Photodimerization of Thymine Derivatives in Single Crystal

Norimitsu Tohnai, Yoshiaki Inaki[†], Mikiji Miyata,
Nobuyoshi Yasui*, Eiko Mochizuki*, and Yasushi Kai*

Department of Materials & Life Science,
*Department of Materials Chemistry,
Osaka University, Yamadaoka 2-1, Suita, Osaka 565, Japan
[†]inaki@chem.eng.osaka-u.ac.jp

Thymine derivatives having long alkyl chain showed a very high photoreactivity in a single crystal and gave only one isomer of photodimer while the photodimerization in solution gave the four isomers. The crystal structure determined by X-ray diffraction method suggested the formation of the *trans-syn* or *cis-anti* photodimer. The photodimer obtained, however, was identified as the *trans-anti* isomer from the NMR spectrum. It was concluded that the *trans-anti* photodimer was formed by disrotatory motion of the thymine in the crystal.
Keywords: thymine, photodimerization, stereo isomer, crystal structure.

INTRODUCTION

Thymine derivatives are known to give photodimers upon irradiation to UV light near the λ_{max} (280 nm) of thymine. The photodimerization is reversible reaction and the photodimer splits to afford the original thymine very efficiently upon irradiation at a shorter wavelength (240 nm) (Fig. 1).

Figure 1 Reversible photodimerization of the thymine derivative.

A variety of thymine derivatives were synthesized and the photoreactivity has been studied in a solid state and a polymer matrix¹⁻⁴. The increase and decrease in the absorbance at 270 nm are repeated as shown in Fig. 2 because of the formation and cleavage of this photodimer.

In order to obtain a high sensitive photo-reversible system, it is necessary to know the reaction

Figure 2 UV spectra of original thymine derivatives and photodimers.

mechanism of the photodimerization in the solid state. The solid of thymine derivatives obtained by spin coating, however, contained amorphous and crystalline parts³. Therefore, the photodimerization of thymine derivatives should be studied in a single crystal in order to know the reaction mechanism. This paper deals with the crystal structure analysis and photodimerization in the single crystal of the thymine compound having long alkyl chain. The photodimerization in the single crystal gave only one stereo isomer (*trans-anti*) of the photodimers, though the reaction in solution gave four isomers. The mechanism of the selective formation of the *trans-anti* isomer will be discussed considering the crystal structure of the thymine derivative.

EXPERIMENTALS

Synthesis of 1-Octyl Thymine (2)

Thymine (31.5 g, 0.25 mol) in hexamethyldisilazane (HMDS) (250 ml, 1.70 mol) was refluxed for 24 hours at 160 ° C with 10 ml of *N,N*-dimethylformamide (DMF) as a catalyst. After the reaction, DMF and HMDS were removed at 40 ° C under reduced pressure. By the distillation under reduced pressure (13 mmHg), 5-methyl-2, 4-bis(trimethylsiloxy)-pyrimidine (1) was obtained (123-125 ° C, yield 98.7%).

A mixture of 1-octyl bromide (27.5 g, 0.142 mol) and 5-methyl-2, 4-bis(trimethylsiloxy)-pyrimidine (23.8 g, 0.088 mol) was stirring for 10 days at 60 ° C. After the reaction, methyl alcohol (200 ml), 6N HCl (20 ml), and water (100 ml) were added to the obtained oil. The mixture was heated and the precipitate was filtered. After removing the solvents under reduced pressure, the residue was extracted with water/chloroform. Chloroform was evaporated and the residue was washed with petroleum ether and recrystallized with benzene to give 2 (nearly quantitative yield). ¹H NMR (270 MHz, CDCl₃) δ 9.04 (1H, s, NH), 6.98 (1H, s, C6-H), 3.69 (2H, t, N-CH₂), 1.92 (3H, s, C5-CH₃), 1.67 (2H, m, N-C-CH₂), 1.25 (10H, m, CH₂), 0.88 (3H, t, C-CH₃).

Scheme 1 Synthesis of thymine derivatives having long alkyl chain.

Crystal Structure Analysis

Crystal data for 1-octyl thymine (2): C₂₆H₄₄O₄N₄, triclinic, space group P1, *a* = 17.08(1), *b* = 17.415(5), *c* = 11.756(3) Å, α = 89.97(2)°, β = 110.02(6)°, γ = 110.27(4)°, *V* = 3053(3) Å³, *D*_{calc} = 1.04 g cm⁻³, *Z* = 8, *R* = 0.139.

X-ray diffraction intensities of 1-octyl thymine were collected by Rigaku RAXIS-CS imaging plate two-dimensional area detector with graphite-monochromatized Mo-Kα radiation at 15 ± 1 °C. The 4455 unique reflections were measured (2θ_{max} = 59.6°). For further calculations, [*I*₀ > 3σ(*I*₀)] reflections were used after Lorenz and polarization corrections. The structure was solved by direct method (SAPI 91), and refined by SHELEX 93. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms attached to carbon atoms were located in the calculated positions. The positions of hydrogen atoms attached to nitrogen atoms were obtained from difference Fourier syntheses. All calculations were performed by using TEXSAN crystallographic software package of the Molecular Structure Corporation.

