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The role of the iron–histidine bridge in the early steps of photosynthesis ☆

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Abstract

The role of the iron–histidine bridge in bacterial photosynthetic reaction centres has been investigated by means of ab initio computations and quantum dynamics of elementary reaction steps. Full geometry optimization and wave packet dynamics show that, upon the arrival of a photo-electron, the primary quinone takes up a proton from the H-bonded histidine in a very fast process, which occurs in a few tens of femtoseconds. The proton transfer step significantly stabilizes the charge separated state, inhibiting the backward charge recombination process. Electron transfer to the secondary quinone can then take place by switching the positions of both the H-bonded hydrogens, in a Bjerrium type mechanism involving whole hydrogen atoms rather than protons.

1. Introduction

Photosynthetic reaction centres (RCs) are the heart of complex molecular machines which, in bacteria and plants, convert low-energy substrates, such as water and carbon dioxide, in chemicals of high free energy content [1,2].

The key step in photosynthesis is a long distance photoinduced electron transfer (ET) from the bacteriochlorophyll special pair (P) to the primary quinone Q_A, which gives rise to a long living charge separated state [3]. Photoinduced ET is a fast process; it occurs in ca. 200 ps, via a well-known exchange mechanism, which involves a bacteriopheophytin (BP) and probably a bacteriochlorophyll (BC) [3–6]. The reduction of the special pair by cytochrome *c* leads to transmembrane charge separation, which triggers the chemical reactions by which biological fuels are produced. That step is comparatively much slower, ca. 270 ns [7], than photoinduced ET, so that the long lifetime of the charge separated state $P^+Q_A^-$ is a key point for an efficient conversion of the solar energy into chemical energy.

The kinetics of the charge recombination reaction has been extensively studied [8–12], but a clear comprehension of the chemical factors which make this process so slow in photosynthetic RCs, a point with important implications for the design and the realization of synthetic systems for artifi-

☆ This paper is dedicated to prof. G. Del Re on the occasion of his 70th birthday.

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cial photosynthesis [13], has yet to be achieved. For bacterial RCs reconstituted with low-potential quinones at the Q_A site, charge recombination can occur also via a thermally activated route involving the P^+BP^- high energy intermediate [8,9], and that uphill process could explain the observed low rates, but for native RCs this route is precluded and the slowness of the backward reaction has been tentatively assigned to a temperature dependent extra stabilization of the $P^+Q_A^-$ state, which can be due either to solvent reorganization energy – there is evidence of conformational changes on microsecond time scale which may solvate Q_A^- [12,14], – or possibly to some chemical modification at Q_A.

In this Letter we will consider the latter hypothesis, focussing attention on proton transfer (PT) reactions which could occur at the Q_A site. It is in fact well known, both from experimental results [15–17] and theoretical analyses [18–21], that ET is often coupled to PT. Remarkably, Hung et al. [15] have shown that, in carotenoid–porphyrin–quinone triads exhibiting photoinduced ET, the addition of a carboxylic group, in a position in which it can form an intramolecular H-bond with a quinone oxygen, significantly stabilizes the charge separated state. That effect was thought to be due to PT from the carboxylic group to the semiquinone anion. As concerns more specifically photosynthetic RCs, Breton and Nabedryk [17] have found that the Q_A/Q_A^- FTIR difference spectrum of photosynthetic RCs, both from *Rhodobacter sphaeroides* and from *Rp. viridis*, shows a broad positive band at 2900–2500 cm^{-1}, shifting at 2200–1800 cm^{-1} upon deuteration. According to previous studies [22], such a band has been tentatively assigned to proton vibrations within a network of polarizable H-bonds, thus suggesting that the formation of Q_A^- could cause a significant rearrangement of the H-bond network around Q_A.

All these pieces of evidence suggest that ET in biosystems can induce fast proton rearrangements and that PT could be a key factor in driving ET reactions in proteins, stabilizing certain products with respects to others. In line with those expectations, it has been proposed that in biosystems where redox cofactors are held together by a chain of H-bonds, a mobile electron, injected on one end of the chain, can be carried to the opposite end by appropriately switching the positions of the H-bonded hydrogens, in a Bjerrium type mechanism which involves not only shifts of protons but also of hydrogen atoms [18–20]. Such a mechanism, called proton assisted electron transfer (PAET), has been applied to ET from Q_A to Q_B in bacterial photosynthetic RCs: semiempirical MNDO/PM3 computations with full geometry optimization and single point ab initio computations on the MNDO optimized geometries suggested the mechanism reported in Scheme 1, in which ET from Q_A to Q_B takes place via a succession of proton (steps 1 and 3 of Scheme 1) and hydrogen atom (step 2) transfers [23].

