28 December 2001

ELSEVIER

Chemical Physics Letters 350 (2001) 515–521

CHEMICAL PHYSICS LETTERS

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Temperature dependence of photoinduced electron transfer within self-associated porphyrin: guanine monophosphate complexes

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Received 31 March 2001; in final form 23 August 2001

Abstract

In the current report, the temperature dependence of photoinduced electron transfer between tetrakis-(4-tetramethylpyridyl)porphine (T4MPyP) and guanine monophosphate (GMP) has been examined. In the presence of GMP the fluorescence lifetime analysis reveals a Lorentzian distribution of lifetimes centered at 0.7 ns with a width of 0.9 ns displaying significant temperature dependence. Fitting temperature dependent data to the Marcus equation gives a reorganizational energy (λ) for the electron transfer reaction of 0.6 eV and an electronic coupling factor (H_{AB}) of 3×10^{-3} eV. These results suggest conformational regulation of electron transfer within the non-covalent porphyrin:nucleotide complex. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

Biological electron transfer reactions are of critical importance in understanding various bioenergetic processes including cellular respiration and photosynthesis [1–5]. The rate of long range electron transfer is governed by a number of factors including distance between electron donor and acceptor, donor–acceptor electronic coupling, and reaction free energy according to

$$k_{ET} = \left[(2\pi/h)|H_{AB}|^2/(4\pi\lambda k_b T)^{1/2}\right] \times \exp\left[-(\Delta G^\circ - \lambda)^2/4\lambda k_b T\right], \qquad (1)$$

where h is Planck's constant, H_{AB} is the electronic coupling factor, λ is the reorganizational

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PII: S0009-2614(01)01313-6

energy, k_b is Boltzmann's constant, T is the temperature, and ΔG° is the reaction free energy [2]. The electronic coupling factor is often expressed as

$$H_{AB} \sim H^\circ_{AB} \exp[-(\beta/2)(r_{AB} - r^\circ)], \tag{2}$$

where H°_{AB} is the electronic coupling factor for the donor/acceptor at a van der Waals contact distance r°, r_{AB} is the actual distance between the donor and acceptor and β is an empirically determined distance decay parameter [6,7]. For proteins, the value of β has been estimated to be between 1 and 1.5 Å^{-1} giving rise to an exponential decrease in reaction rate as the distance is increased [8].

Recently, the ability of DNA to mediate long range electron transfer has been investigated due to the importance of charge migration in DNA damage/repair mechanisms as well as the potential for DNA to function as a conductor in nanodevices [9–13]. The interest in DNA-mediated charge relocation has led to a number of studies designed to probe long range electron transfer in oligonucleotides. Previous studies by Barton and coworkers [9–11] have utilized Ruthenium/Rhodium complexes to the probe distance dependence between intercalated donor/acceptor pairs. A majority of these studies have utilized photoexcita tion of bis-(1,10 phenanthroline)(dipyridophenazine)Ru(II) (Ru(II)(phen)$_2$(dppz)$^{2+}$) which is followed by rapid quenching in the presence of bis(9,10 phenanthroline quinone diimine)(1,10 phenanthroline) Rh(III) (Rh(III)(phi)$_2$(phen)$^{3+}$). Intercalation of the Ru(II)(phen)$_2$(dppz)$^{2+}$/Rh (III)(phi)$_2$(phen)$^{3+}$ into 15-mer double stranded DNA segments resulted in electron transfer rates as high as 3×10^9 s^{-1} over a distance of nearly 40 Å [11]. In contrast, experiments involving ethidium bromide (EB) and N,N'-dimethyl-2,7 diazapyrenium chloride (DAP) or Ru(II)(NH$_3$) (pyridine)/Ru(III) (bipyridine)$_2$ (imidazole) separated by nearly 20 Å both give ET rates on the order of 1×10^6 s^{-1} [12,13]. More recently Priyadarshy et al. [14] performed theoretical calculations for the various donor/acceptor systems described above. The calculated electron transfer rates for the Ru(II) (NH$_3$)$_4$(pyridine)/Ru(III) (bipyridine)$_2$(imidazole) and EB/DAP systems (using $\Delta G^\circ = -0.7$ and -0.26 eV, respectively and $\lambda = 0.9$ eV for both systems) were 7×10^6 s^{-1} and 2×10^6 s^{-1}, respectively, using distance decay factors (β) of 1.0 and 1.6 Å. In contrast, the calculations for the Ru(II)(phen)$_2$(dppz)$^{2+}$/Rh(III) (phi)$_2$(phen)$^{3+}$ system suggested a much lower rate constant than observed. The only way to obtain the experimentally obtained rate constants was to use a value of $\beta \leqslant 0.2$ Å.

