Photoreactive and Inactive Crystals of 1-Alkylthymine Derivatives

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Two kinds of crystal structures were found for 1-octylthymine from X-ray structure analysis: Structure I was obtained from Crystal I recrystallized from ethyl acetate, and Structure II was obtained from Crystal II recrystallized from ethanol. The thymine bases in Crystal I had high reactivity for photodimerization. Crystal II, however, was inactive for the photodimerization of thymine bases. This paper presents the crystal structure of 1-octylthymine crystallized from ethanol (Crystal II) by X-ray crystal structure analysis. Thymines in Crystal II were concluded to be inactive for photodimerization because of unsuitable orientation of thymine bases in the crystal and because of insufficient space for rotation of thymine during photodimerization.

Thymine bases, one of the nucleic acid bases, convert to photodimers upon irradiation with UV light near 280 nm (Fig. 1). This reaction is known to cause damage to DNA by solar UV light.[1,2] The photodimerization is a reversible reaction and the photodimers split to afford the original thymines very efficiently upon irradiation at a shorter wavelength (240 nm). Therefore, the photodimerization of thymine is an equilibrium reaction under the sunlight or by irradiation of white light. The photodimerization and the reverse reaction of thymine, however, can be controlled by monochromatic UV light: 280 nm for photodimerization and 240 nm for the reverse reaction. Irradiation of monochromatic UV light at 280 nm give only photodimers without by-products, although the reaction using white light includes side reactions that are reported for photochemical reactions of DNA under sunlight. The reversible photodimerization of thymine derivatives can be applied to negative or positive type photoresist materials[3—5] and to the photorecording systems by controlling wavelength of irradiating UV light.[6,7]

For applications of the thymine derivatives to the photosensitive materials, it is necessary to investigate the reactivity of thymine derivatives in thin film. It was found that photoreaction of thymine is dependent on the kind of excited state (singlet or triplet),[8—14] the association of the molecules (solutions, thin films, and crystals),[15—17] and the substitution isomers (N^1- or N^3-substituted thymine).[18] We have also reported that the rates of photodimerization in solid thin film were dependent on the length of the alkyl chain of the thymines having long alkyl chains.[19,20]

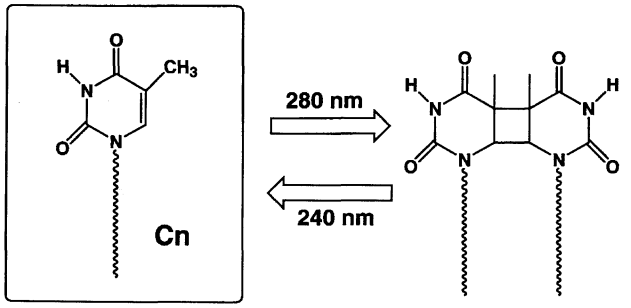

Fig. 1. Reversible photodimerization of the thymine derivatives.

The photodimerization and the reverse reaction of the thymine derivatives in thin films were found to depend on annealing. The photoreactivity of the spin-coated film was high, but decreased remarkably by annealing. This fact indicates that the structure of the thin film changed by annealing. The single crystal from ethyl acetate (Crystal I) gave the photodimer.[21] The study of the photodimerization of 1-octylthymine in single crystal suggested that the thymine base rotated in the single crystal during photodimerization.[21] The single crystal from ethanol (Crystal II), however, did not give the photodimer. Powder X-ray diffraction study revealed that the crystal structure of the spin-coated thin film was the same as that of the single crystal from ethyl acetate (Structure I). On the other hand, the crystal structure of the annealed thin film was the same as that of the single crystal from ethanol (Structure II). This paper presents the crystal structure of 1-octylthymine crystallized from ethanol determined by X-ray crystal structure analysis. Discussions in this paper will be focused on the relationship between the molecular orientation of the thymine bases in the single crystals and the reactivity of the thymine base for photodimerization.

Experimental

Preparation of 1-Octylthymine. The alkylthymine derivative was prepared according to a method reported in the literature.[21] Thymine was reacted with hexamethyldisilazane to give 5-methyl-2, 4-bis(trimethylsiloxy)pyrimidine. A mixture of 1-bromooctane

and 5-methyl-2, 4-bis(trimethylsiloxy)pyrimidine was stirred for 10 d at 60 °C, followed by hydrolysis to afford 1-octylthymine.

Preparation of Thin Films. A chloroform (0.5 ml) solution of 1-octylthymine (10 mg) gives clear thin films by spin coating onto a quartz plate. Annealing of the spin-coated film was carried out by heating at 100—110 °C, below the melting point (122 °C), for various short times, followed by cooling to room temperature.

Photodimerization. Photodimerizations in single crystal were carried out by SUPER CURE-203S UV LIGHT SOURCE SAN-EI ELECTRIC. For the photodimerization in the thin films, JASCO CRM-FA was used. ^{1}H NMR Spectra were recorded with a Varian unity INOVA600 and JEOL GSX270. UV Spectra were recorded with a JASCO UVIDEC 660. IR Spectra were recorded with a JASCO IR-810 infrared spectrophotometer. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were measured by a Rigaku Thermo Plus TG8120 instrument. X-Ray powder diffraction patterns were measured by Rigaku X-ray diffractometer RINT 2000 with Cu $K\alpha$ radiation.