According to the mechanism of Scheme 1, PT from the iron–histidine to the semiquinone anion could be the chemical process which stabilizes the charge separated state, increasing its lifetime. Previous computations have shown that PT is energetically favoured [23], but to better assess its role, it is still necessary to show that it is fast enough to kinetically compete with electron transfer. In this Letter we report the results of a dynamical study of the PT elementary step, using a potential energy surface whose parameters have been obtained, for the first time, from ab initio computations with full geometry optimizations.

$$Q_A^- \cdots \text{H-His-Fe-his-H} \cdots Q_B \rightarrow Q_A\text{-H} \cdots {}^-\text{His-Fe-His-H} \cdots Q_B \qquad 1$$

$$Q_A\text{-H} \cdots {}^-\text{His-Fe-his-H} \cdots Q_B \rightarrow Q_A \cdots \text{H-His-Fe-His}^- \cdots \text{H-}Q_B \qquad 2$$

$$Q_A \cdots \text{H-His-Fe-his}^- \cdots \text{H-}Q_B \rightarrow Q_A \cdots \text{H-His-Fe-His-H} \cdots Q_B^- \qquad 3$$

Scheme 1.

2. Computational details

All the ab initio computations have been performed at UHF level of theory using the Gamess package [24]. Geometries of the stationary points of the potential energy hypersurface have been located by full optimization, using the Hay–Wadt core potential for Zn and the standard 3-21g basis set; all the optimization runs started from the X-ray coordinates of the RC from *Rb. sphaeroides* crystallized under illumination [25]. The energies of the stationary points have been computed at MP2 level of theory, using the standard 6-31g basis set, augmented with polarization functions on all the atoms engaged in H-bonds. Several tests have shown that such a basis performs as well as the larger triple zeta valence Mc Lean/Chandler basis set with additional polarization functions on all atoms.

Wave packet dynamics for proton hopping have been carried out by numerical solution of the time dependent Schrödinger equation of a system consisting of two anharmonic coupled oscillators, one for the N–H stretching coordinate (r), the other for the N–O low frequency stretching motion (R). The potential energy surface has been built up by using the two Morse model [26] for the proton stretching coordinate and a repulsive $(c/R)^{12}$ potential for the heavy atom motion, where c is a constant to be determined from the experimental heavy atom distance of the H-bond complex. Vibrational eigenstates have been computed by the variational method, expanding the true wave function over a basis given by the direct products of harmonic oscillator wave functions; 30 functions on the high frequency mode and 70 on the low frequency one, centred in the most stable minimum, are sufficient to ensure convergence of the computed frequencies in the energy range of interest. All Hamiltonian matrix elements have been computed by Gauss–Hermite numerical integration.

3. Results

A reliable estimate of the potential energy surface for proton transfer can be obtained from the knowledge of the relative energies, geometries and vibrational frequencies of the two equilibrium configurations [26]. The geometries of the two possible stationary points have been obtained by ab initio full optimization on a simplified model of the supramolecular assembly, in which only two of the four histidine residues coordinated to the metal ion have been retained and with a formate anion in place of the glutamic residue, cf. Fig. 1. Moreover, we have replaced Fe^{2+} with Zn^{2+}, since it has been experimentally shown that such a replacement does not affect significantly the ET rate and its efficiency, while it would greatly simplify computations [27].

Full geometry optimization of the supramolecular assembly of Fig. 1 leads only to one minimum of the potential energy surface (PES), that corre-

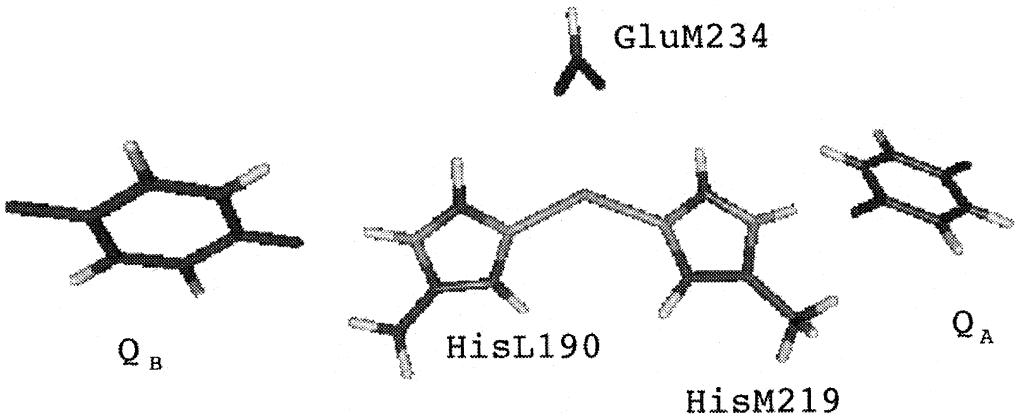

Fig. 1. The model system adopted in this work.