Most recently Lewis et al. [15, and references therein] examined photoinduced charge transfer in a number of synthetic DNA hairpins containing stilbene linkers. These studies revealed β values of 0.6–1.0 Å^{-1} with smaller values reflecting smaller driving forces. These results suggest a single step ET process consistant with a superexchange mechanism in which the electronic coupling is strongly distance dependent.

In an earlier study we examined photoinduced electron transfer between tetrakis-(4-tetramethylpyridyl)porphine (T4MpyP) and guanine monophosphate (GMP) within a self-assembled complex to mimic the initial events in DNA mediated intramolecular electron transfer [16]. Our results demonstrated quenching of the porphyrin singlet excited state by GMP arising from intracomplex electron transfer from ground state GMP to the excited singlet state of the porphyrin. The electron transfer rate of this reaction is on the order of 1.4×10^9 s^{-1} and should represent an upper limit to charge migration in oligonucleotides. In addition, significant dynamics were observed that modulate the electron transfer reaction, as evidenced by the presence of a distribution of quenched porphyrin singlet state lifetimes in the presence of GMP. In the present study, the dynamics of photoinduced electron transfer between T4MpyP and GMP are examined by monitoring the temperature dependence of both the center and width of the lifetime distribution. From the temperature dependence of the distribution center the reorganizational energy (λ) can be obtained. Similarly, the temperature dependence of the distribution width provides information concerning the nature of the dynamics that modulate the electron transfer reaction (see Fig. 1).

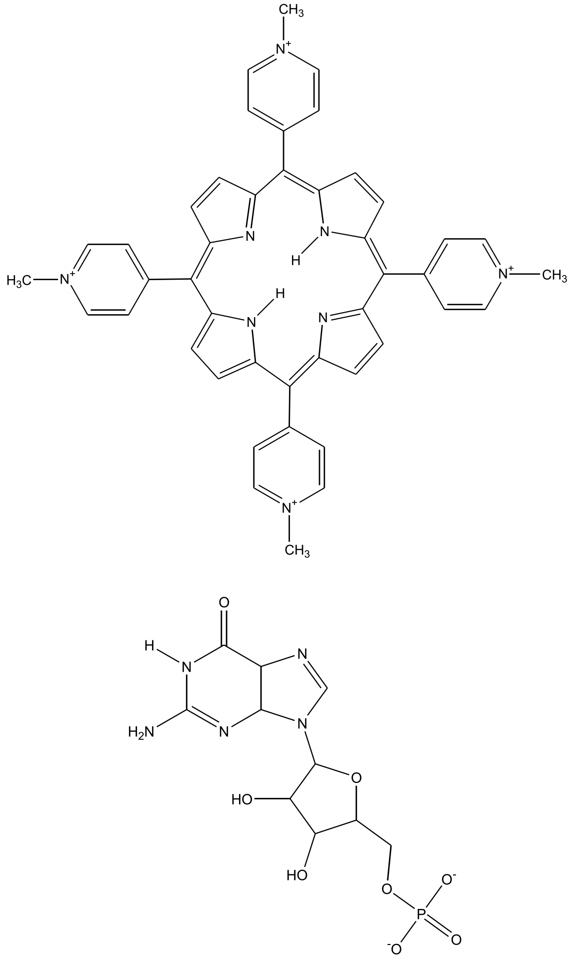

Fig. 1. Structural diagram of tetrakis(tetramethylpyridyl)porphyrin and guanine monosphosphate.