Photodimerization

Photodimerizations in single crystal were carried out with a super high pressure mercury lamp (SUPER CURE-203S, SAN-EI Electric). For the photodimerization in acetone solution, a high pressure mercury lamp (HALOS P1H-500 C/S60, EIKOSHA) was used.

The obtained four isomers were separated by recrystallization. The first crystal was obtained from methanol, and was identified as *trans-anti* dimer from NMR spectra (Table 1). The second crystal (*trans-syn*) was obtained from acetonitrile, and the third crystal (*cis-syn*) was obtained from ethyl acetate. The last crystal (*cis-anti*) was obtained from diethyl ether/petroleum ether (1/9, v/v). The NMR data for the photodimer were identical with the data of dimethylthymine except *trans-syn* dimer⁵.

Table 1. ¹H NMR chemical shifts of four stereo isomeric photodimers (δ in CDCl₃).

Isomers*	C-CH ₃ 6H, t	-CH ₂ - 20H, m	5-CH ₃ 6H, s	N-CH ₂ 2H, m	6-H 2H, s	N-CH ₂ 2H, m	N-H 2H, s
<i>trans-anti</i>	0.87	1.27	1.43	2.84	3.78	3.90	7.82
<i>trans-syn</i>	0.87	1.27	1.44	2.87	3.52	3.87	7.92
<i>cis-syn</i>	0.87	1.26	1.50	2.53	3.79	4.01	8.63
<i>cis-anti</i>	0.87	1.26	1.59	2.99	3.34	3.92	8.26

*Four stereo isomers are shown in Fig. 7.

RESULTS

Crystal Structure

Figure 3 shows (a) the molecular packing in a unit cell and (b) the planar structure of the crystal of 1-octyl thymine. The unit cell contains eight molecules. The crystal structure is consisted of the hydrophilic layers of thymine bases and the hydrophobic layers of long alkyl groups (Fig. 4). The distance between the planes formed by thymine bases was almost 3.3 Å. By considering the van der Waals radius, the thymine bases contact approximately each other. Moreover, the long alkyl chain groups aggregate and form the hydrophobic layer.

The thymine bases are coplanar (Fig. 3b), and are associated by the hydrogen bonds on the plane (Fig.

Figure 3 Crystal structure of 1-octyl thymine recrystallized from ethyl acetate.
(a) Molecular packing in the unit cell,
(b) Planar structure and hydrogen bondings.

Figure 4 Lamella structure of thymine derivative having long alkyl chain.

5). The thymine molecule faces another thymine in the opposite plane with head to head (Fig. 6). As shown in Figure 6, two thymine bases on the opposite plane were in the position of *trans-syn*. Therefore, the distance and the orientation of two thymine bases in this crystal suggest that the *trans-syn* photodimer should be obtained dominantly by photodimerization.

Figure 5 Two thymine derivatives associated with hydrogen bondings.

Figure 6 Two opposite thymine derivatives in the position of *trans-syn*.

Photodimerization

UV light was irradiated to the plate crystal which was obtained by recrystallization from ethyl acetate. The process of the photoreaction was confirmed from UV, IR and NMR spectra. The conversion was calculated by the NMR spectra in CDCl_3 from the peaks of 6-H of photodimers and original thymine. After UV irradiation of the plate crystal for 3 hours, the photodimerization proceeded in yield of 98.3%.

There are four stereoisomeric dimers possible by linkage of two thymines to form a cyclobutane ring across their 5, 6 double bonds: *cis-syn*, *cis-anti*, *trans-syn*, and *trans-anti* (Fig. 7). The *cis-syn* photodimer is known to be formed by UV irradiation on DNA.

Figure 7 Four photodimer structures of 1-alkyl thymine derivatives.

The photodimerization of 1-octyl thymine hardly occurred in solution, where triplet state was the precursor⁶⁻⁹. However, the photodimerization gave all isomers in acetone which was the triplet sensitizer. The isomers were isolated by recrystallization and identified from NMR (Tab. 1). In the solution reaction, the *trans-anti* dimer was a main product and the other isomers were generated with an almost equal ratio (Tab. 2).

Only one isomer was obtained from the UV irradiation of the plate crystal from ethyl acetate. The dimerization was fast because the reaction proceeded

Table 2. Relative photodimer distributions

	Solution	Crystal
<i>cis-syn</i>	0.5	-
<i>cis-anti</i>	0.6	0.07
<i>trans-syn</i>	0.4	0.05
<i>trans-anti</i>	1	1

through the singlet state¹⁰⁻¹². The isomer was identified as *trans-anti* isomer from chemical shifts of 5-CH and 6-H of the NMR spectra. This isomer is different from the *trans-syn* isomer predicted from the crystal structure.

DISCUSSION

Crystal structure showing the nearest neighbor thymine bases in the crystal are picked out from Fig. 4 to give Fig. 8. The distance between thymines in the plane is short, but the formation of the photodimer is difficult because the interaction of double bonds is necessary for the photodimerization.

