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Electron migration in DNA matrix: an electron transfer reaction

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ABSTRACT: This paper brings an active and provocative area of current research. It describes the investigation of electron transfer (ET) chemistry in general and ET reactions results in DNA in particular. Two DNA intercalating molecules were used: Ethidium Bromide as the donor (D) and Methyl-Viologen as the acceptor (A), the former intercalated between DNA bases and the latter in its surface. Using the Perrin model and fluorescence quenching measurements the distance of electron migration, herein considered to be the linear spacing between donor and acceptor molecule along the DNA molecule, was obtained. A value of 22.6 (± 1.1) angstroms for the distance and a number of 6.6 base pairs between donor and acceptor were found. In current literature the values found were 26 angstroms and almost 8 base pairs. DNA electron transfer is considered to be mediated by through-space interactions between the p-electron-containing base pairs.
KEYWORDS: Electron Transfer; DNA matrix; critical distance; Ethidium-Bromide; Methyl-Viologen; Donor-Acceptor

Introduction

In a general chemical view, ET reactions are processes in which reactants change into products. The process, although complex on microscopic scale, has the important simplifying feature that the nuclei do not move while the chemically significant electron changes its location and turns reactants into products. From a practical perspective, ET chemistry is responsible for life as we know it on earth.[8] This is true in face of photosynthesis in plants and bacteria is, at its core, based upon nature's ability to assemble a number of electrochemically active molecules in the right relative locations, so that when they absorb light an ET reaction happens.[11] In fact, much research on photoinduced ET reactions in the past three decades has demonstrated that getting the first charge transfer to occur is not hard.[6,9] The onset of photosynthesis process is that the oxidized donor ($D^{\bullet+}$) and the reduced

acceptor ($A^{\bullet -}$) don't immediately back-react to re-form the starting molecules. In the bacterial photosynthesis environment, almost all of the electrons transferred to the primary acceptor are successfully transferred to secondary acceptor. In consequence of this secondary ET chemistry is that plants, upon which we and most other animals depend, grow. By a reverse process of photosynthesis occurring in our mitochondria, the plant-product carbohydrate provide us with the energy we need to live.

What Is Interesting about ET in DNA?

DNA is a double helix molecule formed by two intertwining strands of deoxyribonucleic acid, nucleic acid bases are stacked in pairs one on top of the other with a slight twist reminiscent of a spiral staircase. The single stacking and overlapping order of the outer n- and p-electron of DNA bases may provide a preferred path for electron transfer. Similarly, the exceptional closeness of the stacked bases may have important consequences for charge motion in DNA duplexes. From a health perspective, both radiation and natural cellular process damage DNA and create reduced and oxidized ET products.[1] Fortunately, much of this damage is repaired shortly after it occurs by DNA repair enzymes. However in some instances this is not the case, and tumors or cancer result. Radiation damage to DNA involves primary ionization steps as well as migration of charges to trap sites where irreversible chemical reactions occur.[10,14] Thus charge migration is a key in both natural photosynthesis and DNA radiation damage.[7]

Theoretical Model of ET in DNA

At this model a steady-state quenching of the donor (D) excited state is performed by the acceptor (A) molecules, both linearly intercalated to DNA. Such arrangement make it possible to use a model to investigate the distance (r_0) dependence of this process between donor and acceptor. The model to be used is commonly applied to an sphere distribution, where the acceptor molecules are randomly distributed surrounding the donor, which is in the center of the sphere. We consider that radius r_0 of this sphere is the own DNA duplexes whereon donor and acceptor are intercalated. At the present example the quenching is due the electron transfer process and r_0 is the critical distance for the electron transfer to occur. Such parameter may be obtained by the Perrin's model[3,12] as follow.

The quenching of fluorescence involves a sequence of steps:

1 $h\nu + D \longrightarrow D^*$ (excitation)

2 $D^* \xrightarrow{w_0} D$ (decay with rate w_0 when no A present)

3 $D^* + A \xrightarrow{w} D + A^*$ (decay with rate w quenched by A)

The fluorescence efficiency is given by

$$\frac{F}{F_0} = \frac{\left[D^*\right]}{\left[D^*\right]_0} = \frac{w_0}{w_0 + w} \qquad (1)$$

where F and F_0 are the fluorescence intensity at the presence and absence of the quencher, respectively.

