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Electron transfer reactions between nucleic acid bases and tetracyanoethylene in acetonitrile

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Abstract

The electron transfer reactions between pyrimidines, e.g. thymine (Thy), uracil (Ura) and cytosine (Cyt), and tetracyanoethylene (TCNE) in acetonitrile were studied using nanosecond laser flash photolysis techniques. Laser photolysis of a mixed solution of TCNE and pyrimidines led to the formation of the anion radical of TCNE, which has an absorption band at 350–500 nm ($\lambda_{max}=430$ nm) and decays following second-order kinetics. The electron transfer reaction occurs through the excited triplet states of the pyrimidines, and the bimolecular quenching rate constants are 7.6×10^9 dm^3 mol^{-1} s^{-1} for thymine and 1.7×10^{10} dm^3 mol^{-1} s^{-1} for uracil. However, a ground state complex between cytosine and TCNE was found. This complex has an absorption band with λ_{max} at 419, 444 and 472 nm. The photoinduced electron transfer between cytosine and TCNE was not observed on a nanosecond time scale during laser photolysis.

Keywords: Electron transfer reactions; Nucleic acid bases; Tetracyanoethylene; Acetonitrile

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1. Introduction

Tetracyanoethylene (TCNE) is a powerful electron acceptor. Since it was first discovered in 1957, numerous investigations concerning its character and reactions have been performed [1–5]. However, only a few studies have been performed on the reactions of TCNE with biological substances. Panov [2, 3] found that TCNE was highly toxic in mice. Machmer [4] observed that adenine and guanine formed charge transfer complexes with TCNE in glacial acetic acid solution at −78 °C. These studies indicate that TCNE can interact with nucleic acid bases, probably leading to damage of DNA. The photochemical properties of DNA, particularly the nucleic acid bases, have been widely studied [6, 7]. The results have demonstrated that the excited triplet state is dominant in the photochemical reaction. It has also been observed that the excited triplet state is an electron donor when it reacts with electrophilic molecules [8]. Therefore an investigation of the electron transfer reactions between TCNE and nucleic acid bases is of interest; laser flash photolysis techniques were used.

2. Experimental details

2.1. Materials

TCNE (Fluka) was recrystallized from redistilled chlorobenzene, and cytosine (BDH), thymine (Sigma) and uracil (Beijing Chemistry Factory) from distilled water. Acetonitrile (spectroscopic grade, 99.9%) was used without further purification.

2.2. Nanosecond laser flash photolysis

Laser flash photolysis experiments were performed using a KrF excimer laser (248 nm; pulse width, 20 ns; energy, 50 mJ). The equipment has been described previously [9].

All experiments were carried out in acetonitrile solutions at room temperature. The solutions were deaer-

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ated by high-purity nitrogen (99.99%) by bubbling for 20 min prior to photolysis.

3. Results

3.1. Absorption spectra

The absorption bands of solutions of pyrimidines or TCNE appear below 300 nm in the UV region; the absorption of mixed solutions of thymine or uracil and TCNE is the sum of the absorptions of the two components; no other bands were found. However, a solution of cytosine and TCNE slowly becomes yellow after mixing, and the colour of the mixed solution becomes constant after approximately 30 min. The yellow solution has a strong absorption band between 350 and 500 nm with λ_{max} at 419, 444 and 472 nm (see Fig. 1); the extinction coefficient (ϵ) is estimated to be greater than 1×10^4 cm^{-1} mol^{-1} l.

3.2. Laser flash photolysis experiments

Laser excitation (248 nm) of mixed solutions of thymine or uracil and TCNE in acetonitrile leads to a transient intermediate, which has an absorption band between 350 and 500 nm with λ_{max} at 430 nm (Fig. 2). The decay of the intermediate follows second-order kinetics. However, laser excitation of a mixed solution of cytosine and TCNE shows no observable signals.

