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Thermochimica Acta, 242 (1994) 77-92

thermochimica
acta

Thermodynamic studies on selective recognition of bases in complex formation between base pairs. Formation of complexes by non-complementary interactions

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(Received 12 July 1993; accepted 28 December 1993)

Abstract

In order to obtain information on non-complementary interaction in polynucleotide mixtures, the molecular conformations of the equimolar mixtures poly(A)-poly(C) (Mixture 1), poly(A)-poly(I) (Mixture 2), poly(C)-poly(U) (Mixture 3), and poly(I)-poly(U) (Mixture 4), in concentrated solutions, were studied by means of microcalorimetry, spectrophotometry, refractive index, and ab initio molecular orbital (MO) calculation. Mixtures 1 and 3 did not form complexes by non-complementary interaction under the experimental conditions of the present work, although possible interactions were considered for both systems from the standpoint of the net charge, using ab initio molecular orbital (MO) calculations.

The phase state for the poly(I)-poly(C) mixture based on complementary interaction between purine and pyrimidine bases was observed using a polarization microscope. This indicated that the poly(I)-poly(C) mixture forms a layered liquid crystal, as reported previously for poly(A)-poly(U) mixture.

It should be noted that Mixture 2 forms liquid crystals with a triple-stranded helical structure by non-complementary interaction between poly(A) and poly(I), both with purine base. Moreover, the change in enthalpy ΔH_t (7.3 kJ) based on the helix-coil transition of Mixture 2 is approximately the same as those of poly(I)-poly(C) mixture ($\Delta H_t = 6.2$ kJ) and poly(A)-poly(U) mixture ($\Delta H_t = 6.4$ kJ), as reported previously. But the change in enthalpy ($\Delta H_{\text{ord-disord}} = 2.7$ kJ) based on the order-disorder transition of the layered liquid crystal for Mixture 2 is different from the value $\Delta H_{\text{ord-disord}} = 5.8$ kJ for an equimolar poly(I)-poly(C)

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SSDI 0040-6031(94)01743-Z

mixture, and $\Delta H_{\text{ord-disord}} = 6.6$ kJ are for equimolar poly(A)–poly(U) mixture, in which liquid crystals are formed by complementary interaction, suggesting that the intermolecular interaction of the liquid crystals formed by non-complementary interaction between the purine bases of poly(A) and poly(I) is very weak compared with that formed by complementary interaction between purine base and pyrimidine base of nucleotides.

Mixture 4 displays an isotropic phase at concentration below 5.0 wt%, and an anisotropic phase with a double-stranded helical structure appears above 5.0 wt%. But the changes in enthalpy ($\Delta H_t = 4.0$ kJ) and $\Delta H_{\text{ord-disord}} = 1.0$ kJ), for Mixture 4 are smaller than those for Mixture 3, poly(I)–poly(C), and poly(A)–poly(U) by complementary interaction, as reported previously, demonstrating that the molecular conformation formed between purine and pyrimidine bases of Mixture 4 is different from that formed between purines or between purine and pyrimidine bases.

Keywords: Anisotropic; Base pair; CDS; Conformation; DTA; Isotropic; Liquid crystal; Non-complementary interaction; Polynucleotide; Refractive index; UVS

1. Introduction

It is well known that equimolar mixtures of poly(A) and poly(U), and poly(G) and poly(C) and/or poly(I) and poly(C) form poly(A) · poly(U), poly(G) · poly(C), and poly(I) · poly(C) duplexes, respectively, by complementary interactions between purine and pyrimidine bases, and that these duplexes bring about a helix–coil transition with increasing temperature [1–4].

In our previous work [5–9], the thermal behaviour of these duplexes in dilute solutions was studied by calorimetric measurements such as microcalorimetry and adiabatic differential scanning calorimetry, and their thermodynamic quantities were estimated. However, it is very difficult to understand the mechanism of functional development in vivo from the behaviour in vitro in dilute solutions, due to a paucity of information.

Therefore, for a more realistic understanding, these duplexes should be studied in concentrated solutions, because DNA in cells in vivo exists as a solution with a concentration of 70% [10–12].

In a study of biopolymers in concentrated solutions, we reported [13, 14] that DNA and equimolar poly(A)–poly(U) mixtures in concentrated solutions show various liquid crystal phases depending on the concentration. The interactions between these liquid crystals and dye, drug, and/or metallic ions were investigated by a differential thermal analysis (DTA) apparatus equipped with a laser which was designed to allow simultaneous measurement of thermal change and of the optical properties of biopolymer solutions.