Assuming that there is a distribution *p(w)* of decay rate constants w, with

$$\int_0^\infty p(w)dw = 1 \qquad (2)$$

it's necessary to substitute equation (1) by the average with respect to *w*

$$\frac{F}{F_0} = \int_0^\infty \frac{w_0}{w_0 + w} p(w)dw \qquad (3)$$

Working with a small donor concentration, it may be considered that the quenching of donor excited state only happens with its nearest neighbor. Naming $p_1(r)$ the probability of the donor finding the first nearest acceptor molecule in a distance *r* and normalizing this probability, one has

$$\int_0^\infty p_1(r)4\pi r^2 dr = 1 \qquad (4)$$

It also means that the rate of decay is unique and a function of the distance *r*, i.e., *w = K(r)*. So, the distribution *p(w)dw* becomes $p_1(r)4\pi r^2 dr$. The equation (3) is rearranged giving

$$\frac{F}{F_0} = \int_0^\infty \frac{w_0}{w_0 + K(r)} p_1(r)4\pi r^2 dr \qquad (5)$$

To calculate $p_1(r)$, means to find the first neighbor in the distance *r* which represents the absence of any other neighbor inside the distance $\mathbf{r} < r$. Now it is necessary to find an expression for considering uniform the distribution of quencher.

The probability of finding a quencher inside the element of volume $dV = 4\pi\mathbf{r}^2 d\mathbf{r}$ is $4\pi C\mathbf{r}^2 d\mathbf{r}$; where *C* is the number of acceptor molecules per volume unit. The probability of absence of a quencher in *dV* is $1 - 4\pi C\mathbf{r}^2 d\mathbf{r}$.

Assuming that different elements of volume are uncorrelated, the event of not finding a quencher in $4\pi C_i \mathbf{r}_i^2 d\mathbf{r}_i$ is independent of the event of not finding a quencher in $4\pi C_i \mathbf{r}_i^2 d\mathbf{r}_i$, $\mathbf{r}_i^{-1}\mathbf{r}_i$ hence the product of the individual probabilities gives the probability of not finding a quencher in $\mathbf{r} < r$.

$$p = \prod_{i=1}^{N} \left(1 - 4\pi C\rho_i^2 d\rho_i\right) \qquad (6)$$

where $\mathbf{r}_i = 0,\ldots, \mathbf{r}_N = r$, taking logarithms and using $In(1-c) \approx -c$ for small *x* one has

$$\ln p = \sum_i \ln\left(1 - 4\pi C\rho_i^2 d\rho_i\right) \approx -4\pi C \sum_i \rho_i^2 d\rho$$

when *N* tends to infinity, one has

$$\ln(p) = -4\pi C\int_0^r \rho^2 d\rho = -\frac{4}{3}\pi C r^3$$
$$p(\rho(r) = e^{\frac{-4}{3}\pi C r^3} \qquad (7)$$

the probability that there is an acceptor at distance **r** < *r* is equal to *C* times the volume element *dV* = 4p$r^2 dr$ is:

$$p_1(r)dV = C4\pi r^2 dr e^{-\left(\frac{4}{3}\pi C r^3\right)}$$

Rewriting the equation (5) one has

$$\frac{F}{F_0} = \int_0^\infty \frac{w_0}{w_0 + K(r)} C e^{-\left(\frac{4}{3}\pi C r^3\right)} 4\pi r^2 dr \qquad (8)$$

Finally in order to be coherent with the Perrin[3,12] model one considers an sphere of ray r_0, in which the electron transfer occurs when $r < r_0$ and does not occur when $r > r_0$.

$$K(r) = \begin{cases} \infty \rightarrow r \langle r_0 \\ 0 \rightarrow r \rangle r_0 \end{cases}$$

The equation (8) becomes

$$\frac{F}{F_0} = \int_{r_0}^\infty 4\pi C e^{-\frac{4}{3}\pi C r^3} r^2 dr = e^{-\frac{4}{3}\pi C r_0^3} \qquad (9)$$

Plotting F/F_0versus *C* which is the number of acceptor molecules per volume unit, one obtains the value of r_0, the critical distance for the electron transfer process take place.