Crystal Structure Analysis. Data of X-ray diffraction for 1-octylthymine were collected by Rigaku AFC5R using graphite-monochromatized Mo $K\alpha$ radiation (λ = 0.71069 Å) at 23.0±1 °C. Unique reflections of 6632 were measured up to 2θ of 55.2°. For further calculations, [$|F_o| > 3\sigma(F_o)$] reflections were used after Lorenz and polarization corrections. Each crystal structure was solved by the direct methods (LODEM), and refined by the full-matrix least squares. 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 the difference Fourier syntheses. All the crystallographic calculations were performed by using TEXSAN software package of the Molecular Structure Corporation.

Results and Discussion

Photodimerization of 1-Octylthymine in Thin Film. The photodimerization of 1-octylthymine in thin film was reversible by alternate irradiation of UV light at 280 and 240 nm. Figure 2 shows the repeated reversible photodimerization of 1-octylthymine in spin-coated thin film. The photoreactive thin film was obtained by spin coating on a quartz plate from a chloroform solution of 1-octylthymine, followed by drying under reduced pressure at room temperature. The absorbance at 270 nm decreased under irradiation of UV light at 280 nm, followed by increase of absorbance by irradiation of UV light at 240 nm. The formation of the photodimer and the splitting of the photodimer were confirmed by NMR spectra.[21] Under the conditions used here using monochromatic light for several minutes, we confirmed that only photodimers were formed without any by-products. The slight decrease of conversion by repeating the reaction in Fig. 2 may be caused by the structural changes of the thin film.

Little photodimerization, however, occurred after annealing of the thin film as shown in Fig. 3. The annealing process was done at 100 °C, below the melting point of thymine derivative (122 °C). The annealing times had been varied: (**a**) 0, (**b**) 30, (**c**) 60, and (**d**) 90 s. The appearances of thin films and UV spectra were not changed essentially by annealing. Figure 3 shows the relative absorbance at 270 nm after irradiation at 280 nm for about 400 s (3.52 J cm^{-2}),

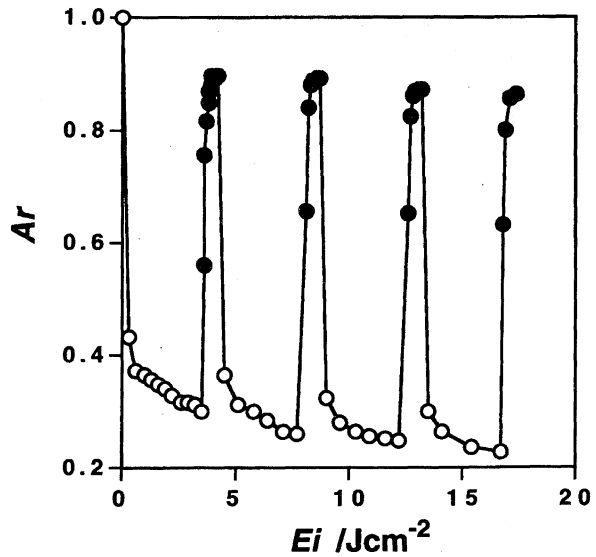

Fig. 2. Reversible phtodimerization of 1-octylthymine in spin coat thin film.
(Relative absorbance at 270 nm (*Ar*) vs. irradiated energy (*Ei*).) (○) Irradiation at 280 nm, and (●) at 240 nm.

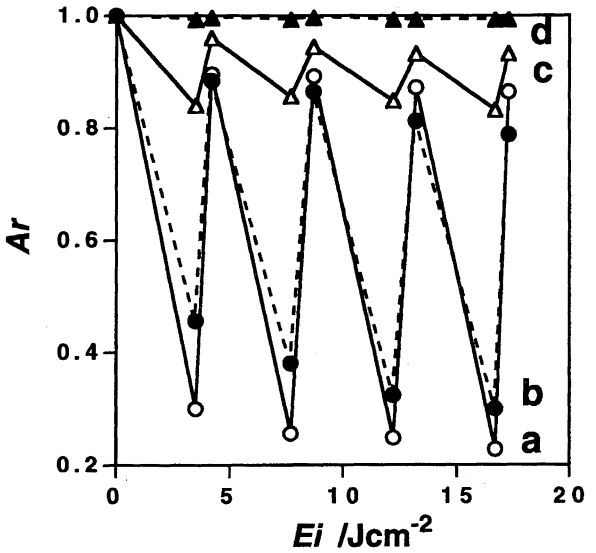

Fig. 3. Effect of annealing on the reversible photodimerization of 1-octylthymine.
(Relative absorbance at 270 nm (*Ar*) vs. irradiated energy (*Ei*).) Annealing at 100 °C for (**a**) 0, (**b**) 30, (**c**) 60, and (**d**) 90 s.

and after irradiation at 240 nm for about 500 s (0.66 J cm^{-2}). The photodimerizations and the reverse reactions were repeated 4 times. The conversion of the reaction decreased with increase of annealing time (**a** to **c** in Fig. 3). After annealing for 90 s (**d** in Fig. 3), the photodimerization occurred very slowly. This result indicated that the structure of 1-octylthymine in the thin film was changed by annealing.