2. Materials and methods

Nucleotides (sodium salts, Sigma) and T4MPyP (tosylate salt, Porphyrin products) were used as received. The stock solutions were prepared in 100 mM phosphate ($\mu = 240$, no salt added) buffer (pH 7.1). Time-resolved fluorescence measurements were obtained using an ISS K2 multifrequency and phase modulation spectrofluorometer (ISS, Champaign, IL). Excitation of each sample was accomplished using the 488 nm line from an argon ion laser (Spectra-Physics model 2045). Excitation at 488 nm is at the blue edge of the porphryin soret absorption band. The resulting emission above 550 nm was observed through a Schott OG570 cut-on filter. The exciting light was polarized parallel to the vertical laboratory axis, while the emission was viewed through a polarizer oriented at 55°. The experimental protocols and analysis methods are as described in [16].

3. Results and discussion

We have previously shown that in the absence of GMP the T4MpyP exhibits a discrete lifetime of 5.9 ns while in the presence of GMP (with GMP:T4MPyP molar ratios of >1000) two lifetime components are present, one with a lifetime of 5.9 ns and a second component that can be fit to a Lorentzian distribution of lifetimes centered at 0.69 ns with a width of 0.9 ns (see Fig. 2, trace d) [16]. The center of the distribution, as well as the width, varies with temperature ranging from 0.91 to 0.5 ns at 280 K (Fig. 2, trace a) to 0.48–0.21 ns at 323 K (Fig. 2, trace g). The data are summarized in Table 1.

The corresponding Arrehenius plot for the quenching reaction is shown in Fig. 3, top panel. The rate constants were obtained from the center of the lifetime distribution and are taken as the average of the intracomplex electron transfer reaction. From the Arrehenius plot an activation

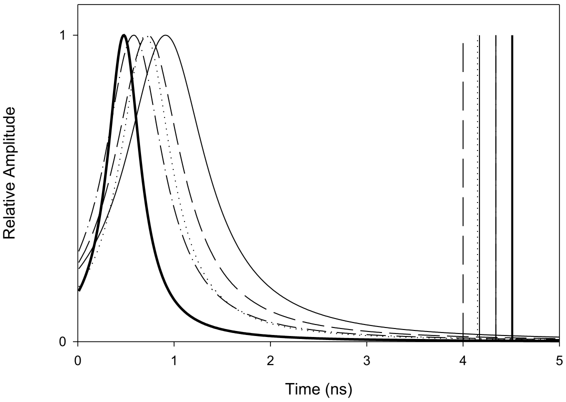

Fig. 2. Plot of lifetimes of T4MpyP in the presence of GMP at various temperatures. The temperatures are, in order of decreasing lifetime: 323, 307, 290, 285, and 280 K. [T4MpyP] = 10 μM, [GMP] = 1 mM in 100 mM pottasium phosphate buffer, pH 7.1.

Table 1
Summary of fluorescence lifetime data

Temp (K)	τ_1 (ns)	Width (ns)	Fraction	τ_2 (ns)	Fraction
280	0.91	0.50	0.67	4.3	0.33
284.8	0.73	0.42	0.6	4.0	0.4
289.9	0.71	0.32	0.54	4.2	0.46
298	0.60	0.32	0.42	4.2	0.58
306.5	0.58	0.37	0.32	4.5	0.68
318	0.49	0.21	0.17	4.4	0.83
323	0.48	0.21	0.14	4.5	0.86

energy of 2.5 kcal/mol is obtained. Fitting the rate constants versus temperature to the Marcus Equation (Eq. (1)) gives a reorganizational energy, λ, of 0.6 eV and an electronic coupling factor of 3×10^{-3} eV (Fig. 3, bottom panel) [2]. The temperature dependence of the electron transfer rates can also be evaluated using:

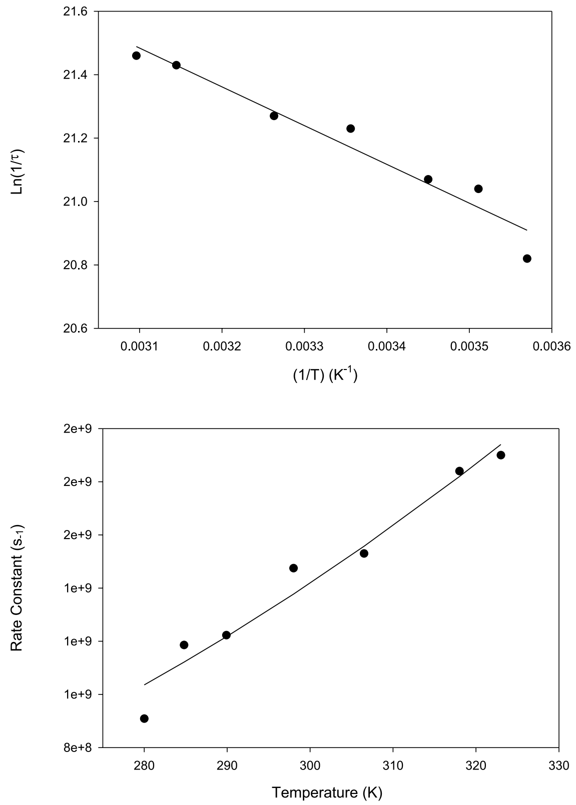

Fig. 3. Top panel: Arrhenius plot for the rate of intra-complex electron transfer. Rate constants were obtained using the center of the lifetime distributions for the short lifetime component. Bottom panel: Fit of the intra-complex electron transfer rate constants to temperature using the Marcus equation (Eq. (1) in the text).

$$K_{\mathrm{ET}} = k^{\circ} \exp[-(\beta/2)(r_{\mathrm{AB}} - r^{\circ})] \times \exp[-(\Delta G^{\circ} - \lambda)^2/4\lambda k_{\mathrm{b}}T], \qquad (3)$$

where k° is the maximum rate constant for activationless electron transfer, r° is the van der Waals distance of closest approach, β is an empirically determined parameter, and r_{AB} is the distance between electron transfer partners. Using this equation with $r^{\circ} = 3.6$ Å, $k^{\circ} = 2 \times 10^{11}$ s^{-1}, and $\beta = 1.4$ Å gives $\lambda = 0.6$ eV and $r_{\mathrm{AB}} = 3.8$ Å. It should be kept in mind that the use of Eqs. (1) and (2) assumes no temperature dependence in ΔG°. Although the temperature dependence of ΔE° for T4MpyP is not known it has been shown that for ET reactions with low driving forces (i.e., $\Delta G^{\circ} < \lambda$) the change in ΔG° would have to be quite significant (i.e., >100 mV) to have a significant effect on H_{AB} and λ [17].

The width of the lifetime distribution is related to the number of different conformations present and indicates conformational flexibility within the complex [16]. The changes in the width as a function of temperature provides important information regarding the potential surface describing the energetics of the interconversion between the different conformations. As the temperature is lowered the width of the distribution increases (see Table 1). This increase suggests that, in the present system, the potential surface describing the complex displays a number of shallow potential wells in dynamic equilibrium (see Figs. 4 and 5). At higher temperatures, these wells become equivalent (due to a low activation barrier) thereby