Experimental

Preparation of Solutions

Calf-Thymus DNA (20 mg) purchased from Pharmacia was dissolved in Milli-Q water (10 ml) under agitation at -3°C during 24h until complete dissolution. Absorption measurements at the UV region, 260 and 280 nm determined the degree of contamination of the DNA by proteins. The ionic strength of the solution was 0.100 mol/L of NaCl. Aqueous solutions of ethidium bromide (D) and methyl viologen (A) purchased from Sigma, were prepared by dissolution of the substances under the conditions as before. The donor concentration was determined by absorption measurements at 480 nm (**e** = 5700 mol^{-1} L cm^{-1}),[4] and acceptor concentration was defined through gravimetric measurement and dissolving 25 mg in 5 ml of Milli-Q water, considering that its formula weight is 257.17 g/mol.[2]

Fluorimetry

Determination of Fluorimeter Settings

The fluorescence measurements were done using a HITACHI 4500 spectrofluorimeter. The fluorescence cuvette was filled with 1 ml of the ethidium bromide stock solution. Excitation wavelength was set to 525 nm, the excitation and emission slits to 5 nm. The emission maximum was determined by a slow scan of the emission wavelength from 540 to 700 nm, and the measurements were obtained in this particular wavelength. A gaussian fitting was used to determine the fluorescence intensity with its center fixed at 600 nm.

Results and discussion

Fluorescence measurements of ethidium binding to DNA are well known.[15] However, herein its luminescent property was used as a probe and the quenching of the excited state by an acceptor molecule (Methyl Viologen), mediated through the DNA matrix, raised questions about the behavior of electron transfer process in DNA. In recent paper Beratan[13] discussed the earliest studies of electron-transfer proteins and its application to DNA, emphasizing the contribution of the p-electron system of the DNA base pairs.

The following results do not confirm exactly the migration path of the electron using the p-electron system of the DNA base pairs but confirm the existence of such reaction. The data of Figure 1 were obtained with the ethidium (D) at a concentration of 1.53×10^{-4} M and the addition of DNA aliquots (**m**l). The fluorescence intensity increment was expected in face of the intercalative binding of ethidium to DNA. The DNA addition stopped when no modification in intensity was observed confirming that all intercalant disposable in bulk solution was bound. The second step was the addition of methyl viologen (A) a quenching promoter, so the fluorescence intensity was expected to decrease (see Figure 2).