Powder X-Ray Diffraction of 1-Octylthymine. The powder X-ray diffraction studies were employed to clear the crystal structure of the thin film. Figure 4 shows the powder X-ray diffraction patterns of 1-octylthymine thin

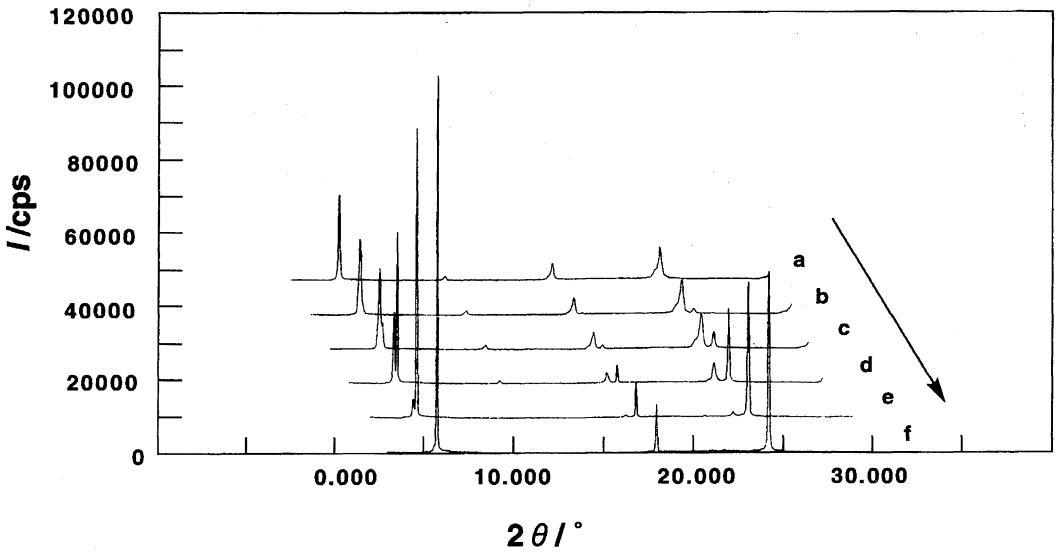

Fig. 4. Effect of annealing on powder X-ray diffraction pattern of 1-octylthymine thin film. Annealing time at 100—110 °C: [**a**] 0, [**b**] 10, [**c**] 20, [**d**] 30, [**e**] 60, and [**f**] 90 s.
(Intensity (I) vs. 2θ. The numbers on the abscissa and the vertical axis are for (**f**). The patterns for (**a**) to (**e**) are shifted.)

film at various annealing times. The spin-coated thin film without annealing (**a** in Fig. 4) was not a glassy state but had the crystal structure. The peak at $2\theta = 5.70°$ disappeared, and the new peak at $2\theta = 5.74°$ appeared during annealing of the thin film when the annealing time was made longer (from **b** to **e** in Fig. 4). Moreover, the peaks at $2\theta = 11.64°$, 17.60°, and 23.60° went down gradually and the peaks at $2\theta = 17.98°$ and 24.16° grew. After annealing for 90 s (**f** in Fig. 4), the original crystal structure shifted almost completely to the new structure.

The pattern of powder X-ray diffraction of the plate crystal from ethyl acetate[21] (Crystal I) (**a** in Fig. 5) coincided with the pattern of the spin-coated thin film without annealing (**b** in Fig. 5). On the other hand, the pattern of powder X-ray diffraction for the plate crystal from ethanol (Crystal II) (**c** in Fig. 5) coincided with the pattern of the annealed thin film (annealing for 90 s, **d** in Fig. 5).

Crystal Structure of 1-Octylthymine. The crystal structure of 1-octylthymine crystallized from ethyl acetate (Structure I) was reported previously.[21] In this paper, the crystal structure of the compound crystallized from ethanol (Structure II) will be reported.

Crystal data for 1- octylthymine (from ethanol): $C_{13}H_{22}O_2N_2$, monoclinic, space group $P2_1/c$, $a = 15.063(5)$, $b = 12.080(4)$, $c = 16.424(5)$ Å, $\beta = 106.38(2)°$, $V = 2867(1)$ Å^3, $D_{calcd} = 1.104$ g cm^{-3}, $Z = 8$, $R = 0.083$, $R_w = 0.126$.

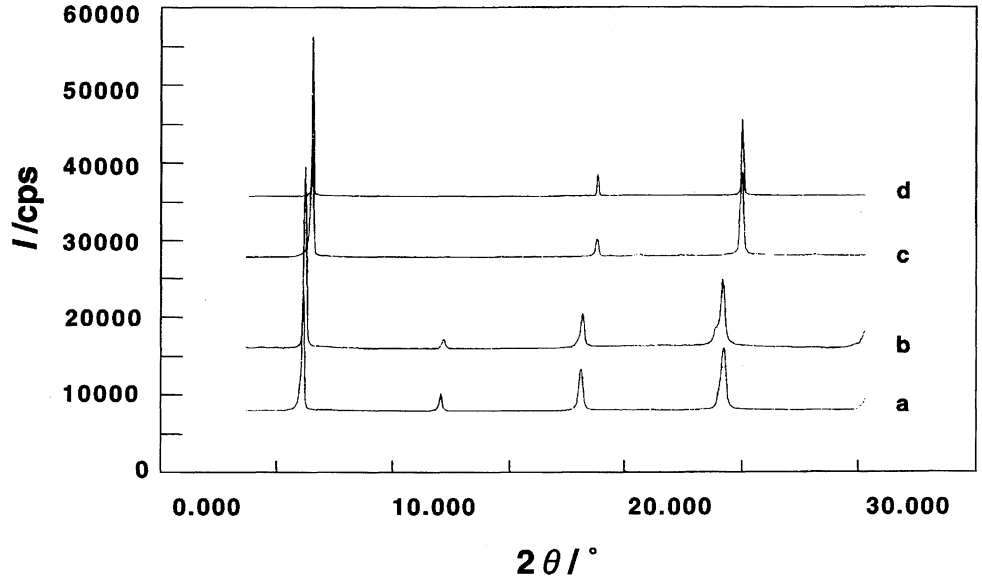

Fig. 5. Powder X-ray diffraction pattern of 1-octylthymine. [**a**]; Crystal I (ethyl acetate), [**b**]; Thin film before annealing, [**c**]; Crystal II (ethanol), [**d**]; Thin film after annealing for 90 s.
(Intensity (I) vs. 2θ. The base lines of patterns for (**b**) to (**d**) are shifted.)