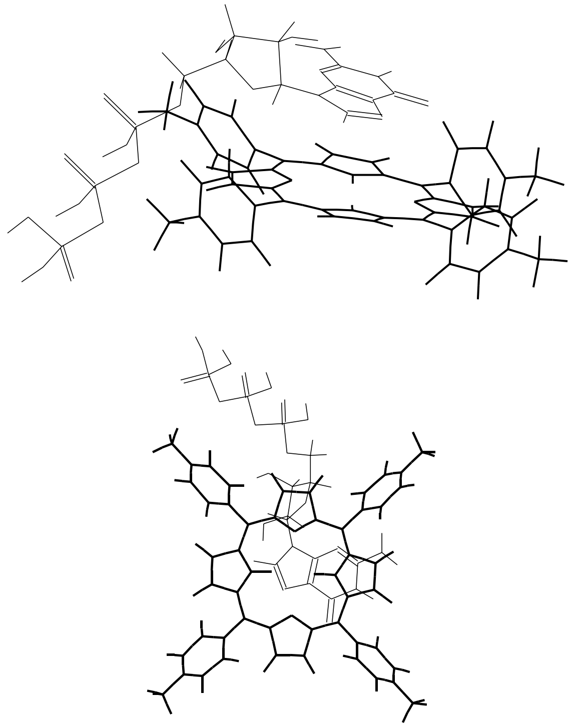

Fig. 4. Geometry optimized complex between GTP and T4MpyP. Models were constructed using HyperChem™ with an MM+ force field.

collapsing the width of distributions of rates (i.e., the observed rates would be an average of the rates of the various potential wells due to rapid interconversion). On the other hand, as the temperature is lowered the interconversion is gradually reduced and since there is no single energetically favorable conformation, all of the complexes become trapped in distinct shallow wells. Eventually, further lowering of the temperature should resolve the wide distribution of lifetimes into either several distributions or discrete components (Fig. 5). If the potential surface is defined by a single deep well and multiple shallow wells surrounding it, the number of accessible conformations should decrease as the temperature is decreased. This decrease, in turn, should reduce the width of the observed lifetime distributions and ideally, at very low temperatures (depending on the relative depths of the wells) the distribution should collapse to a discrete lifetime component.

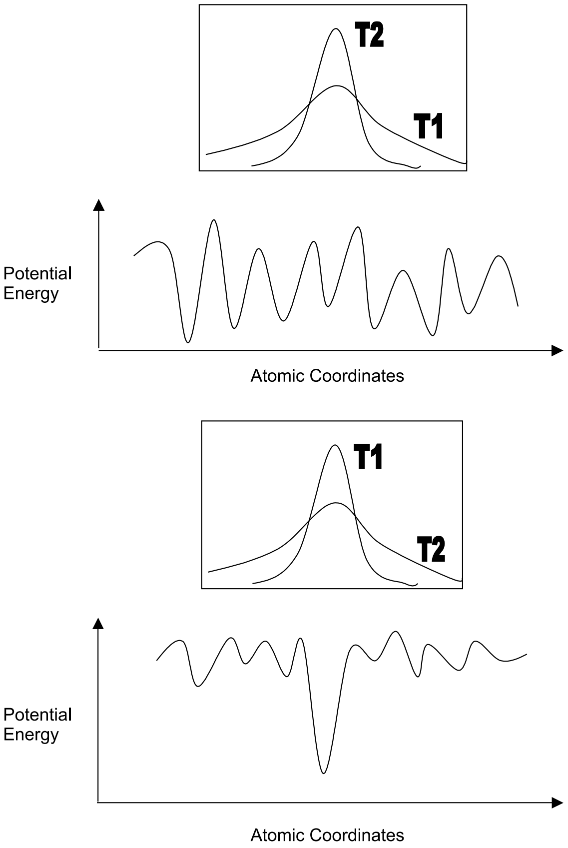

Fig. 5. Diagram showing two scenarios for temperature dependence of the width of the lifetime distribution. Top panel: many potential energy wells all with similar energies giving a broad distribution of lifetimes. As the temperature is increases the complexes rapidly interconvert between wells giving a single average lifetime and a narrow distribution. Bottom panel: single low potential well surrounded by many lower potential wells with relatively low barriers. As the temperature is lowered, the lowest energy well becomes favored resulting in a narrowing of the lifetime distribution.