To confirm whether or not non-complementary interactions between purine and purine, and pyrimidine and pyrimidine in concentrated solutions contribute to complex formation is a very interesting problem in the study of DNA replication and of the base sequence, because it seems that there is a possibility of cancer developing if non-complementary interaction takes place in the process of RNA syntheses in cells.

A mismatch of polynucleotide duplexes as a result of non-complementary interactions has been studied by some investigators [15–23].

No information, however, is available for mismatched intermolecular interactions, such as poly(A)–poly(C), poly(A)–poly(I), poly(C)–poly(U), and poly(I)–poly(U) mixtures, by non-complementary interactions in concentrated solutions.

In this paper, in order to obtain information about non-complementary interaction, the phase states of equimolar poly(A)–poly(C), poly(A)–poly(I), poly(C)–poly(U), and poly(I)–poly(U) mixtures were studied by means of a DTA set-up equipped with a laser and a polarization microscope. And, furthermore, in order to obtain information about a possible binding position based on non-complementary interactions, ab initio MO calculations for these systems were carried out using IMSPACK and GAUSSIAN 86 programs with the STO-3G minimal basis set.

We will discuss non-complementary interactions from the standpoint of a possible intermolecular interaction between non-complementary base pairs derived from the results from the laser–DTA apparatus, considering the possible binding position expected from ab initio MO calculations.

2. Experimental

2.1. Materials

The samples used in this study were poly(riboadenylic acid) (poly(A)), poly(ribouridylic acid) (poly(U)), poly(ribocytidylic acid) poly(C)), and poly(riboinosinic acid) (poly(I)). They were purchased from Yamasa Shoyu Co. Ltd., Japan, and were used after further purification according to the usual method.

The concentrations of each polynucleotide were determined by the phosphorous analysis method [24].

The solvent used in this study was Ringer solution at pH 7.00.

The water used was passed through an inverse osmotic membrane, deionized by ion exchange, and then distilled.

Mixtures were prepared from equimolar quantities of poly(A) and poly(C), poly(A) and poly(I), poly(C) and poly(U), and poly(I) and poly(U) as non-complementary bases, and poly(I) and poly(C) as complementary bases, respectively. Samples of the equimolar polynucleotide mixtures in the concentration range 1.0–10.0 wt% were used following one month's storage in a refrigerator at 278 K after preparation.

The abbreviations for the equimolar mixtures of polynucleotides used in this text are: Mixture 1 for an equimolar mixture of poly(A) and poly(C); Mixture 2 for an equimolar mixture of poly(A) and poly(I); Mixture 3 for an equimolar mixture of poly(C) and poly(U); and Mixture 4 for an equimolar mixture of poly(I) and poly(U).

3.2. Apparatus and procedure

The apparatus used in this study was a differential thermal analysis (DTA) set-up equipped with a laser which was constructed to allow simultaneous measurement of the thermal and optical properties of biopolymer solutions, as reported previously [25].

The heating rate employed was about 1 K min^{-1}. The standard samples used to calibrate the apparatus were benzophenone, naphthalene and benzal.

The estimation of the change in enthalpy from the area of an endothermic peak on the DTA curve was determined from the value 2.42×10^{-5} kJ cm^{-2}, determined from standard samples.

The polarization microscope used was an XTP-11 (Nikon Co. Ltd., Japan). The ultraviolet absorption spectrophotometer used in this study was a 220A Hitachi, Japan. The UV spectra of the mixtures were measured at room temperature using a quartz cell (GL Science, Inc., Japan), with a polypropylene film of 15 μm (light-pass length) as a spacer.

The circular dichroism spectropolarimeter, CD, used in this study was a JASCO, J-20A. CD spectra of the mixtures were measured at 298-363 K using a quartz cell with a light-pass length of 0.05 mm. For the CD spectral measurement, to prevent the evaporation of solvent with increasing temperature, laboratory-film (American National Can) was wounded tightly around the cell.

The apparatus used for the refractive index measurements was Abbe's refractometry (2T, ATAGO Co. Ltd., Japan).

Ab initio MO calculation was carried out using the IMSPACK [26] and GAUSSIAN 86 [27] programs with the STO-3G minimal basis set [28] in order to obtain information about a possible binding position based on interaction between non-complementary base pairs.