The final R and R_w indices are rather high because of the disordered structure for the long alkyl chains, judged from the anisotropic temperature factors. Thus, it was difficult to locate the ends of long alkyl groups accurately. The thymine base part, which played an important role for photoreaction, however, could be located effectively.

Figure 6 shows a molecular packing structure of 1-octylthymine crystallized from ethanol (Crystal II). The unit cell contains eight molecules. The crystal is a double layer structure consisting of the hydrophilic layer of thymine bases and the hydrophobic layer of long alkyl groups (Fig. 7). The distance between the two planes formed by thymine bases was about 3.4 Å. The van der Waals radius of the thymine base might be touched. Moreover, the long alkyl chain groups aggregate and form a hydrophobic layer. Therefore, hydrogen bonding of thymine bases and van der Waals forces of thymine bases and the alkyl chains were the main driving forces to form the crystal. The lamella structure was a structure peculiar to thymine derivatives having long alkyl chains. The overall crystal structure of Crystal II was

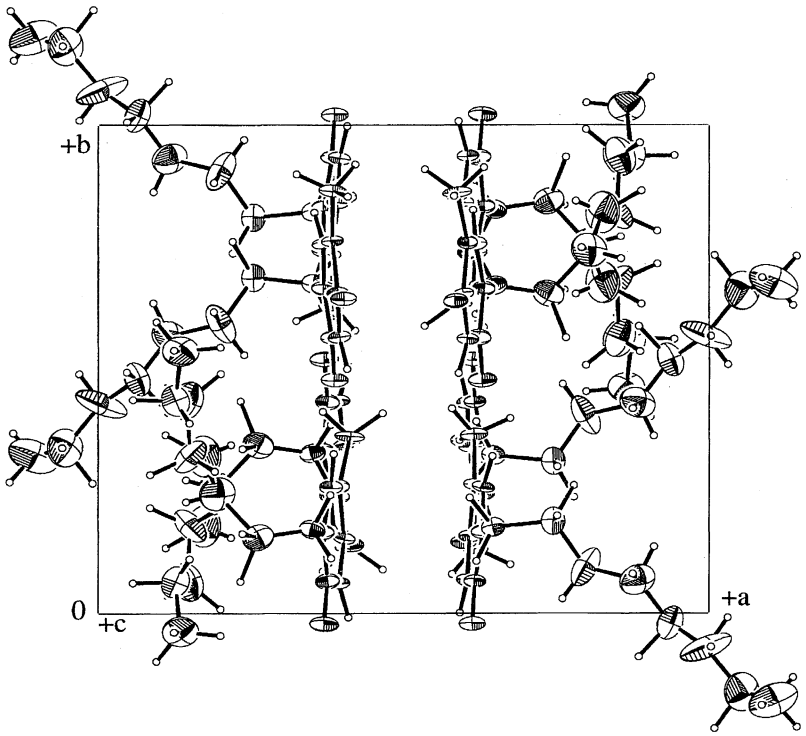

Fig. 6. Molecular packing structure of 1-octylthymine crystallized from ethanol (Crystal II).

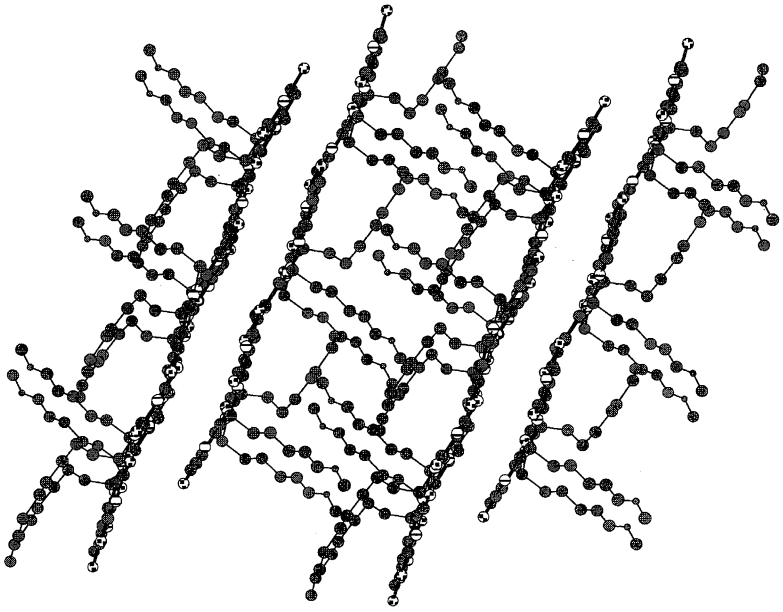

Fig. 7. Lamella structure of 1-octylthymine crystallized from ethanol (Crystal II).

the same as that of Crystal I.

Structural Comparison between Two Forms of Crystals. Figure 8a shows the plane structure in Structure II (ethanol). The orientation of the thymine bases in Fig. 8a is the same as that of Structure I (ethyl acetate) (Fig. 8b).[21] Moreover, the distances of hydrogen bonding are almost equal for both structures. It should be noted that the plain structure formed by hydrogen bonding in Structure II was almost identical to that of Structure I.