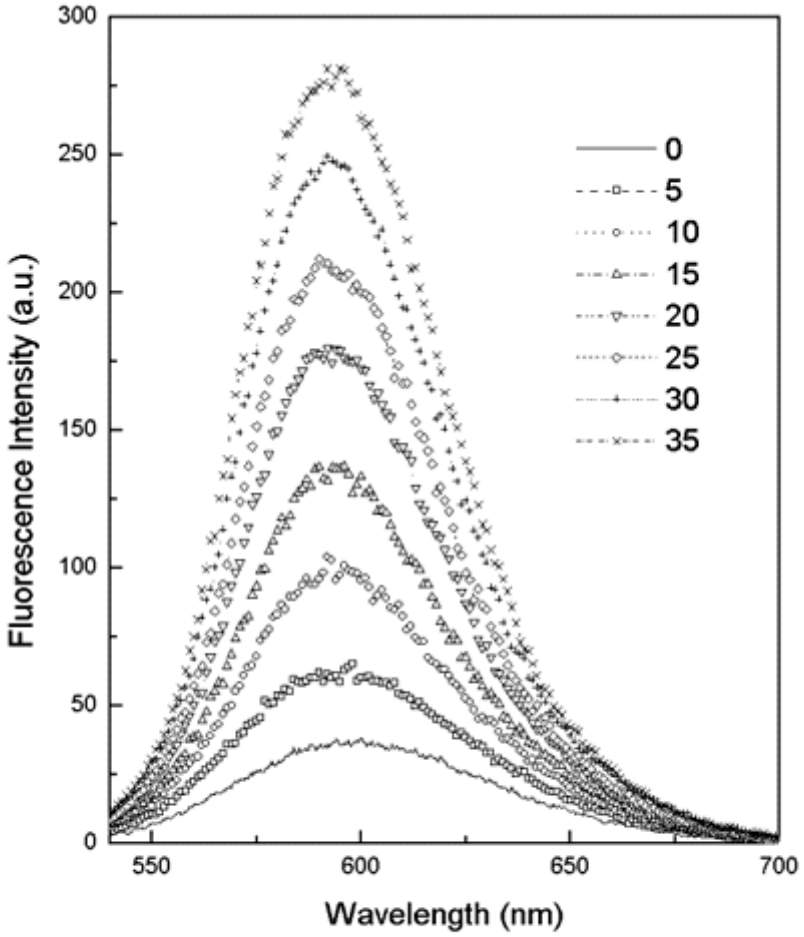

FIGURE 1 - Variation of fluorescence of ethidium (D) 1.53×10^{-4} mol/L as a function of added Ct-DNA. The spectra are numbered from 0 to 35, the number corresponding to the added volumes of stock solution of Ct-DNA, expressed in μl.

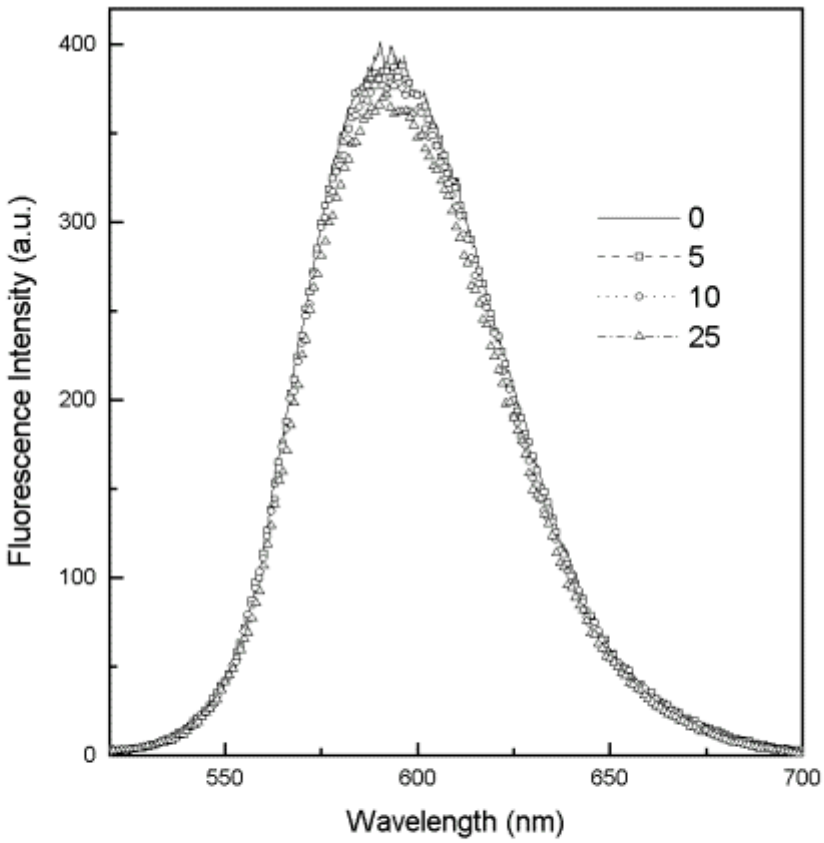

FIGURE 2 - Fluorescence quenching measurements of ethidium (D) intercalated to Ct-DNA (55 μl) in NaCl solution 0.100 mol/L, as a function of added Methyl Viologen (A). The spectra are numbered from 0 to 25, the number corresponding to the added volumes of stock solution of Methyl Viologen, expressed in μl.

Addition of Methyl Viologen solution was continued until no modification on fluorescence intensity was detected, which may be understood by considering that all Methyl Viologen added was anchored to DNA surface. The absence of free D and A molecules at the bulk solution avoided the quenching process occurring by random collision, what should restrain the use of the Perrin model and confirmed that the distribution of donor and acceptor molecules happened along the DNA matrix. Using the fluorescence quenching data a linear plot of $\ln(F/F_0)$ vs. number of acceptor molecules per volume unit, shown in Figure 3, was obtained, as predicted by Perrin model. A linear regression fit of these data gave a result of 22.6± 1.1 Å for the distance between a donor and an acceptor molecule along the DNA duplexes, and a number of about 6.6 base pairs involved in the process.

