Comparative studies of triplet states of thymine components by acetone sensitization and direct excitation in aqueous solution at room temperature

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Abstract

The transient absorption spectra and the kinetic parameters of decay of the triplet states of thymine and its nucleoside, nucleotide, were obtained from acetone sensitization and direct excitation in aqueous solution at room temperature. The kinetic parameters from two methods are equivalent, thus time-resolved acetone sensitization method was verified by the laser photolysis experiment. Furthermore, advantages of acetone sensitization method were clarified by the characteristics of triplet acetone and enhanced yields of triplet states of thymine components. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Thymine; Thymidine; TMP; Triplet states; Laser flash photolysis; Direct excitation; Acetone sensitization

1. Introduction

Using laser flash photolysis, Salet and Bensasson [1] and Salet et al. [2] obtained successfully the triplet–triplet (T–T) absorption spectra and determined kinetic parameters of the triplet state of thymine components whose yields are highest in nucleic acid bases by direct photolysis in 1970s. After this, the study of triplet state behavior of DNA and its components is hampered by the very small yields produced by direct excitation.

Utilizing acetone sensitization method, a breakthrough for detection of triplet–triplet absorption spectra and triplet state kinetics of DNA components was performed at our laboratory [3–6]. Very recently similar studies of triplet states of DNA components from acetone sensitization have been reported by Gut et al. [7] and Wood and Redmond [8]. Triplet acetone ($^3Ac^*$) has distinct advantages over a variety of triplet sensitizer for kinetic studies of triplet state behavior of nucleic acid and its component nucleotides. First, acetone with very high triplet energy [9] can sensitize the triplet states of all the nucleotides [7]. Secondly, the quantum yield (Φ_{ISC}) of $^3Ac^*$ [10] is 2–3 orders of magnitude higher than those of the nucleotides [11], so the quantum yields of the triplet

nucleotides should be enhanced to a most extent under acetone sensitization. Finally, owing to the fact that the absorption band of $^3Ac^*$ appearing in the narrow wavelength region of 300–380 nm with very short life time, acetone–nucleotide interaction allowed the nucleotide T–T absorption spectra to be measured accurately. In short, kinetic studies of photophysical and photochemical process of DNA and nucleotide system have been rendered readily by acetone sensitization [7,12]. Thus, time-resolved acetone-sensitization method has been accepted as a tool for studies of triplet state behavior of DNA and its components [7,8,13]. However, comparative studies of triplet states of DNA components from acetone sensitization and direct excitation at similar condition have not been reported yet. In this work, the kinetic parameters of the decay of triplet states of thymine (Thy), thymidine (Thd) and thymidine 5'-monophosphate (TMP) have been obtained from both acetone sensitization and direct excitation using 248 nm, 20 ns excimer laser pulse in aqueous solution at room temperature.

2. Materials and methods

Thy, Thd and TMP obtained from Sigma were used as received. Acetone (analytic grade reagent) was distilled before use. NaOH and phosphate (analytic grade reagent) were used as received. The pH values of solutions were

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adjusted by NaOH and H_3PO_4 solutions. All samples were prepared in triply distilled water and deaerated by high purity nitrogen (99.99%) bubbling for 20 min. All experiments were carried out at room temperature.

Laser photolysis experiments were performed using a home-made excimer laser which provided 248 nm (KrF) light pulse with a duration of 20 ns. The maximum laser energy was 50 mJ per pulse. The signals were collected using an HP 54510B 300 MHz transient recorder, then processed with an PC-486 personal computer. Detailed descriptions of the equipment and experimental conditions were given elsewhere [4].