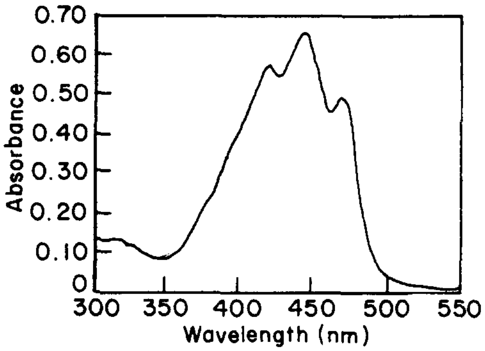

Fig. 1. Absorption spectrum of the mixed solution of cytosine and TCNE in the range 300–550 nm (the concentrations of cytosine and TCNE were 7×10^{-5} mol l^{-1} and 4.8×10^{-3} mol l^{-1} respectively).

Laser photolysis of solutions of pure pyrimidines or TCNE was also performed for comparison. Photolysis of a solution of thymine in acetonitrile produces an absorption band with $\lambda_{max}=330$ nm and a lifetime of 1.8 μs, which is assigned to the excited triplet state of thymine; photolysis of uracil in acetonitrile gives an absorption band with $\lambda_{max}=370$ nm and a lifetimes of 0.6 μs, which is assigned to the excited triplet state of uracil (Fig. 3). The two excited triplet states have been confirmed by many investigators [7, 8]. Excitation of a solution of TCNE does not lead to observable signals.

3.3. Quenching of the excited triplet state

The addition of TCNE to solutions of thymine or uracil leads to a decrease in the lifetime of the excited triplet state of the base in a systematic manner according to

$$k_{obs}=k_0+k[\mathrm{TCNE}] \qquad (1)$$

where k_{obs} is the observed first-order decay constant of the excited triplet base, k_0 is the triplet decay rate constant in the absence of TCNE and k is the bimolecular quenching rate constant. The kinetic experiments were carried out at 330 nm. The observed values of k_{obs} and the corresponding concentrations of TCNE are listed in Table 1. The two data groups show good linearity. The bimolecular quenching rate constants obtained are $k_{Thy}=7.6\times10^9$ dm^3 mol^{-1} s^{-1} and $k_{Ura}=1.7\times10^{10}$ dm^3 mol^{-1} s^{-1} (the concentration of the base used in the kinetic experiments was 2.0×10^{-4} mol l^{-1}).

4. Discussion

The absorption spectra show that cytosine and TCNE form a ground state complex, but no ground state complex is observed between TCNE and thymine or uracil, probably due to the lower π-electron density of thymine and uracil.

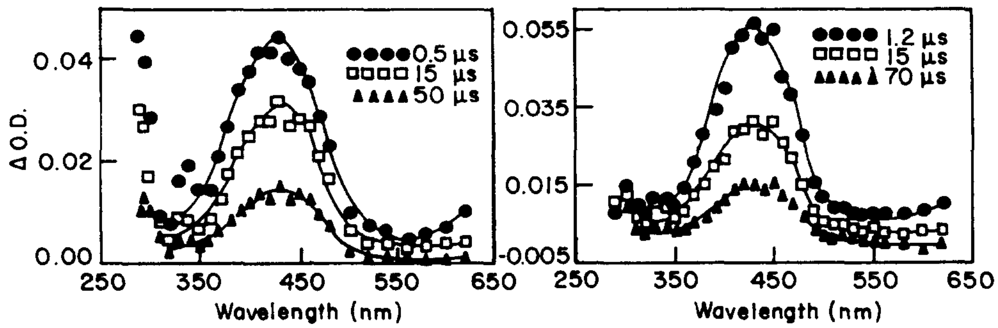

Fig. 2. Time-resolved transient absorption spectra of mixed solutions of thymine or uracil and TCNE after excitation at 248 nm (the concentration of pyrimidine was 1.2×10^{-4} mol l^{-1} and that of TCNE was 8.6×10^{-5} mol l^{-1}): left, thymine and TCNE; right, uracil and TCNE.

