

Distance-Dependent Activation Energies for Hole Injection from Protonated 9-Amino-6-chloro-2-methoxyacridine into Duplex DNA

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The recent investigation¹ of the apparently anomalous attenuation factor ($\beta > 1.5 \text{ \AA}^{-1}$)² for photoinduced hole injection into DNA duplexes modified by protonated 9-amino-6-chloro-2-methoxyacridine (X^+) led to the conclusion that in addition to the electronic couplings, the activation energy must also be distance-dependent. Here we report the verification of this postulate by direct measurements of the activation energies for a series of (X^+)-modified DNA duplexes which sample an appreciable range of donor–acceptor distances ($\sim 4\text{--}11 \text{ \AA}$). The resulting changes in thermal activation energy can be explained within the framework of a distance-dependent reorganization energy.

The chemical structure of the intercalated hole donor X^+ and the DNA sequences investigated are shown in Chart 1. The forward and backward charge shift rates have been studied by femtosecond to nanosecond pump–probe spectroscopy, as described previously in detail.^{1,3} The activation energies E_a defined in terms of eq 1

$$k_{\text{ET}}\sqrt{T} = A \exp\left[-\frac{E_a}{k_B T}\right], \quad (1)$$

obtained for oxidation of the two nucleobases studied, guanine (G) and 7-deazaguanine (Z), are summarized in Table 1. Each successive insertion of an A:T pair between X^+ and G or Z leads to an increase in the activation energy for forward charge shift of about 0.1 eV (Figure 1).⁴ In contrast, the activation energies for backward charge shift show a somewhat weaker distance dependence that explains why for X^+AG and X^+AAZ only the forward charge shift can be observed.¹ We thus conclude that the anomalously steep distance-dependence of hole injection rates in X^+ /DNA duplexes carrying zero, one, or two A:T pairs between X^+ and G or Z is dominated by distance-dependent activation energies. Independent of the energetic parameters involved, the activation energy can explain the large changes in rates in a self-consistent way. Therefore temperature-dependent structural effects on the coupling are most likely quite minor. This view is also supported by the monoexponentiality of the injection rates, the linearity of the Arrhenius plots, and the invariance of the CD spectra down to 263 K (not shown).

We restrict our analysis to the activated hole injection reactions and employ the classical Marcus expression:⁵

$$E_a = \frac{(\Delta G + \lambda)^2}{4\lambda} \quad (2)$$

This relates E_a to the thermodynamic driving force ΔG and the

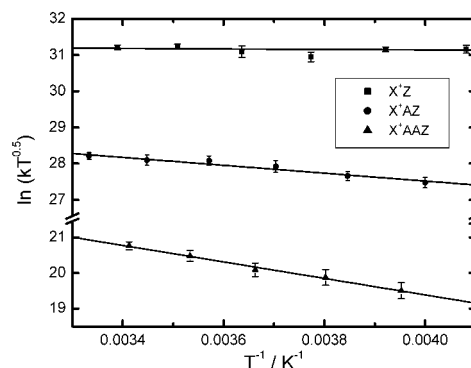


Figure 1. Effect of temperature on the rate constants for the forward charge shift in X^+Z (■), X^+AZ (●), and X^+AAZ (▲).

Chart 1. Structure of the Intercalated X^+ Dye and the Studied DNA Sequences

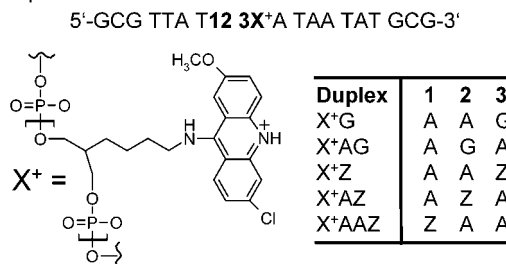


Table 1. Forward and Backward Hole Transfer Rates k_1 and k_2 at 283 K and the Corresponding Activation Energies³

duplex	k_1 [s ⁻¹]	E_a [eV]	k_2 [s ⁻¹]	E_a [eV]
X^+G	2.0×10^{11}	0.08 ± 0.04	1.9×10^{10}	0.14 ± 0.02
X^+AG	6.5×10^7	0.20 ± 0.04		
X^+Z	2.0×10^{12}	<0.015	1.0×10^{11}	0.08 ± 0.01
X^+AZ	9.3×10^{10}	0.09 ± 0.01	6.9×10^9	0.13 ± 0.02
X^+AAZ	4.6×10^7	0.20 ± 0.02		

reorganization energy λ , which in the present situation is due primarily to the medium contribution λ_s (see below). Equation 2 is a useful starting point since quantum effects are expected to be small in the Marcus normal region ($-\Delta G < \lambda$) near room temperature.⁶ This behavior is in contrast to the back-transfer reactions, which occur under strongly exergonic conditions ($-\Delta G > \lambda$) in the inverted regime. For these latter reactions, a quantum mechanical treatment of the vibrational degrees of freedom is essential.