sponding to the proton bonded to the semiquinone anion. In order to estimate the relative energy of the nuclear configuration with both protons on the histidine sites, the two N–H distances have been frozen. The constrained optimization leads to a nuclear configuration which is ca. 12 kcal/mol higher than the equilibrium point, with one of the two N–O intermolecular distance, that involving the semiquinone oxygen, significantly shorter than the other, 2.5 and 2.8 Å, respectively. The SCF computed energy difference for PT are probably overestimated: the experimental ΔG for ET from P^+BP^- to $P^+Q_A^-$ in the RC from *Rb. sphaeroides* is 0.61 eV [28], so that the SCF computed energy for PT would be just within the experimental limit. The inclusion of correlation effects at MP2 level lowers the energy gain for PT from histidine to Q_A^- to 8.66 kcal/mol, which is now well within the limit suggested by experiments.

No significant changes have been obtained, at least at SCF level, by considering both the whole coordination sphere of the metal ion and the H-bond formed by Q_A with a NH group of a protein skeleton [29]. In both cases, full geometry optimizations predicted that the nuclear configurations with both the H-bonded protons on the histidine sites are not stationary points of the PES, thus suggesting that, upon formation of the semiquinone anion for photoinduced ET from P^*, PT from the H-bonded histidine to Q_A^- is a spontaneous, barrierless process. The results of all the ab initio computations are summarized in Table 1.

In order to build up the PES for PT, we have followed the analytical procedure recently reported in the literature [26], according to which the potential energy for the proton coordinate (r) is written as the sum of two Morse potentials, each representing the proton stretching mode of the isolated moieties of the H-bond complex. For the heavy atom stretching coordinate (R) we assume a repulsive $(c/R)^{12}$ potential, so that the PES is written as:

$$V(r,R) = D_{\mathrm{His}}\left[1 - \mathrm{e}^{-\alpha\left(r+R/2-r^0_{\mathrm{His}}\right)}\right]^2 + D_Q\left[1 - \mathrm{e}^{\beta\left(r-R/2+r^0_Q\right)}\right]^2 + \left(\frac{c}{R}\right)^{12}. \quad (1)$$

The parameters of $V(r,R)$ have been fixed as suggested in [26]. For dissociation energies we started from the computed values of the N–H and O–H bond in imidazole and semiquinone at DFT/B3LYP/6-311G+(2p,2d), 92.9 [30] and 66.7 kcal/mol (our computations) respectively, and changed them of an equal amount in order to reproduce the MP2 computed energy difference. The resulting values are: 72.9 and 86.7 kcal/mol, for the N–H and O–H bond, respectively. The values of α and β have been set from the computed vibrational frequencies of N–H and O–H bonds in imidazole and semiquinone (DFT/B3LYP/6-31g), 3673 and 3692 cm^{-1} respectively, leading to $\alpha = 2.68$ and $\beta = 2.58$ $\mathrm{Å}^{-1}$. Both r^0's, 0.98 Å for N–H and 0.95 Å for O–H, and c, 3.3 $(\mathrm{kcal\ Å/mol})^{-12}$, have been determined from the optimized geometries of Table 1.

Table 1
Summary of the results of ab initio computations

	ΔE (kcal/mol)		Bond lengths (Å)		Charge (a.u.) Q_A
	3-21g	MP2	$O_{Q_A}\cdots N$	O_{Q_A}–H	
Q_A–H$\cdots br_1^-\cdots Q_B$	0.0	0.0	2.681	1.006	−0.362
$Q_A^-\cdots$H–$br_1\cdots Q_B$	12.798	8.66	2.469	1.432	−0.815
Q_A–H$\cdots br_2^-$	0.0	–	2.642	1.020	−0.116
$Q_A^-\cdots$H–br_2	12.035	–	2.538	1.432	−0.796
R–H$\cdots Q_A$–H$\cdots br_2^-$	0.0	–	2.616	1.029	−0.065
R–H$\cdots Q_A^-\cdots$H–br_2	9.426	–	2.532	1.440	−0.789

Notes. Relative energies refer to the following values: Q_A–H$\cdots br_1^-$: $E = -1529.84110$ and -3255.17474 a.u. at UHF/3-21g and MP2/6-31g** level respectively; Q_A–H$\cdots br_2^-$: $E = -1716.33274$ a.u.; R–H$\cdots Q_A$–H$\cdots br_2^-$: $E = -1884.32747$ a.u. br_1 and br_2 stand for the molecular bridges with two and four histidines coordinated to the metal ion. R–H indicates the *N*-methyl formamide molecule added to model the H-bond formed by Q_A with the protein backbone.