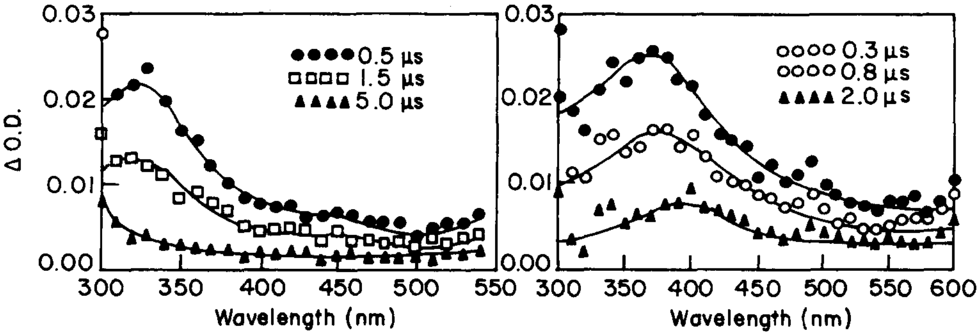

Fig. 3. Time-resolved transient absorption spectra of solutions of thymine and uracil in acetonitrile: left, thymine; right, uracil.

Table 1
The observed values of k_{obs} (s^{-1}) and the corresponding concentrations of TCNE (mol l^{-1})

Thy	[TCNE] $\times 10^5$	0	0.68	2.5	6.3	9.5
	$k_{obs} \times 10^{-6}$	0.376	0.425	0.476	0.760	1.12
Ura	[TCNE] $\times 10^5$	0	2.0	4.0	6.3	8.2
	$k_{obs} \times 10^{-6}$	1.11	1.34	1.79	2.01	2.50

The absorption band of the complex between cytosine and TCNE is very similar to that of the TCNE radical anion reported in the literature [10], which suggests that charge transfer occurs in the complex. Moreover, the absorption of the complex arises mainly from the TCNE anion radical. This is probably due to the very weak absorption of the cation radical. A similar situation has been reported in nucleic acids [11].

The photolysis of uracil and thymine at 248 nm has been studied previously [12]. From the transient absorption spectra, the intermediate produced after laser photolysis of mixed solutions of TCNE and thymine or uracil should be assigned to the TCNE anion radical, as the absorption spectrum is similar to that reported for the TCNE anion radical in the literature [10]. Therefore electron transfer reactions between TCNE and thymine or uracil are successfully induced by 248 nm laser photolysis.

No other absorption bands are found in the transient spectrum; the corresponding radical cations of the bases are not observed, probably due to their small extinction coefficient [11–13]. The latter seems to be more reasonable since the absorption of the TCNE anion radical is also detected for the cytosine–TCNE complex.

The lifetime of the TCNE anion radical reaches about 15 µs; a lifetime of 50–60 ps has been reported in the literature [10, 14]. The large difference may be due to the solvent cage effect and the different excitation wavelength. In refs. [10, 14], ground state complexes of TCNE and substituted anthracenes and indenes were photolysed at 532 nm. After excitation, the charge of the complex was separated; therefore, the absorption band of the TCNE anion radical was observed. In this case, the distance between the radical anion and cation is small and they remain in the same solvent cage as a solvated ion radical pair. At the end of the laser pulse, the separated charges recombine very rapidly, and thus the TCNE anion radical is very short lived. In our experiments, no ground state complex is formed and the excitation wavelength of 248 nm can excite the nucleic acid base. The excited base molecules collide with ground state TCNE and lead to electron transfer. In this case, the generated TCNE anion radical is surrounded by polar solvent molecules and is stabilized rapidly. Recombination can only be achieved after collision with the cation radical of the pyrimidines. Therefore the process of decay will be far slower.