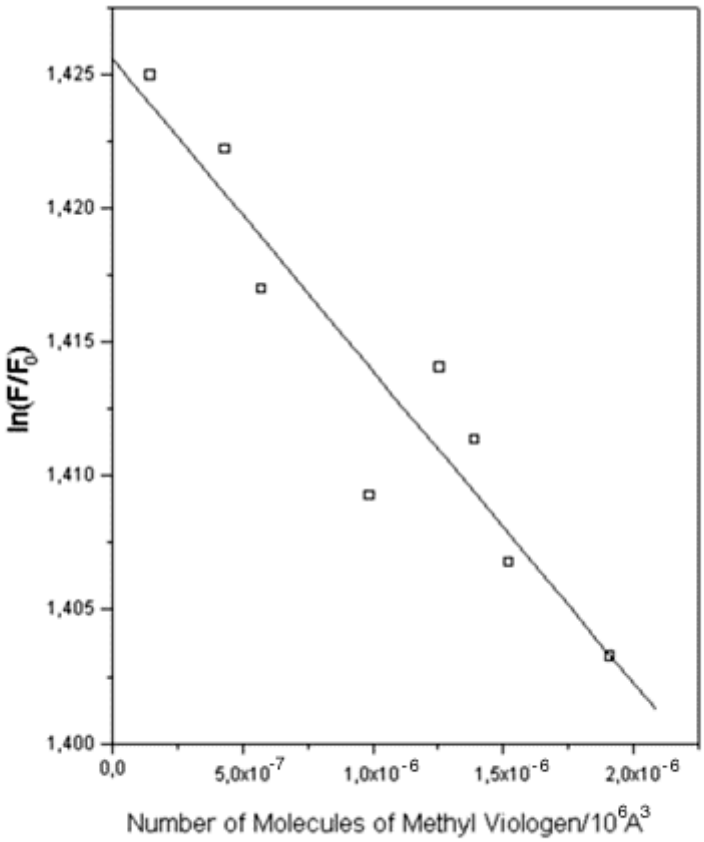

FIGURE 3 - Plot of $\ln(F/F_0)$ as a function of the number of acceptor molecules per unit volume.

Comparing these results with those found in the literature[12] of 26 Å for the distance and a number of 8 base pairs, the differences are almost insignificant. The theoretical model here applied is limited to the information of the distance between donor and acceptor molecules do not being able to give more information about the electron pathway on the DNA duplexes, by using the p-electron system of the base pairs or the DNA backbone. The p-electron system contribution may be verified by this simple experiment just modifying the ionic strength what should change the DNA compactness moving closer the base pairs what is important to the system over mentioned. The process in consideration and mentioned in literature do not take in account the importance of the hydrogen bridges formed between the bases, the ET reaction in DNA being as a reaction where the DNA behaves like a "molecule wire". The next step following these results is to test the importance of the distance between the stacked bases and how the hydrogen bridges play an important role in this chemical reaction.

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RESUMO: Este artigo apresenta uma área de pesquisa atual, ativa e interessante. Descreve a investigação da química de transferência de elétrons (TE) de um modo geral e resultados de TE em DNA em particular. Dois intercalantes de DNA foram utilizados: Ethidium Bromide como doador

(D) e Methyl-viologen como receptor (A), o primeiro intercala-se entre as bases do DNA e o último na sua superfície. Utilizando o modelo de Perrin e medidas de Supressão de Fluorescência obteve-se a distância de migração do elétron; aqui a distância foi considerada o espaçamento linear entre as moléculas de doador e receptor ao longo da molécula de DNA. O valor determinado foi de 22,6 ± 1,1 angstrons e o número de pares de bases entre doador e receptor de 6,6. Na literatura os valores encontrados foram de 26 angstrons e de quase 8 pares de bases. Considera-se que a transferência de elétrons em DNA seja mediada através das interações através do espaço entre os elétrons do tipo p contido nos pares de bases.

PALAVRAS-CHAVE: Transferência de elétrons; matriz de DNA; distância crítica; ethidium-bromide; methyl-viologen; doador-receptor

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