Figure 9 shows the orientation of thymine bases for two planes. In the photoreactive Crystal I (ethyl acetate) (Fig. 9b), the two double bonds (C5 and C6 of thymine) overlapped with each other. This orientation of thymine ring may be suitable for the photodimerization. In the inactive Crystal II (ethanol) (Fig. 9a), however, thymine rings were arranged alternatively to avoid the steric hindrance between two opposite planes. Therefore, the double bonds did not overlap in Crystal II. Consequently, the packing form of the plane structures in Crystal II is different from that of Crystal I, though the orientation of the thymine bases in the plane showed no differences between the two crystals.

Crystal data suggested that the molecules packed together more closely in Crystal II than the molecules in Crystal I. The unit cell of both crystals contained 8 molecules, but the volume of the unit cell for Crystal II (V = 2867(1) Å^3) was smaller than the volume for Crystal I (V = 3053(3) Å^3).[21] The calculated density of crystal for Crystal II (D_{calcd} = 1.10 g cm^{-3}) was also different from the value for Crystal I (D_{calcd} = 1.04 g cm^{-3}).[21]

Photodimerization of 1-Octylthymine in Single Crystals. Irradiation of UV light (280 nm) to Crystal I (ethyl acetate) gave the photodimer with 98.7% conversion.[21] On the

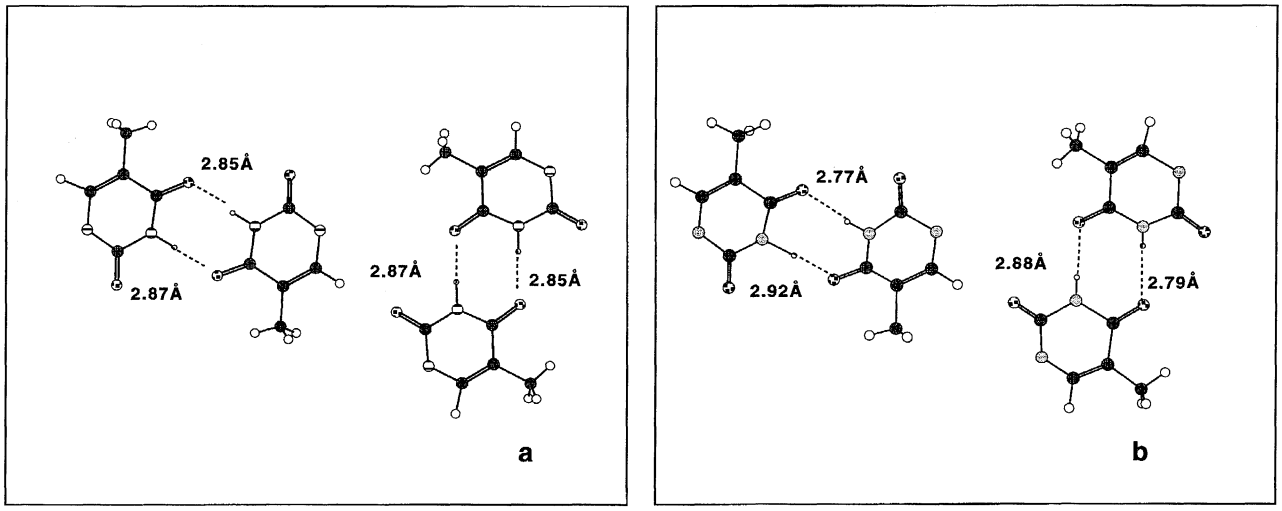

Fig. 8. Plane structures and hydrogen bonds of 1-octylthymine. [**a**]; Crystal II (ethanol), [**b**]; Crystal I (ethyl acetate).[21]