Because the electron transfer reaction only occurs after collision, the excited state must possess a long lifetime. The fluorescence lifetime of the bases is very short at room temperature, only 1–10 ps [15–18]; however, in our experiment, the lifetime of the intermediate is on the microsecond time scale. Therefore it is more probable that the electron transfer reaction occurs through the excited triplet state of the bases. This process can be represented by

$$\begin{matrix}\text{Thy}\\ \text{Ura}\end{matrix} \xrightarrow[\text{2) ISC}]{\text{1) }h\nu} \begin{matrix}{}^{3}\text{Thy}^{*}\\ {}^{3}\text{Ura}^{*}\end{matrix} + \text{TCNE} \longrightarrow \begin{matrix}\text{Thy}^{\cdot +}\\ \text{Ura}^{\cdot +}\end{matrix} + \text{TCNE}^{\cdot -}$$

Figure 4 gives the decay curves of the transients at 430 and 330 nm. The decay curve at 430 nm is due to the TCNE anion radical, and that at 330 nm is due to the excited triplet states of thymine and uracil. From Fig. 4, it can be seen that the decay of the excited triplet states of the pyrimidines and the increase in the absorption of the TCNE anion radical occur simultaneously. On the basis of this deduction, the measurement of the bimolecular quenching rate constant becomes possible. The good linearity of a plot of k_{obs} vs. the concentration of TCNE is in accordance with this.

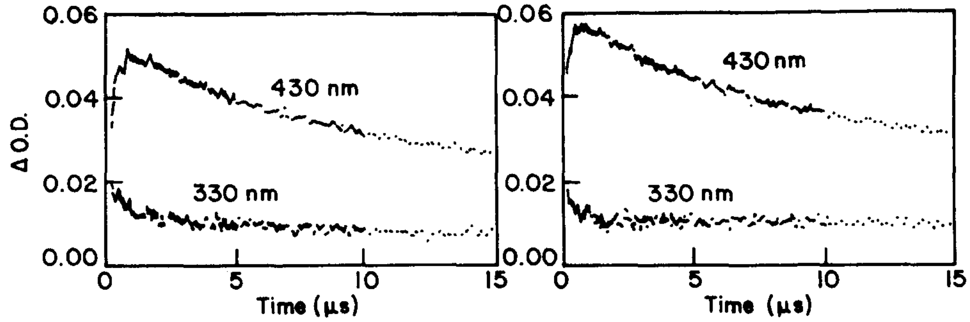

Fig. 4. Decay curves at 430 and 330 nm: left, thymine and TCNE; right, uracil and TCNE.

The free energy change ΔG (kJ mol^{-1}) of the electron transfer process can be calculated approximately according to the equation [8]

$$\Delta G^\circ = 96.48(E^\circ_{ox} - E^\circ_{red} - e^2/\varepsilon r - \Delta^3 E_{0,0}) \qquad (2)$$

where E_{ox} and E_{red} are the polarographic half-wave oxidation potential of the electron donor and the reduction potential of the acceptor in volts respectively, e^2/r is the coulombic term and $\Delta^3 E_{0,0}$ is the zero–zero spectral energy of the triplet base in electronvolts. The values of E_{ox} of thymine and uracil in acetonitrile are 1.75 V and 2.05 V respectively, the $\Delta^3 E_{0,0}$ values are 313 and 328 kJ mol^{-1} respectively and $e^2/r = 0.06$ eV [8]. The reduction potential of TCNE in acetonitrile is 0.24 V vs. a saturated calomel electrode (SCE) [19–23]. The values obtained from eqn. (2) are $\Delta G_{Thy} = -127$ kJ mol^{-1} and $\Delta G_{Ura} = -113$ kJ mol^{-1}, which show that electron transfer occurs easily in the excited state.

For cytosine, it is believed that electron transfer occurs only within the solvent cage, since cytosine and TCNE form a ground state complex. This leads to rapid recombination which cannot be observed with our apparatus.

5. Conclusions

Our experiments demonstrate that the electron transfer reactions between TCNE and pyrimidines can occur in the ground or excited state. Since TCNE is phototoxic to biological tissue, and DNA is readily damaged by TCNE on exposure to sunlight [2, 3], TCNE is probably carcinogenic.

Acknowledgements

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