Figure 8 Hydrogen bonding network and photoreacting base pairs on the opposite plane.

Two stacked thymines in Fig. 8 are illustrated in Fig. 9. The cyclobutane photodimer can be formed by the reaction between two double bonds of C5 and C6. When the first bond is formed between C5 and C5', the *syn* type photodimer should be formed after formation of the second bond between C6 and C6'. On the other hand, when the first bond is formed between C5 and C6', the *anti* type photodimer should be formed after formation of the second bond between C6 and C5'. The crystal data suggested that the nearest carbons are C5 and C6' in the distance of 3.36 Å as suggested in Fig. 9. Therefore, the photodimer formed by UV irradiation in the single crystal should be the *anti* type isomer.

Figure 9 Photoreacting sites of the nearest neighbor thymine bases in crystal.

If the photodimerization occurs face to face between the stacked thymines, the photodimer should be the *cis-anti* isomer. However, the photodimer obtained was the *trans-anti* isomer, suggesting another mechanism of photodimerization. The photocyclization of a diene compound is known to occur by disrotatory motion, although the thermal cyclization occurs by conrotatory motion. If the photodimerization of thymine bases in crystal occurred by disrotatory motion, the product should be the *trans-anti* isomer. This mechanism of disrotation may be possible because the motion of the molecule is fixed in the crystal by the interaction of the long alkyl chain.

The probable mechanism of the photodimerization is illustrated in Fig. 10. When the thymine bases in crystal rotate disrotatory (path a), the first bond is formed between C5 and C6' in *cis* conformation. After rotation of the thymine on the plane, the second bond is formed between C6 and C5' giving the *trans-anti* photodimer. By the conrotatory motion of thymine bases (path b), however, the *cis-anti* photodimer should be formed through the *trans* type intermediate.

Though the *trans-anti* isomer is the main product of the photodimerization in crystal, small amounts of another isomer (*cis-anti* and *trans-syn*) are formed (Tab. 2). These minor isomers may be formed from

Figure 10 Probable mechanism of the photodimerization in the single crystal.

Figure 11 Powder X-ray diffraction patterns of thymine derivatives having long alkyl chain recrystallized from ethyl acetate with different photoreaction conversion.

[a] conversion=0%, [b] conversion=98.7%

collapsed crystals. The powder X-ray diffraction suggested that the sharp peak observed at the beginning of the reaction became a broad peak at the end of reaction (Fig. 11). The formation of the *trans-anti* photodimer may accompany collapse of the crystal structure. The transparent crystal was observed to become cloudy as the progress of the reaction. It was concluded that the minor isomers obtained were formed from the collapsed crystals at the end of reaction.

CONCLUSION

The plate single crystal of 1-octyl thymine recrystallized from ethyl acetate showed very high photoreactivity and gave only one isomer of the photodimer. The crystal structure suggested the formation of the *trans-syn* or *cis-anti* photodimer. The photodimer obtained, however, was identified as the *trans-anti* isomer from the NMR spectrum. It was concluded the *trans-anti* photodimer was formed by disrotatory motion of the thymine in the crystal. The mechanism for the intermolecular photodimerization of thymine in the crystal was found to be the same as that for the intramolecular photocycloaddition of dienes because the thymine derivative was fixed with the alkyl group in the crystal.

References

1. Y. Inaki, Y. Wang, M. Kubo, and K. Takemoto, "Chemistry of Functional Dyes", Z. Yoshida and Y. Shirota, Eds, Mita, Osaka, p.365 (1993).
2. N. Tohnai, T. Sugiki, E. Mochizuki, T. Wada, and Y. Inaki, *J. Photopolym. Sci. Technol.*, **7**, (1994) 91.
3. T. Sugiki, N. Tohnai, E. Mochizuki, T. Wada, Y. Inaki, *Bul. Chem. Soc. Jap.*, **69**, (1996) 1777.
4. N. Tohnai, M. Miyata, Y. Inaki, *J. Photopolym. Sci. Technol.*, **9**, (1996) 63.
5. R. Kleopfer and H. Morrison, *J. Am. Chem. Soc.*, **94**, 255 (1972).
6. G. J. Fisher, and H. E. Johns, *Photochem. & Photobiol.*, **11**, (1970) 429.
7. J. Eisinger, and A. A. Lamola, *Mol. Photochem.*, **1**, (1969) 209.
8. D. Wienblum, *Photochem. Photobiol.*, **12**, (1970) 509.
9. B. H. Jennings, S. C. Pastra, and J. L. Wellington, *Photochem. & Photobiol.*, **11**, (1970) 215.
10. S. Y. Wang, *Nature (London)*, **200**, (1960) 879.
11. D. Weinblum, and H. E. Johns, *Biochim. Biophys. Acta.*, **114**, (1966) 450.
12. R. Lisewski, and K. L. Wierzchowski, *Photochem. & Photobiol.*, **11**, (1970) 327.