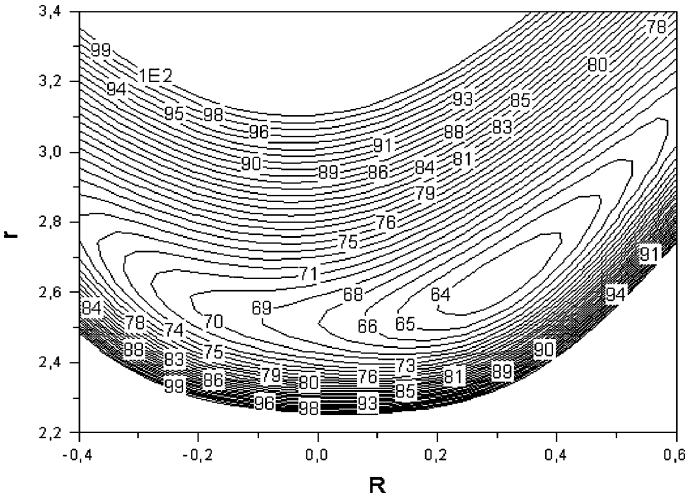

Fig. 2. The potential energy surface for proton transfer from the histidine ($r \leqslant -0.2$) to quinone ($r \geqslant 0.25$). Contour line spacing 1 kcal/mol; distances in Å.

The resulting PES is shown in Fig. 2. As expected it exhibits only a single minimum, for the proton bonded to the quinone oxygen, so that PT from the histidine to the semiquinone anion is expected to occur readily, on the timescale of the vibrational N–H or N···O oscillations.

To confirm that expectation, we have analyzed the dynamical features of the PT elementary step by performing wave packet dynamics on the PES of Fig. 2. The initial state has been taken as the ground vibrational state of the system before the injection of an electron on Q_A. For that situation, we assume a single well potential with the proton localized on the histidine site and harmonic approximation for both coordinates, with frequencies of 3000 and 400 cm^{-1} for r and R, respectively. Snapshots of the vibrational wavefunction at different times are portrayed in Fig. 3. At $t = 0.0$, the wave function is completely localized in the region of the PES corresponding to the proton bonded to the histidine nitrogen. After a few femtoseconds the wave packet elongates along the r coordinate, and reaches the region of PT products in ca. 10 fs. After that, the wave packet begins to broaden over the whole permitted region of the PES and, since no mechanism allowing for energy dissipation has been included in the dynamics, it oscillates between PT reactants and products.

4. Conclusions

The results discussed in this Letter lead to the conclusion that the iron–histidine bridge could play an important role in the early steps of photosynthesis. As far as theoretical computations can tell, the iron–histidine bridge is expected to stabilize the charge separated state $P^+Q_A^-$ by protonating the semiquinone anion and delocalizing the negative charge on itself. The backwards charge recombination process is inhibited because it would yield the protonated quinone cation, which, in the ground state, is a high energy intermediate. Beyond that, the iron–histidine bridge is also expected to play an active role in the ET process from the primary to the secondary quinone. In fact, since PT is much faster than ET between quinones, the latter cannot take place by the usual tunnelling or superexchange mechanism, because ET from Q_A–H to Q_B would be endergonic. Therefore, unless neutral Q_B is protonated by some other amino acidic residues, which appears to be quite improbable, the only mechanistic hypothesis which is not ruled out by PT is the PAET mechanism reported in Scheme 1, namely that ET from Q_A–H to Q_B occurs by hydrogen atom transfer. Of course, all the above conjectures are based on computations in which the effects of the environment have