3. Results and discussion

3.1. Direct excitation

The transient absorption spectra of the intermediates produced from laser photolysis of Thy, Thd and TMP aqueous solution are shown in Figs. 1–3, respectively. At the end of laser pulse, an absorption band appears and can be quenched by O_2, which decays following first-order kinetics (see insets
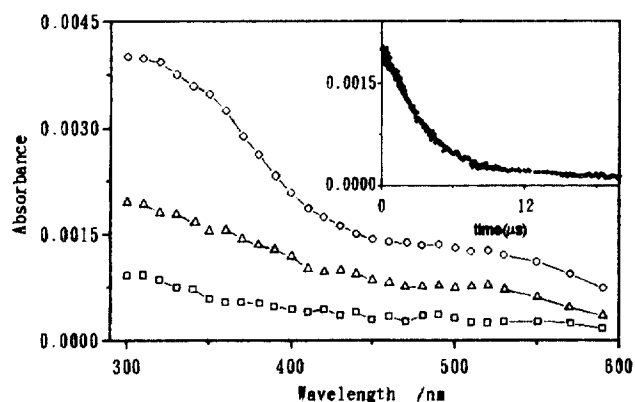

Fig. 1. Transient absorption spectra from photolysis of 1 mM Thy aqueous solution saturated with N_2 at pH 7: 0 μs (O), 2 μs ($\triangle$), 6 μs ($\square$). Inset: trace at 400 nm.
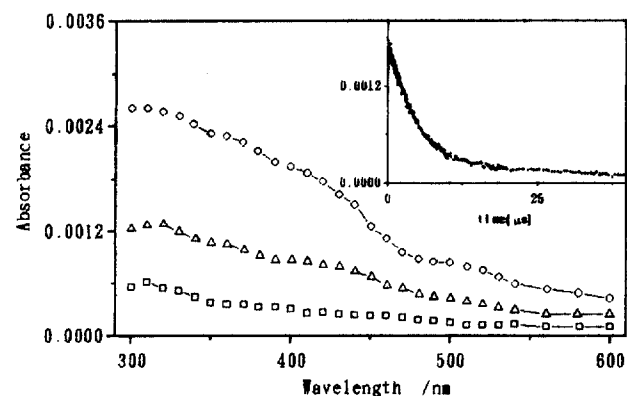

Fig. 2. Transient absorption spectra from photolysis of 1 mM Thd aqueous solution saturated with N_2 at pH 7: 0 μs (O), 5 μs ($\triangle$), 15 μs ($\square$). Inset: trace at 400 nm.
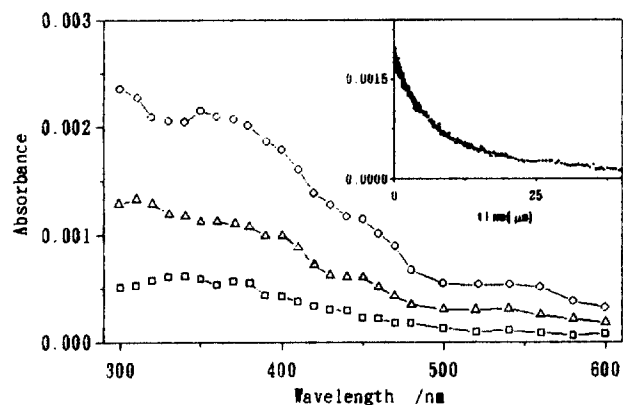

Fig. 3. Transient absorption spectra from photolysis of 1 mM TMP aqueous solution saturated with N_2 at pH 7: 0 μs (O), 5 μs ($\triangle$), 20 μs ($\square$). Inset: trace at 400 nm.

of Figs. 1–3), thus should be assigned to the absorption of triplet Thy, Thd and TMP (symbolized as $^3T^*$). The scheme of formation and decay as follow:

$$T \xrightarrow{h\nu} {}^1T^* \xrightarrow{ISC} {}^3T^* \tag{1}$$

$$^3T^* \rightarrow T \quad k_0 \tag{2}$$

$$^3T^* \rightarrow 2T, T_2 \quad k_s \tag{3}$$

The triplet states of thymine components ($^3T^*$) decay according to pseudo-first-order kinetics. Its apparent decay rate constant can be expressed as

$$k_{obs} = k_0 + k_s[T]$$

in which $k_0 = k_r + k_{nr}$, [T] expresses the concentration of thymine component. A series of k_{obs} values were obtained from changes of the concentrations of thymine components. The plot of k_{obs} against the concentration of thymine component is a straight line. The self-quenching rate constant (k_s) and the rate constants of the unimolecular decay (k_0) are obtained from the value of the slope and the intercept of the straight line respectively, and are listed in Table 1.