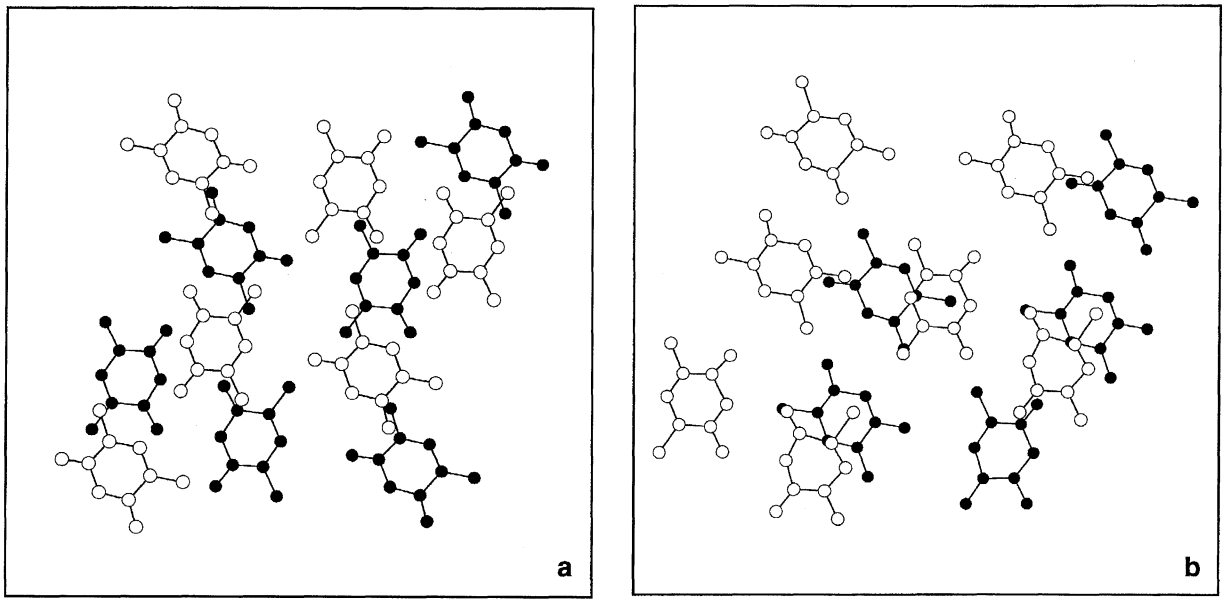

Fig. 9. Orientations of thymine bases in two layers. White circles are on this side, and black circles are on the inside. **a**; Crystal II (ethanol), [**b**]; Crystal I (ethyl acetate).[21]

other hand, Crystal II (ethanol) gave no photodimers under the same conditions. These results of the photodimerization in single crystals corresponded to the results of photoreaction in the thin film. Structure I of the spin-coated film gave the photodimer, but Structure II formed by annealing gave no photodimer. The differences of these photoreactivities will be clarified by the molecular orientation in the crystal structure obtained from X-ray structure analysis studies.

Two facing thymines in Crystal I and Crystal II are shown in Fig. 10. In Crystal I (Fig. 10b), the distance between two planes of the thymines was about 3.3 Å, and the distance between the nearest carbon atoms of the double bond (C6 and C5′) was 3.36 Å. This distance of 3.36 Å for Crystal I was suitable for photodimerization. In Crystal II (Fig. 10a), on the other hand, the distance between two planes of the thymines was about 3.4 Å, but the distance between the nearest carbon atoms of the double bond (C5 and C6′) was 4.34 Å. The distance of 4.34 Å for the Crystal II was too far for the photodimerization. The long distance between thymines alone is not the reason for inactivity of Crystal II for photodimerization, because the amorphous thin film of 1-alkylthymine has photoreactivity.

Thermal Analysis of the Crystals. The transition points of the Structure I to Structure II were obtained by differential thermal analysis (DTA) study (Fig. 11). A large endothermic peak at 122 °C and a small and broad endothermic peak around 100 °C were observed for the DTA thermogram of Crystal II (ethanol) (Fig. 11a). On the DTA thermogram of Crystal I (ethyl acetate), a small endothermic peak was found at 106 °C and an exothermic peak was found at 111 °C (Fig. 11b). To make sure that the peaks at 106 and 111 °C for Crystal I are the transition points, DTA was measured for the annealed crystal. Crystal I was heated to 110 °C and cooled to 50 °C. The DTA thermogram for the annealed crystal showed a quite similar DTA thermogram to that of Crystal II, as shown in Fig. 11a. This result indicated that Structure I (ethyl acetate) transformed to Structure II (ethanol) by annealing.

A large endothermic peak at 122 °C should indicate a melting point. The thymine bases associated strongly with two hydrogen bonds on the plane, as shown in Fig. 8. The

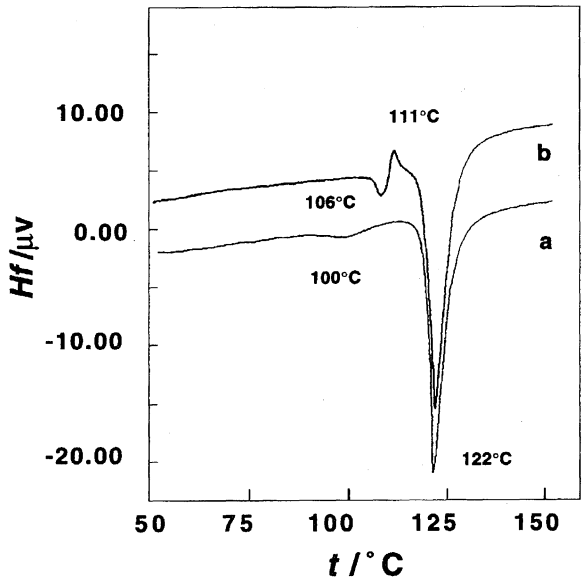

Fig. 11. DTA of 1-octylthymine crystals.
(Scanning rate was 5 K min^{-1}. Heat flow (H_f)). [**a**]; Crystal II (ethanol), [**b**]; Crystal I (ethyl acetate).

structure of the plane for Crystal II (ethanol) (Fig. 8a) was the same as the structure of Crystal I (ethyl acetate) (Fig. 8b). Therefore, both crystals showed the same melting point at 122 °C.

The small peaks from 100 to 111 °C below melting point (122 °C) may be related to the association of the octyl chain. The assemblies of the long alkyl chain are compared for the two crystals in Fig. 12. The long alkyl chains cross each other in Crystal II (Fig. 12a), but the chains are parallel in Crystal I (Fig. 12b). The distance between the planes across the alkyl chain was 10.5 Å for Crystal II, and 11.4 Å for Crystal I. The data indicated that the alkyl chains packed more compactly in Crystal II than in Crystal I. In Crystal II, moreover, the thymine rings were arranged alternatively to avoid the steric hindrance between two opposite planes (Fig. 9a). From these data, the small peaks at 106 and 111 °C for Crystal I in Fig. 11b may be explained as follows. The octyl groups in Crystal I melted at 106 °C while keeping