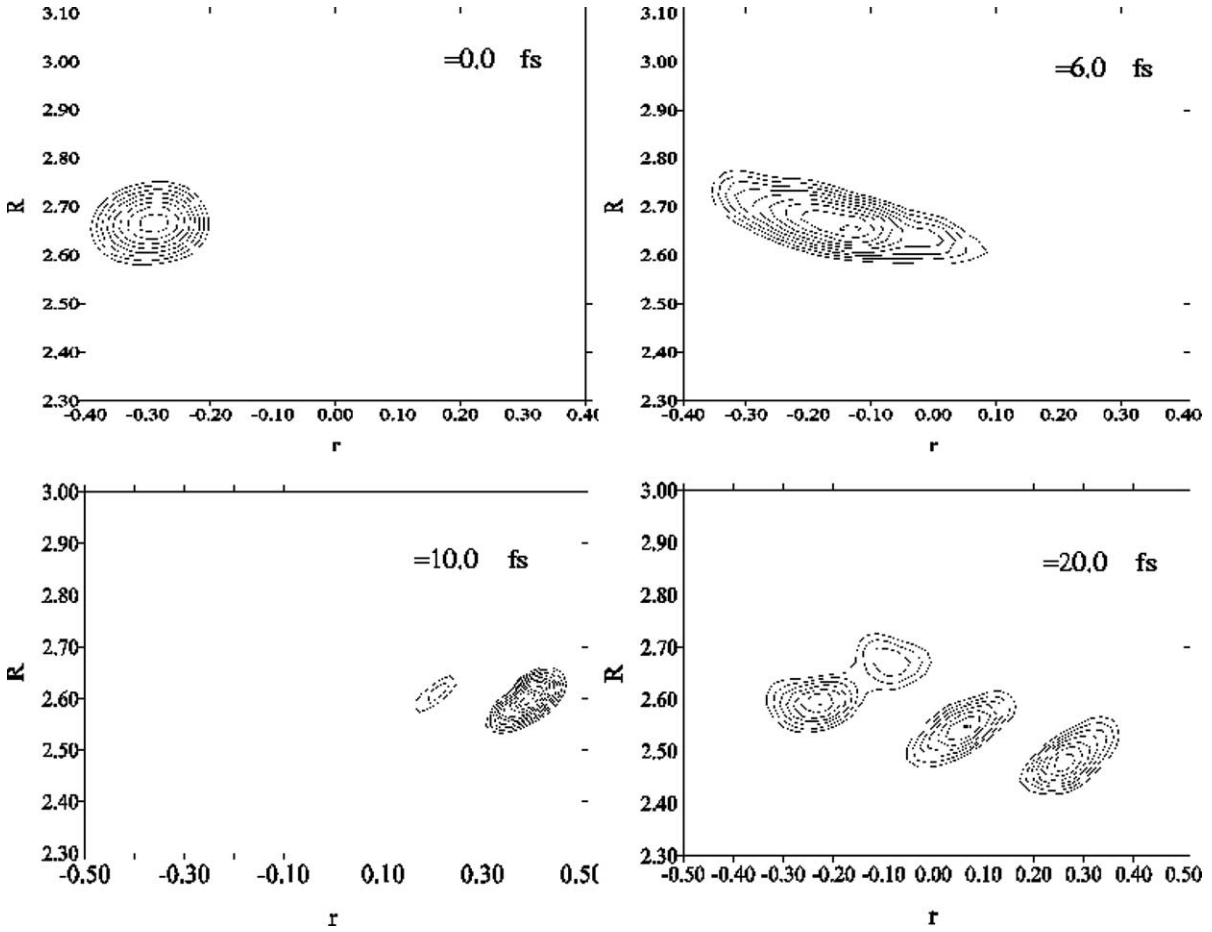

Fig. 3. Snapshots of the vibrational wavefunction at different times. Coordinates in Å.

been neglected, but for the H-bond at the Q_A site; the latter can affect the computed relative energies, even though that effect is expected to be small since Q_A is placed in a non polar pocket of the protein. Thus, our results should be considered as guide lines for further experimental work.

Finally, the question about how proton transfer from the bridge histidine to Q_A^- could be experimentally detected has to be addressed. In [23] it has already been suggested the use of time resolved pump–probe spectroscopy, the most suitable technique for detecting fast (barrierless) proton transfer [31]. In this specific context, the detection of Q_A–H can be associated to the appearance of a band at 300 nm as a transient signal in a time resolved electronic spectrum, corresponding to the absorption band of neutral semiquinones, shifted of about 20 nm at shorter wavelengths with respect to those of semiquinone anions [32,33]. Moreover, the broad positive band at 2900–2500 cm^{-1}, shifting at 2200–1800 cm^{-1} upon deuteration, observed in the Q_A/Q_A^- FTIR difference spectrum of photosynthetic RCs [17], can potentially provide important pieces of information about the possible protonation of Q_A^-. In particular, suitable isotopic substitutions should lead to a better assignment of the broad absorption band, thus clarifying the role of the iron–histidine faced to Q_A. Work in this direction is already in progress, encouraged by the fact

that rapid-scan FTIR has already proved to be able to provide very useful information about proton transfer equilibria at the Q_B site [34], and that the step-scan technique, combined with use of inhibitors for Q_B, should be still more powerful.

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