The structures of the hydrated polynucleotides in aqueous solutions were optimized using the STO-3G basis set [29, 30], and the structure of water was referred to the experimental value [31].

3. Results and discussion

3.1. Phase states of mixtures between non-complementary base pairs

The phase states of Mixtures 1-4 between non-complementary base pairs were observed at room temperature using a polarization microscope. The polarizing microphotographs obtained are shown in Fig. 1, (a)-(d′), together with those between complementary base pairs in poly(A)-poly(U) (Fig. 1(e)) and poly(I)-poly(C) (Fig. 1(f)) mixtures, as reported previously [14].

It can be seen from Fig. 1 that the phase states for all mixtures, except Mixture 3, are different from those for poly(A)-poly(U) and poly(I)-poly(C), the equimolar mixtures of complementary interactions, Fig. 1(e) and (f). As seen in Fig. 1(a), the polarizing microphotograph for Mixture 1 over the concentration range 1.0-

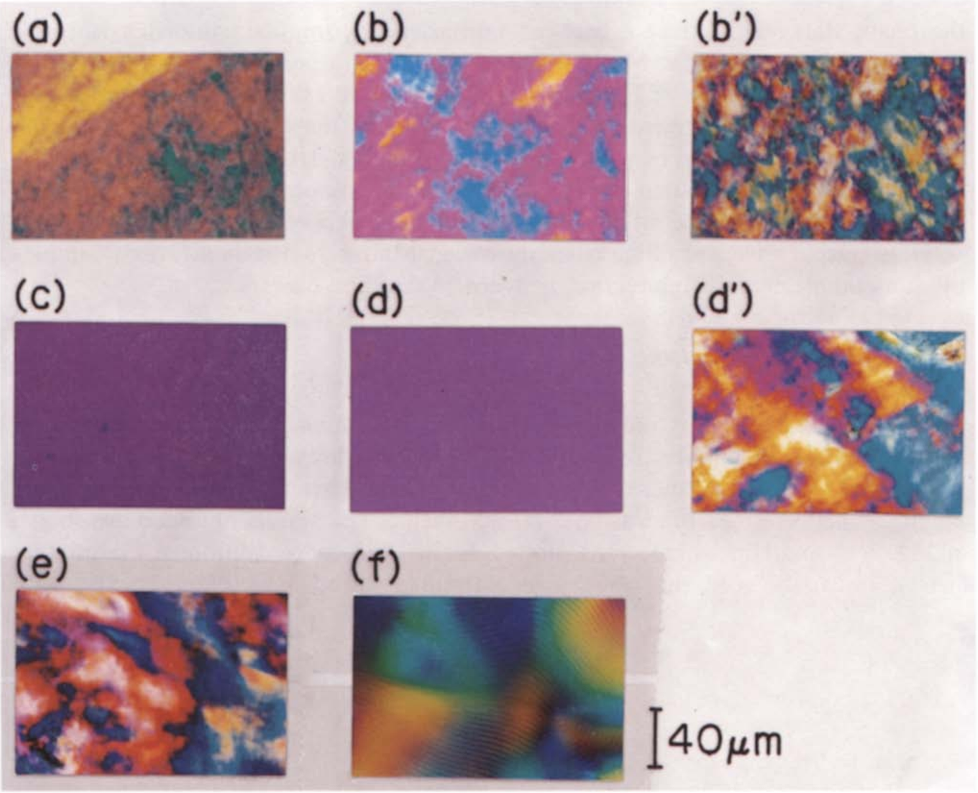

Fig. 1. Polarizing microphotographs of the mixtures with non-complementary, (a)–(d), and complementary, (e) and (f), interactions. (a) Mixture 1 in the concentration range 1.0–10.0 wt%. (b) Mixture 2 in the concentration range 1.0–6.0 wt%. (b′) Mixture 2 in the concentration range 7.0–10.0 wt%. (c) Mixture 3 in the concentration range 1.0–10.0 wt%. (d) Mixture 4 in the concentration range 1.0–6.0 wt%. (d′) Mixture 4 in the concentration range 7.0–10.0 wt%. (e) poly(I)–poly(C) mixture in the concentration range 7.0–10.0 wt%. (f) Poly(A)–poly(U) mixture in the concentration range 7.0–10.0 wt% (see ref. 14).