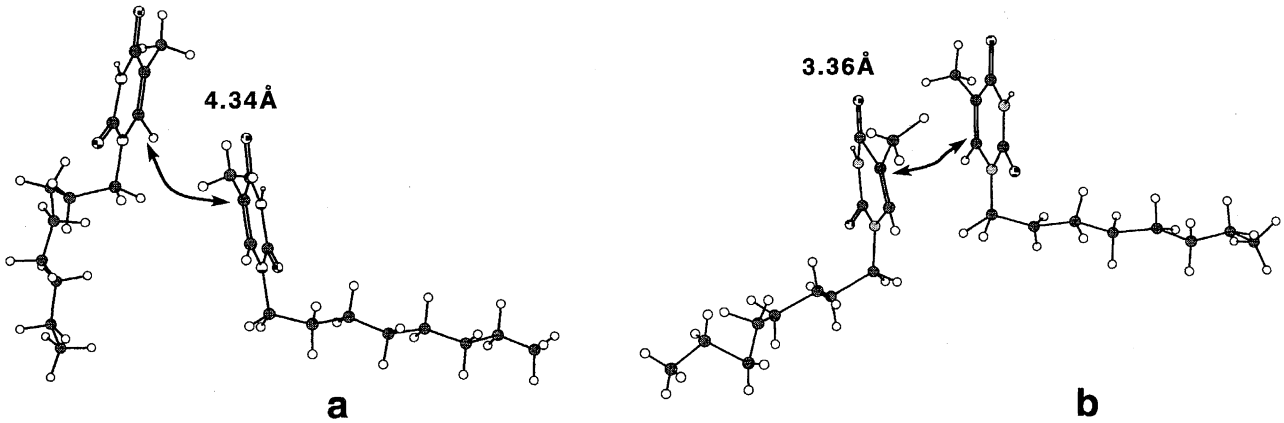

Fig. 10. Two facing thymine derivatives in the nearest position. [**a**]; Crystal II (ethanol), [**b**]; Crystal I (ethyl acetate).[21]

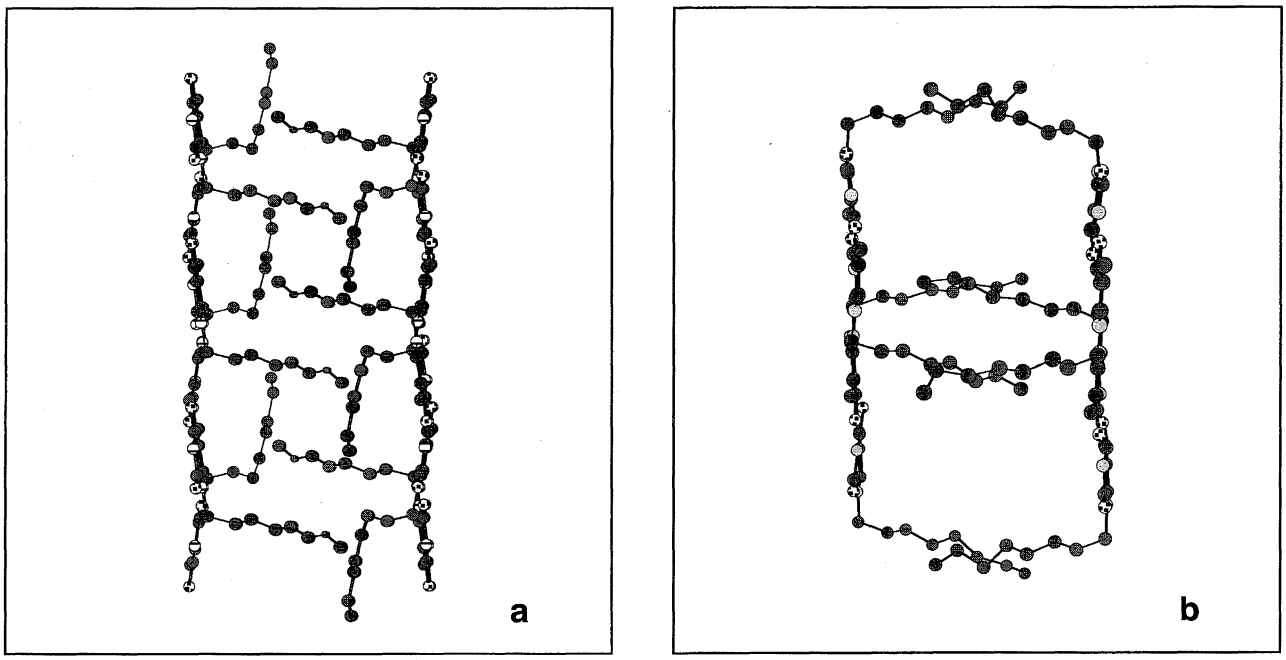

Fig. 12. Packing of long alkyl chains in crystals. [**a**]; Crystal II (ethanol), [**b**]; Crystal I (ethyl acetate).[21]

the plane structures by hydrogen bonding, and the planes moved to the more packed and stable structure of Crystal II at 111 °C. The small and broad peak at around 100 °C for Crystal II (Fig. 11a) may arise from imperfections or defects in the crystal, because the annealed Crystal II had no peak at around 100 °C. The same phenomenon of the crystal structure transition should be caused by annealing of the spin-coated thin film.

Photoreactive and Inactive Crystals. Irradiation of UV light at 280 nm to the Crystal I obtained from ethyl acetate solution was reported to give the photodimer.[21] The thymine bases in the crystal that are in *trans-syn* orientation were concluded to rotate disrotatory during photodimerization reaction to give the *trans-anti* photodimer (Fig. 13). Therefore, thymines in crystals should have sufficient space around thymines to rotate for the photodimerization.