The reorganizational energy for intracomplex electron transfer is found to be 0.6 eV for the T4MpyP:GMP system. This value is smaller than value obtained for the Ru(II)$(NH_3)_4$(pyridine)/Ru(III)$(bipyridine)_2$(imidazole) system ($\lambda = 0.9$ eV) but is considerably larger than the experimentally determined value obtained for the EB/DAP system ($\lambda = 0.2$ eV) [12,13]. The corresponding value for the Ru(II)$(phen)_2(dppz)^{2+}$/Rh(III)$(phi)_2(phen)^{3+}$ system is also on the order of 0.4 eV [9]. Interestingly, in the case of the EB/

DAP system theoretical calculations give rate constants quite close to experimentally determined values if λ is $\sim$0.9 eV but predict a rate constant nearly an order of magnitude faster using the experimentally determined value of λ (i.e., 0.2 eV) [13,14]. The value of λ for the T4MpyP:GMP system is also in the range of values observed for other porphyrin ET systems including.

The overall reorganizational energy is the sum of two components; λ_{I}, the inner sphere reorganizational energy is due to nuclear distortion due to the change in redox state of the donor/acceptor and λ_{o}, the outer sphere reorganizational energy, is due to the reorientation of the solvent cage subsequent to the formation of the products. The value of λ_{o} can be estimated using a simplified two sphere model:

$$\lambda_{\mathrm{o}} = e_{\mathrm{o}}^2[(1/2r_{\mathrm{D}}) + (1/2r_{\mathrm{A}}) - (1/r_{\mathrm{DA}})] \times [(1/\varepsilon_{\mathrm{op}} - 1/\varepsilon_{\mathrm{s}}], \tag{4}$$

where ε_{s} and $\varepsilon_{\mathrm{op}}$ are the static and optical dielectric constants, respectively, $r_{\mathrm{A}}/r_{\mathrm{D}}$ are the radii of the acceptor/donor, r_{DA} is the distance between the donor and acceptor, and e_{o} is the electron charge. This model assumes two spheres separated by a van der Waals distance and any temperature dependence is accounted for in the ε_{s} and $\varepsilon_{\mathrm{op}}$ terms. Although the T4MpyP:GMP ET occurs within the complex (i.e., excluding solvent molecules between the donor and acceptor) Eq. (4) can serve as an initial estimate of λ_{o}. Using $r_{\mathrm{GMP}} = 1.95$ Å, $r_{\mathrm{T4MPyP}} + = 9$ Å, $r_{\mathrm{cplx}} = 3.7$ Å, $\varepsilon_{\mathrm{op}} = 1.77$, and $\varepsilon_{\mathrm{s}} = 78.3$ (for water) a value of 0.33 eV is obtained for λ_{o} which is consistent with other porphyrin based donor/acceptor complexes [18–21] [4]. In addition, since the overall value of λ is 0.6 eV and the value of λ_{o} is estimated to be 0.33 eV the value of λ_{I} must also be roughly 0.3 eV. This value is also similar to λ_{I} for other free-base porphyrin ET systems [18–21].

4. Summary

In the present study we have examined the thermodynamics of electron transfer within self-assembled T4MpyP:GMP complexes and used this system as a model for DNA mediated electron transfer. Our data demonstrates significant conformational flexibility within the complex itself which regulates the electron rates. In addition, the value of the reorganizational energy for electron transfer within the model complex was used to probe the role oligonucleotide in modulating the thermodynamics of electron transfer between intercalated donor/acceptor pairs. These results further demonstrate the importance of conformational dynamics in regulating electron transfer rates in biological molecules.

Acknowledgements

The authors would like to acknowledge the National Science Foundation (NSF-REU Fellowship to SHL, NSF MCB 9904713 to R.W.L., and NSF MCB 9808427 to D.M.J.), and the American Cancer Society for an Institutional Seed Grant to R.W.L. We would also like to acknowledge the Laboratory for Fluorescence Dynamics which is supported by the National Institutes of Health Grant RR03155.

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[4] It should be pointed out that the values of λ and H_{AB} determined using Eqs. (1) and (4) should be viewed with some caution. Eqs. (1) and (4) assume that neither λ or ΔG° are temperature dependent within the range used here. A recent study by Kumar et al. [22] demonstrated that temperature dependent ΔG° and λ_{o} could have a significant effect on the magnitude of the electronic coupling factor. In addition, Eq. (4) assumes spherical reactants which could introduce additional uncertainty into the value of λ_{o}.

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