10.0 wt% shows layered liquid crystal, in contrast with the spherical liquid crystals of poly(A)–poly(U) and poly(I)–poly(C) mixtures, Fig. 1(e) and (f), as reported previously [32], demonstrating that Mixture 1 seems to represent complex formation by non-complementary interaction between poly(A) with purine base and poly(C) with pyrimidine base. Figure 1(b) and (b′) shows polarizing microphotographs for Mixture 2 in two concentration ranges, 1.0–6.0 and 7.0–10.0 wt%, respectively. Evidently, Mixture 2 shows two different anisotropic phases at concentrations below 6.0 wt% and above 7.0 wt%, demonstrating that Mixture 2 forms liquid crystals by non-complementary interaction between the purine bases of poly(A) and poly(I). Figure 1(c) shows isotropic phases in the concentration range 1.0–10.0 wt%, demonstrating that Mixture 3 does not form a complex by non-complementary interaction between the pyrimidine bases of poly(C) and poly(U). And finally, in the concentration range 1.0–6.0 wt%, as shown in Fig. 1(d) and (d′), the phase states of Mixture 4 between purine and pyrimidine exhibit an isotropic phase, demonstrating that Mixture 4 does not form a complex at concentrations below 6.0 wt% as shown in Fig. 1(d). However, above 7.0 wt%, Mixture 4 forms a complex by non-complementary interaction between the pyridine base of poly(I) and the pyramidine base of poly(U), as shown in Fig. 1(d′).

From the results obtained above, it is interesting to note that the mixtures form liquid crystals by non-complementary interactions between two purine bases, or between purine and pyrimidine bases; however, Mixture 3 does not form a complex by non-complementary interaction between pyrimidine bases.

3.2. Refractive indices of the mixtures

The phase states of Mixtures 1–4 were observed using a polarization microscope; all the mixtures have anisotropic phases, except Mixture 3.

In order to obtain further characterization of the mixtures, the refractive indices n_D^{25} at 298 K were measured using a refractometer. The results obtained are shown in Fig. 2(a) and (b), where n_D^{25} is plotted against the concentration of the mixture (wt%). It can be seen that the n_D^{25} of Mixtures 1 and 3 increases linearly with

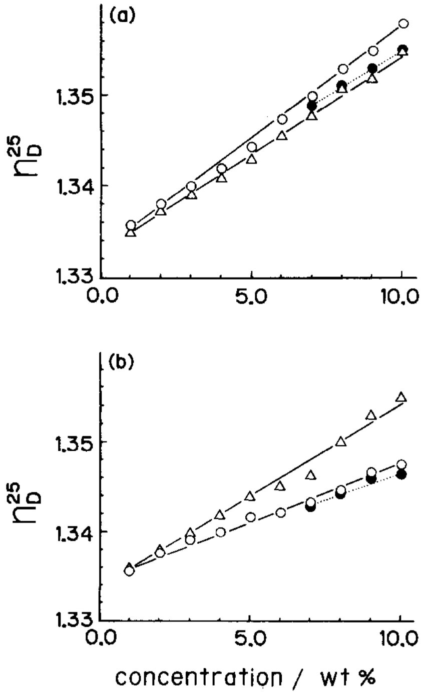

Fig. 2. Plots of refractive indices n_D^{25} of the mixtures at 298 K against the concentration of the mixture (wt%). (a) △, Mixture 1; ○, Mixture 2; ●, Δn_D^{25} of Mixture 2. (b) △, Mixture 3; ○, Mixture 4; ●, Δn_D^{25} of Mixture 4.

increasing concentration, demonstrating that complexes are not formed by non-complementary interactions between poly(A) with purine base and poly(C) with pyrimidine base, or between poly(C) with pyrimidine base and poly(U) with pyrimidine base. However, the n_D^{25} values of Mixtures 2 and 4 show a linear increase, and, in concentrations above 7.0 wt% for both systems, birefringence Δn_D^{25} (a difference between the refractive indices parallel and perpendicular to the prism plane) appears, suggesting that Mixtures 2 and 4 form liquid crystals at concentrations above 7.0 wt% by non-complementary interactions between poly(A) and poly(I), both with purine bases, and between poly(I) with purine base and poly(U) with pyrimidine base. However, formation of liquid crystals in both systems seems to be very weak in comparison with complementaty interactions, as with poly(A)–poly(U) and poly(I)–poly(C) mixtures.

However, the fact that Mixture 1, despite having an anisotropic phase, does not show birefringence, cannot be explained due to a lack of information under the present experimental conditions.

