the special pair dimer [8]. The detailed mechanism proposed provides a straightforward explanation for fast forward chemistry and slow backward chemistry while the superexchange mechanism is operative. We note that such a model incorporates four molecules, a dimer special pair donor, a superexchange site of the bridging Bchl and the primary acceptor. The occurrence of four molecules instead of three provides a weakly coupled dimeric species essential for the singlet charge resonance state ($P^+B_L H_L$), (Figure 2). The singlet charge resonance states within the special pair quantum mechanically connect the primary donor special pair to the primary acceptor Bphea via the bridging Bchl. The same analysis shows that the triplet state of the primary donor, i.e., the triplet of site 1, is not significantly coupled to the charge separation state and thus the back reaction to the triplet state is also not significant.

However, the problem of such a mechanism for artificial photosynthesis is severe. One major difficulty is the requirement of four molecules and the complications for their synthesis. Moreover, until the precise details of this four molecule superexchange mechanism have been established, the energetic and geometrical requirements for the synthesis of such a four molecule complex will be unknown.

Summary

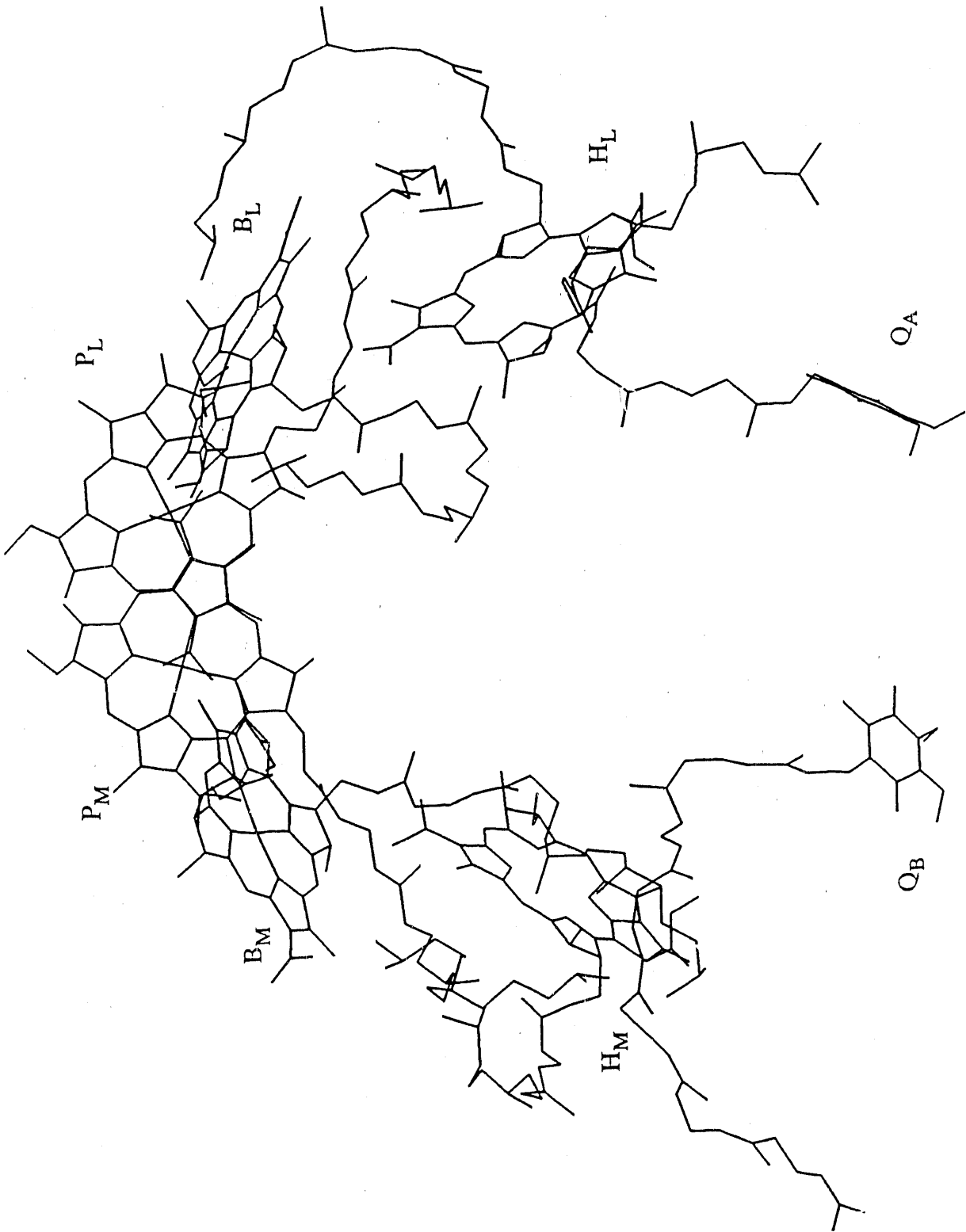
If a multiple discrete jump model is operative in the primary events of photosynthesis, then various model systems have been synthesized with many of the properties of bacterial photosynthesis [9,10]. Such a model prevents back reactions by a series of downhill chemical reactions using well established charge separation principles. Each downhill step in energy gains charge separation distance. The back reaction, even to the less energetic triplet state, involves intermediate states uphill in energy and thereby is not favorable. On the other hand, if superexchange is a correct mechanism of photosynthesis, then no model system has been developed that mimics properly the natural process. The back reaction to the triplet state of the primary donor is downhill in energy and thus can occur in principle. In nature this downhill back reaction appears to be prevented by the use of four molecules in the donor-acceptor complex. Thus, we suggest that superexchange artificial photosynthesis requires four molecules to mimic the natural event sufficiently. In this view superexchange artificial photosynthesis is the more difficult challenge.