It is well-known that distance-dependent activation energies can arise from the distance-dependence of both ΔG and λ_s on purely

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electrostatic grounds.^{5,7} An important simplification in the present situation is that for charge shift reactions, ΔG is expected to have little or no distance-dependence, in contrast to charge separation reactions. Therefore, E_a for charge shift reactions should normally be distance-dependent due solely to λ_s . Recent measurements of the temporal evolution of the stimulated emission spectrum in an X^+ -labeled duplex on the 200 fs to 60 ps time scale exclude any time-dependent (and therefore distance-dependent) change of ΔG ,⁸ in contrast to X^+ analogues in free solution which can display picosecond to nanosecond excited-state relaxation.⁹ Thus, we attribute the present distance-dependent activation energy solely to the reorganization energy.

In the absence of absolute values for ΔG , the analysis will start from a comparison of E_a values for the systems X^+AG and X^+AZ , assuming the oxidation potential of the easier-to-oxidize Z to be separated from G by $\Delta\Delta G = \Delta G_{X^+AG} - \Delta G_{X^+AZ} \approx 0.3$ eV.¹⁰ Utilizing $\Delta G_{X^+AG} = -0.15$ eV and $\Delta G_{X^+AZ} = -0.45$ eV, a fit of eq 2 to the corresponding E_a values in Table 1 provides a range of permissible values: 0.85 eV $\leq \lambda \leq 1.20$ eV.¹¹ Importantly, the calculated reorganization energy seems to be mainly dependent on the donor–acceptor distance and almost independent of the nature of the hole acceptor, G or Z. Applying the above values of ΔG to X^+G and to X^+AAZ yields respectively $\lambda = 0.6 \pm 0.1$ and 1.6 ± 0.1 eV. Extending this procedure to X^+Z is problematic, since the (almost) zero activation energy does not allow discrimination between an activationless or (slightly) inverted charge shift reaction. However, assuming that hole injection in X^+Z can be treated within the classical Marcus formalism (eq 2), one obtains $\lambda = 0.6 \pm 0.1$ eV, in good agreement with X^+G . In view of the uncertainties associated with the experimental estimate of $\Delta\Delta G = 0.3$ eV,¹⁰ it should be explicitly stated that only a small range of $\Delta\Delta G$ values, 0.15 – 0.3 eV, leads to self-consistent fits of the type cited above.

The λ values estimated above contain, of course, a contribution from molecular (λ_v) as well as medium (λ_s) modes. Since λ_v is not expected to depend appreciably on donor–acceptor separation, and since the overall λ for the “contact” cases studied here (X^+G and X^+Z) is estimated to be rather small (~ 0.5 eV), we conclude that λ_v is also likely to be quite small (a few tenths of an electronvolt), a result consistent with previous work showing that for an organic donor–acceptor pair, λ_v can be as small as 0.1 eV.¹² We also considered quantum mechanically the extent to which λ_v is likely to contribute to the activation energy. Calculations using the semiclassical electron-transfer model of Bixon and Jortner¹³ indicate that this contribution is small,¹⁴ and the λ estimates cited above can be taken as being dominated by λ_s .

The magnitude of λ_s , as well as the degree of distance-dependence, relies on a number of factors including the effective size of the donor and acceptor sites, their effective separation distance, and the effective polarity of their environment.⁵ The increase of ~ 1 eV as the donor–acceptor distance changes from ~ 4 (contact) to ~ 11 Å (two intervening A:T base pairs) is consistent with recent model calculations of λ_s in duplex DNA based on consideration of the dielectric heterogeneity of the surrounding medium.¹⁵ Quantitative modeling of λ_s for the present DNA duplexes would require detailed information about the structure of the X^+ chromophore in its binding site, including also any specific perturbations of the DNA structure.

It is still an open question as to the contribution of distance-dependent activation energies to the hole-transfer kinetics measured for other DNA-based systems.¹⁶ In general, distance-dependent activation energies for ET in molecular donor–acceptor systems may be expected if the medium reorganization energy (λ_s) is

appreciable, if the charge-transfer step is relatively short-range (i.e., occurring over ≤ 10 – 15 Å), and if the driving force is in the normal region ($-\Delta G < \lambda$) of the Marcus expression, eq 2.¹⁷

It is a task for the future to test different donor–acceptor systems derived from proteins or DNA duplexes for the contribution of distance-dependent Franck–Condon factors to the empirical attenuation factor β . In such systems, β is often discussed exclusively in terms of electronic couplings, although the potentially crucial role of the Franck–Condon factor has been noted in the general electron-transfer literature.^{17,18}

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- (3) All strands were purchased from Eurogentec Köln (Germany) and the samples were prepared as previously described.¹ The sequences shown in Chart 1 were hybridized with their Watson–Crick complements in a buffer solution (10 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, 500 mM NaCl, pH 7.2), and an adenine base was placed opposite to X^+ . The temperature range studied was 245–305 K. Sucrose (3.5 M) was added for freezing point depression to maintain the samples in the liquid phase. The invariance of the CD spectra and only minor changes of the rates at 283 K (Table 1) upon addition of sucrose indicate that the binding features of X^+ and the overall B-form of the duplex are maintained.
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