3.3. *CD spectra of the mixtures*

In order to obtain further information concerning why Mixtures 2 and 4 form liquid crystals whereas Mixtures 1 and 3 do not, the interaction between non-complementary bases was studied using a circular dichroism spectropolarimeter (CD). The results obtained are shown in Fig. 3. Figure 3(a) shows the CD spectra of poly(A), poly(U), and of Mixture 1 in solutions of concentration 0.05 wt%. The CD spectrum of Mixture 1 corresponds fairly well with the sum of the spectra of pure poly(A) and pure poly(C), indicating that there is no interaction between non-complementary bases. These results correspond to those obtained from the polarizing microphotographs and the refractometer.

Figure 3(b) and (b′) shows CD spectra of poly(A), poly(I), and Mixture 2 in solutions of concentrations 0.05 wt% (b) and 5.0 wt% (b′) at room temperature. The CD spectra of Mixture 2 at both concentrations are different from those that result from an addition of those of pure poly(A) and pure poly(I), demonstrating that Mixture 2, in solutions of 0.05 and 5.00 wt%, forms liquid crystals by molecular arrangement resulting from the repulsive forces between phosphate groups in non-complementary bases.

Figure 3(c) shows that the CD spectrum of Mixture 3 represents an addition of the CD spectra of pure poly(C) and pure poly(U), as seen in the poly(A)–poly(C) system.

Finally, Fig. 3(d) and (d′) shows CD spectra of poly(I), poly(U), and Mixture 4 in solutions of concentrations of 0.05 wt% (d) and 5.00 wt% (d′) at room temperature. It can be seen that the CD spectrum of Mixture 4 with a concentration of 5.00 wt% (d′) is different from the result of the addition of the CD spectra of poly(I) and poly(U). However, the CD spectrum of Mixture 4 in solution with a concentration of 0.05 wt% (d) is in good agreement a simple addition of the CD spectra of the two polynucleotides. It is very interesting that the formation of liquid crystals in Mixture 4 depends on the concentration.

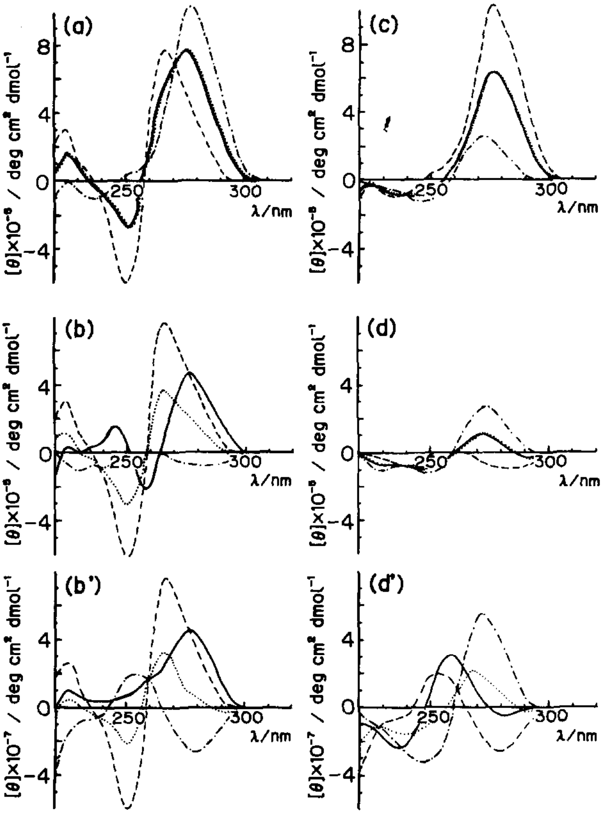

Fig. 3. CD spectra for pure polynucleotides and mixtures, with an addition of the spectra of each polynucleotides. (a) ——, Mixture 1; – – – , poly(A); – · – · – , poly(C); - - -, additive curve. (b) and (b′) ——, Mixture 2; poly(A); – · – · – , poly(I); - - -, additive curve. (c) ——, Mixture 3; – – – , poly(C); – · – · – , poly(U); - - -, additive curve. (d) and (d′) ——, Mixture 4; poly(I); – · – · – , poly(U); - - - , additive curve.

3.4. *UV spectra of the mixtures*

In order to confirm the molecular conformations suggested by the CD spectra for each system, the absorbance mixing curves of mixtures with varying mole fractions of poly(A) X_A and poly(U) X_U were studied at room temperature using a spectrophotometer.