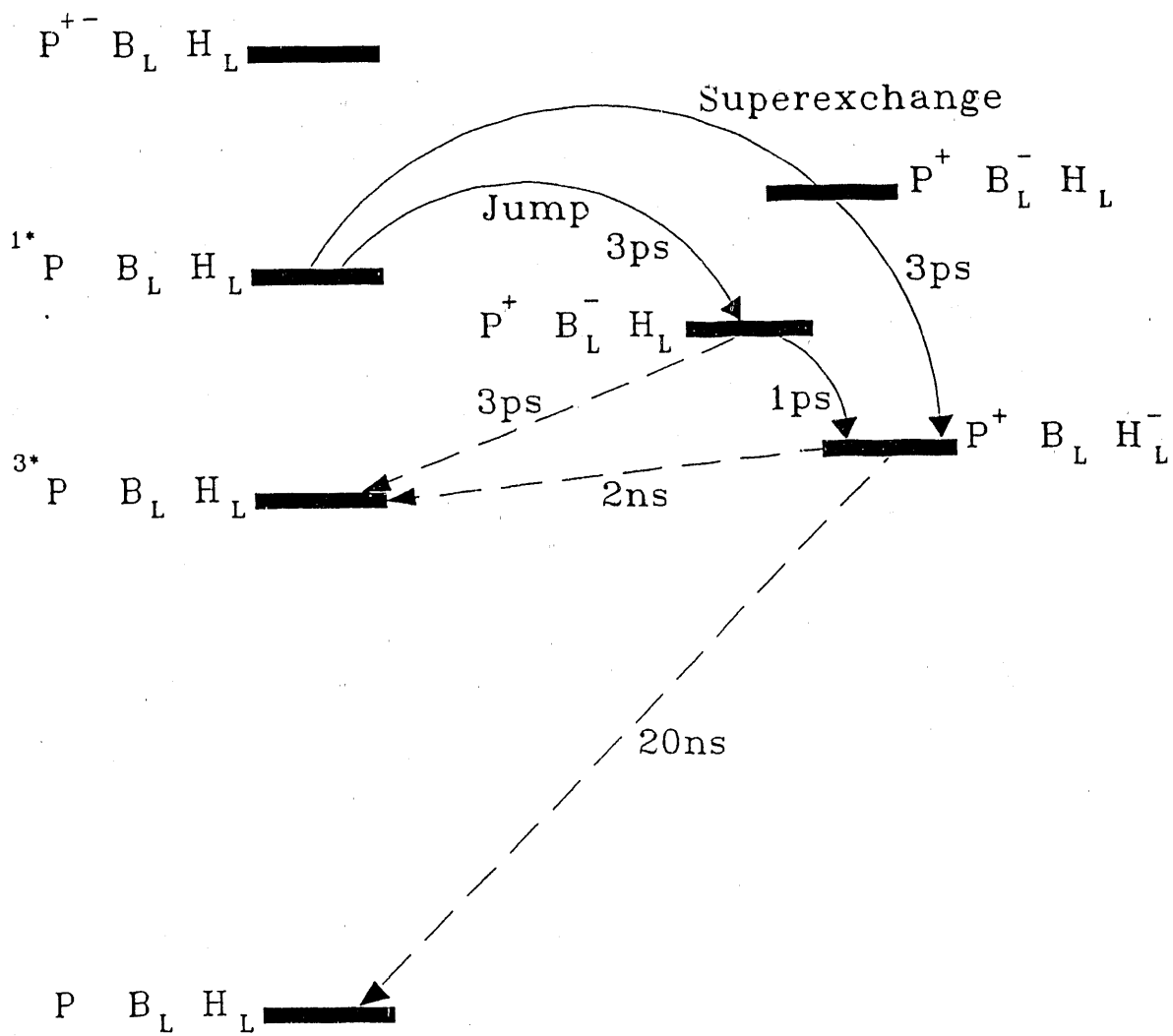
Acknowledgement

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