3.2. Acetone sensitization

Figs. 4–6 show the transient absorption spectra obtained from the photolysis of Thy, Thd and TMP in the presence of

Table 1
Rate constants for decay of triplet Thy, Thd and TMP obtained from acetone sensitization (S) and direct excitation (D)

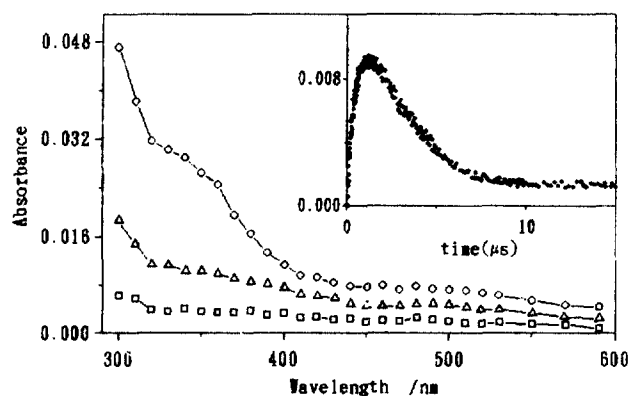| Thymine component	Excitation method	k_0 ($10^5 s^{-1}$)	k_s ($10^8 dm^3 mol^{-1} s^{-1}$)	k_T ($10^9 dm^3 mol^{-1} s^{-1}$)
Thy	S	0.31	12	1.0
	D	0.22	7.9	
Thd	S	0.50	2.8	0.93
	D	0.42(0.4)[a]	1.9(1)[a]	
TMP	S	0.42	0.30(0.27)[b]	1.6
	D	0.40	0.17	

[a,b] From Refs. [2,7], respectively.

Fig. 4. Transient absorption spectra from photolysis of 0.2 mM Thy aqueous solution containing 0.13 M acetone saturated with N_2 at pH 7: 0.8 μs (O), 5 μs (△), 10 μs (□). Inset: growth and decay trace at 400 nm.
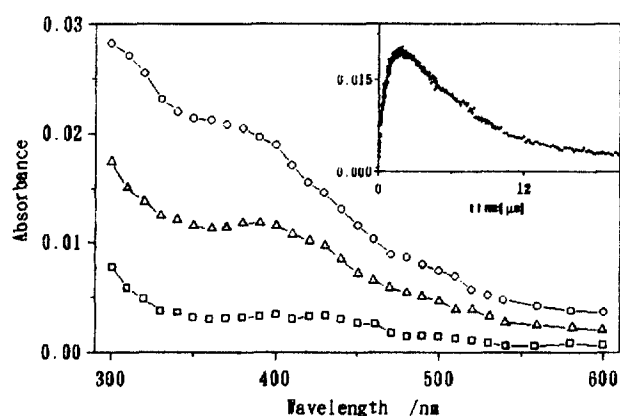

Fig. 5. Transient absorption spectra from photolysis of 0.2 mM Thd aqueous solution containing 0.13 M acetone saturated with N_2 at pH 7: 2 μs (O), 6 μs (△), 15 μs (□). Inset: growth and decay trace at 400 nm.

acetone respectively. At the end of laser pulse, an absorption band appears in the wavelength region of 300–380 nm, which is similar to that from photolysis of neat acetone aqueous solution, thus should be assigned to the absorption of triplet acetone ($^3Ac^*$). Another absorption spectrum different from $^3Ac^*$ appears subsequently, and decays with a first order rate law. It should be attributed to $^3T^*$.