The results obtained are shown in Fig. 4(a)–(d). The absorbance mixing curve of Mixture 1 at 260 nm (A_{260}) is plotted against X_A in Fig. 4(a); the result is linear, practically independent of X_A, demonstrating that there is no interaction between non-complementary bases.

Figure 4(b) shows the absorbance mixing curves at 249 nm (A_{250}) in solutions of concentrations 0.2, 1.0, 5.0, and 10.0 wt%. The appearance of discontinuities in the vicinity of $X_A = 0.3$, for all concentrations, demonstrates the formation of com-

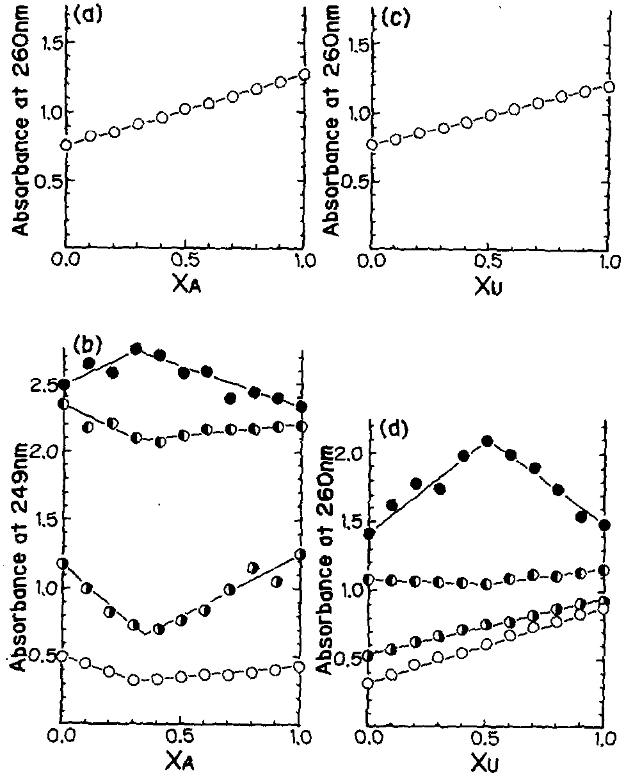

Fig. 4. Plots of absorbance mixing curves for mixtures against mole fractions of poly(A) X_A and poly(U) X_U. (a) Mixture 1. (b) Mixture 2. (c) Mixture 3. (d) Mixture 4.

plexes by non-complementary interaction between purine bases, suggesting that Mixture 2 consists of a poly(A) · 2poly(I) triplex with a triple-stranded helical structure.

Figure 4(c) shows a plot of the absorbance mixing curve at 260 nm (A_{260}) for Mixture 3 against X_U. The plot is linear, virtually independent of X_U, indicating that there is no interaction.

Finally, the plots of the absorbance mixing curves at 260 nm (A_{260}) for Mixture 4 in solutions of varying concentrations of 0.05, 1.00, 5.00, and 10.0 wt%, against X_U are shown in Fig. 4(d). In the concentration range 0.05–1.00 wt%, A_{260} shows a linear relationship which is practically independent of X_U, demonstrating that there is no interaction in this range of concentration. However, at 5.00 wt% concentration, a discontinuity appears in the vicinity of $X_U = 0.5$, whereas, at 10.0 wt%, there is a distinct discontinuity at $X_U = 0.50$, suggesting that a poly(I) · poly(U) duplex with a double-stranded helical structure is formed by non-complementary interaction between purine base and pyrimidine base.

3.5. Thermal and optical behaviours of the mixtures

The characterization of the phase states for the mixtures was achieved from the results of polarized light microscopy, refractive indices, and CD and UV spectra. It is suggested that Mixture 2 forms a liquid crystal with a triple-stranded helical structure by non-complementary interaction between purine bases, whereas Mixture 4 forms a liquid crystal with a double-stranded helical structure at concentrations above 5.0 wt%; however, Mixture 4 shows an isotropic phase at concentrations below 5.0 wt%.

Polarized light microscopy indicates that Mixture 1 exhibits an anisotropic phase although it does not show birefringence, thus implying a weak interaction under the present experimental conditions.

Mixture 3 does not form a complex by non-complementary interaction between poly(C) and poly(U), both with pyrimidine base.