Figure 14 shows an illustration of hydrophilic thymine layer for photoreactive and inactive crystals from Fig. 7. In this figure, rectangles represent the thymines in the plane formed by hydrogen bonding shown in Fig. 8. Photodimerizations of the thymine bases occurs between the planes, but can not occur in a plane. A photoreactive crystal obtained from ethyl acetate (Crystal I) has the Structure I, where the planes overlap each other with slight deviation (2.4 Å). The distance between the nearest carbon atoms of two double bonds was 3.36 Å. The planes of the photoreactive crystals slide by annealing to give the Structure II of the photoinactive crystal crystallized from ethanol (Crystal II). The Structure II has the spacing of 3.4 Å between planes, which is similar to 3.3 Å of Structure I, but has no overlapping of the thymine bases. The distance between the nearest carbon atoms of two double bonds in Crystal II was 4.34 Å. Inactivity of Crystal II (ethanol) may be caused both by no overlapping of the thymine bases and by the longer distance between two reacting double bonds.

The overlapping thymine bases in Crystal I (white rectangles with an arrow in Fig. 14) move into the space between thymines (black rectangles) by annealing to give Crystal II. The crystal data indicated also the compact arrangement of the molecules in Crystal II. Density of Crystal II

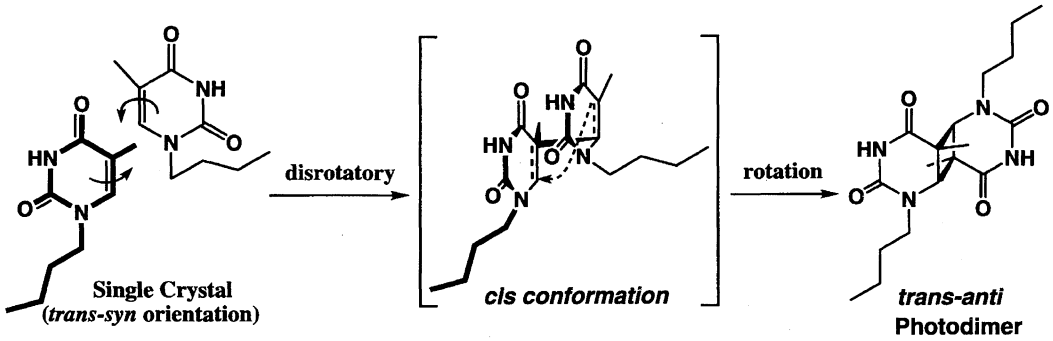

Fig. 13. Mechanism of photodimerization for 1-octylthymine in Crystal I (ethyl acetate).[21]

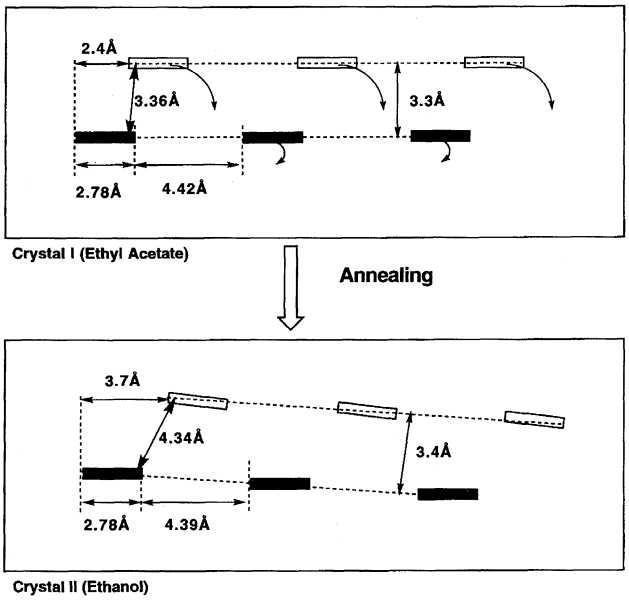

Fig. 14. Transformation of structure by annealing (Illustration).

(D_{calcd} = 1.10 g cm^{-3}) was higher than density of Crystal I (D_{calcd} = 1.04 g cm^{-3}).[21] Therefore, the molecules in Crystal II were concluded to arrange compactly in the crystal compared with the Crystal I. In addition, Crystal II dose not have sufficient space in the hydrophobic layer of alkyl chains because the alkyl chains were folded (Fig. 12a). From these results, one can conclude that the rotation of thymine bases in Crystal II is difficult during the photodimerization reaction. The inhibition of rotation in Crystal II should be another reason for the inactivity of photodimerization.

In conclusion, the Crystal II obtained from ethanol solution is inactive for the photodimerization, because the distance between the thymines is too far and the rotation of the thymines is impossible.

Conclusion

Reactivity for the photodimerization of 1-octylthymine in the spin-coated thin film was very high. The photoreactivity, however, disappeared by annealing at 100—110 °C. The crystal structure of the thin film before annealing corresponded to Structure I for photoreactive Crystal I crystallized from ethyl acetate solution. The crystal structure of the thin film after annealing corresponded to Structure II of inactive Crystal II recrystallized from ethanol solution. The powder X-ray diffraction and the differential thermal analysis indicated that the annealing of the thin film caused the transformation of Structure I to Structure II, while keeping the plane structure by the hydrogen bonding. The Crystal II obtained from ethanol solution was inactive for the photodimerization, because the distance between the thymines was too far and the rotation of the thymines was impossible.

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