$$Ac \xrightarrow{h\nu} {}^1Ac^* \xrightarrow{ISC} {}^3Ac^* \qquad (4)$$

$$^3Ac^* + T \rightarrow Ac + {}^3T^* \quad k_T \qquad (5)$$

$$^3Ac^* \rightarrow Ac \quad k_0' \qquad (6)$$

$$^3Ac^* + Ac \rightarrow 2Ac \quad k_s' \qquad (7)$$

The kinetic parameters of decay of triplet Thy, Thd and TMP were obtained through the same data processing and summarized in Table 1. Additionally, rate constant of T–T energy transfer (k_T) from $^3Ac^*$ to Thy, Thd and TMP were also determined according to the following procedure; since triplet acetone decays following the pseudo-first-order kinetics via unimolecular decay, self-quenching by its ground state molecular and T–T energy transfer from $^3Ac^*$ to thymine
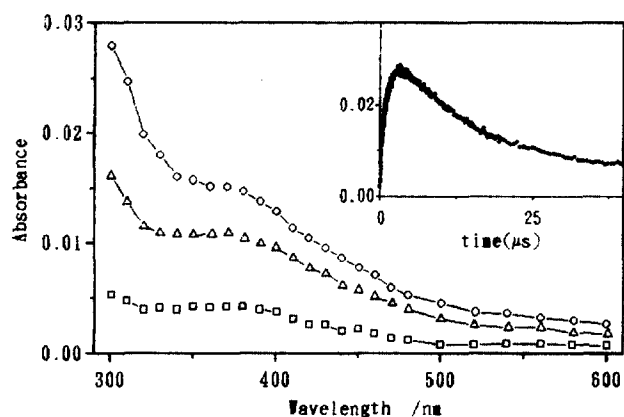
component, the observed rate constant (k_{obs}) for decay of $^3Ac^*$ can be expressed as [4]

$$k_{obs} = k_{obs}' + k_T[T] \qquad (8)$$

in which $k_{obs}' = k_0' + k_s'[Ac]$, $[Ac] = 0.13$ M. k_T can be obtained from the linear plot of k_{obs} against the concentration of thymine component (see Table 2). Furthermore, T–T energy transfer efficiency (ρ_T) from $^3Ac^*$ to T can be expressed as

$$\rho_T = \frac{k_T[T]}{k_{obs}} \qquad (9)$$

When set [T] as 0.2 mM, ρ_T was obtained as 0.11 for Thy, 0.10 for Thd and 0.23 for TMP.

From Table 1, it is evident that the rate constants from acetone sensitization are in reasonable agreement with those from direct excitation. Therefore, acetone sensitization method is verified by the experiment.

To our knowledge, the k_0, k_s values for Thy and TMP in aqueous solution from direct excitation and that for Thy and Thd via acetone sensitization are reported for the first time. Literature values are given in Table 1 for comparison. Salet and Bensasson [1] obtained k_0 and k_s values of triplet Thy in acetonitrile solution, and afterwards determined the values of Thd in aqueous solution and that of TMP in ethanol solution [2].

The acetone sensitization can produce the excited states of DNA components in high concentration, which improve signal amplitude of the transients and can be detected accurately. Comparison of inset of Fig. 3 and that of Fig. 6 shows that the maximum absorbance of $^3TMP^*$ in aqueous solution from acetone sensitization is four times of that from direct excitation, while the concentration of T in direct excitation is five times of that in acetone sensitization.

Fig. 6. Transient absorption spectra from photolysis of 0.2 mM TMP aqueous solution containing 0.13 M acetone saturated with N_2 at pH 7: 2.5 μs (O), 8 μs (△), 20 μs (□). Inset: growth and decay trace at 400 nm.

4. Conclusion

On the basis of above comparison, it is concluded that the kinetic parameters of decay of triplet thymine components

from acetone sensitization are likely to be more correct and reliable than direct excitation due to the enhanced signal amplitude arising from the triplet states in high yields. These findings may be ones of the interesting advances taking place during the last two decades.

Acknowledgements

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