In order to obtain further information about non-complementary interaction, the thermal and optical behaviours of these systems were studied by differential thermal analysis (DTA), using an apparatus with a laser. Typical DTA and laser-transmitted curves are shown in Fig. 5. As seen in Fig. 5, DTA and laser-transmitted curves do not appear for Mixture 1 in the concentration range 1.0–10.0 wt%, indicating

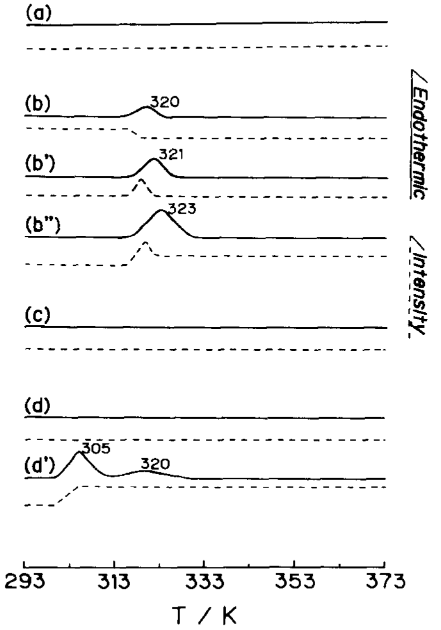

Fig. 5. DTA (solid line) and laser transmittance (broken line) curves for the mixtures. (a) Mixture 1 in the concentration range 1.0–10.0 wt%. (b), (b′), and (b″) Mixture 2 in the concentration ranges 1.0–3.0 wt%, 4.0–6.0 wt%, and 7.0–10.0 wt%, respectively. (c) Mixture 3 in the concentration range 1.0–10.0 wt%. (d) and (d′) Mixture 4 in the concentration ranges 1.0–5.0 wt% and 7.0–10.0 wt%, respectively.

that there is no interaction in this system. In the concentration range 1.0–10.0 wt%, the DTA curves of Mixture 2 show an endothermic peak at 320–323 K, with increasing concentration, demonstrating that an interaction between non-complementary bases exists; this endothermic peak corresponds to the helix–coil transition of the complex formed by non-complementary interaction between poly(A) and poly(I), both with purine base. However, from the polarizing microphotographs and the refractive indices, the DTA curves of Mixture 2 indicate two phase states, depending on the concentration: in the first, in the concentration range 1.0–6.0 wt%, Mixture 2 exists as an anisotropic phase, the phase state showing a slight colour, although the birefringence is not evident; in the second, at concentrations above 7.0 wt%, Mixture 2 exhibits liquid crystals having a triple-stranded helical structure formed by the repulsion between the phosphate groups, bearing negative charges, in the main chains of poly(A) and poly(I). However, the molecular conformations of these complexes depend on the concentration, as evidenced from the change in intensity of the laser-transmitted curves. That is, in the concentration range 1.0–3.0 wt%, the change in intensity of laser transmittance decreases, corresponding to the start of the thermal change of the DTA curve, demonstrating that the decrease in intensity of laser transmittance corresponds to the helix–coil transition of Mixture 2. In concentrations above 4.0 wt%, the change in intensity of laser transmittance first increases, corresponding to the start of the thermal change of the DTA curve, and then decreases with increasing temperature. The increase and decrease in the change in intensity of the laser transmittance correspond to the conformational change based on the order–disorder transition of the triple-stranded helical structure of Mixture 2, as pointed out above.

However, there is no thermal change or change in the intensity of laser transmittance of Mixture 3, demonstrating that an interaction between non-complementary bases such as poly(C) base and poly(U), both with pyrimidine bases, does not exist under the present experimental conditions.

For Mixture 4 in the concentration range 1.0–5.0 wt%, there is no thermal change in the DTA curve.

It is suggested that any interaction between non-complementary bases such as poly(I) with a purine base and poly(U) with a pyrimidine base does not form a complex. However, the DTA curves for concentrations above 5.0 wt% exhibit two endothermic peaks at 305 and 320 K. The endothermic peak at 305 K corresponds to the changes in enthalpy derived from the helix–coil and order–disorder transitions of the liquid crystal, with the double-stranded helical structure being formed from the molecular arrangement resulting from the repulsive forces between negatively charged phosphate groups in the main chains of poly(I) and poly(U).

With further increase in temperature, a broad endothermic DTA peak is seen at 320 K; it is not possible to analyse this peak without more information.

Assuming that an endothermic DTA peak corresponds to the helix–coil transitions of Mixture 2 with a triple-stranded helical structure, and of Mixture 4 with a double-stranded helical structure, formed by non-complementary interactions between purines or between purine and pyrimidine, the transition temperature T_t and the change in enthalpy ΔH_{obs} of the transition can be estimated from the peak and

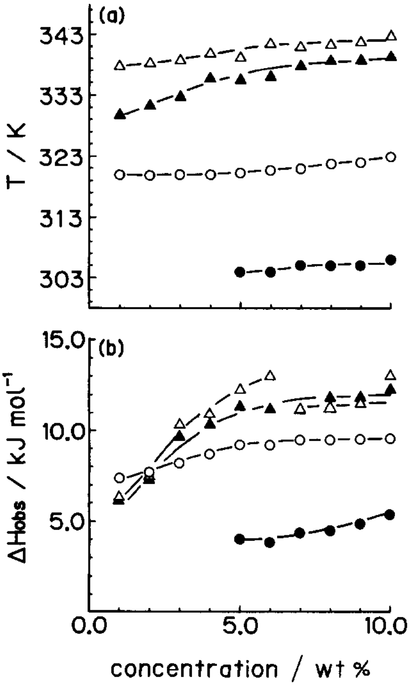

Fig. 6. Plots of (a) transition temperature T_t and (b) the change in enthalpy ΔH_{obs} observed, based on the helix-coil transition, against the concentrations of the mixtures: △, Mixture 1; ○, Mixture 2; ▲, Mixture 3; and ●, Mixture 4.

the area of the DTA curve, respectively. Plots of T_t and ΔH_{obs} against the concentration of the mixtures are shown in Fig. 6(a) and (b), together with those of equimolar poly(I)-poly(C) and poly(A)-poly(U) mixtures [14] formed by complementary interactions between purine and pyrimidine.

In addition, the poly(I)-poly(C) mixture was investigated under the same experimental conditions as the poly(A)-poly(U) mixture; its behaviour is the same as for poly(A)-poly(U). Only the result for the complementary interaction of poly(I)-poly(C) mixture is shown in Fig. 6(a) and (b), respectively.

As seen in Fig. 6, the T_t and ΔH_{obs} values of non-complementary systems are lower than those of systems with complementary interaction, demonstrating that liquid crystals formed by non-complementary interaction are thermodynamically less stable than those formed by complementary interaction.

The difference between ΔH_{obs} at 10.0 wt% and the change in enthalpy $\Delta H^{\ominus}_{trans}$ based on the helix-coil transition of the mixtures at 1.0 wt% gives the change in enthalpy $\Delta H_{ord-disord}$ for the order-disorder transition of liquid crystals, i.e. $\Delta H_{ord-disord} = \Delta H_{obs} - \Delta H^{\ominus}_{trans}$. In addition, in Mixture 4, ΔH_{obs} at 5.0 wt% is assumed to correspond to $\Delta H^{\ominus}_{trans}$.

The magnitude of $\Delta H_{ord-disord}$ corresponds to the degree of order-disorder of the liquid crystals formed by non-complementary interactions between purines or purine and pyrimidine.

Table 1
The changes in enthalpy based on the helix-coil transition $\Delta H^{\circ}_{\text{trans}}$, the observed heat ΔH_{obs}, and $\Delta H_{\text{ord-disord}}$ based on the order-disorder transition of the liquid crystal formed by non-complementary and complementary interactions

	$\Delta H^{\circ}_{\text{trans}}$/ (kJ mol^{-1}) [a]	ΔH_{obs}/ (kJ mol^{-1}) [a]	$\Delta H_{\text{ord-disord}}$/ (kJ mol^{-1}) [a]
Mixture 1	–	–	–
Mixture 2	7.3	10	2.7
Mixture 3	–	–	–
Mixture 4	4.0	5.0	1.0
Poly(I) · poly(C)	6.2	13	6.6
Poly(A) · poly(U) [b]	6.4	12	5.8

[a] Here mol means mole of nucleotide.
[b] See ref. 14.

The results obtained are listed in Table 1, together with those of equimolar poly(A)-poly(U) and poly(I)-poly(C) mixtures, as reported previously [32]. As seen in Table 1, $\Delta H_{\text{ord-disord}}$ for non-complementary interactions is smaller than for complementary interactions, demonstrating that interaction between non-complementary bases is very weak, more weak than that between complementary bases.

3.6. Possible interactions between non-complementary bases

In order to obtain information about a possible binding position, the possibility of interaction between non-complementary bases was studied by means of ab initio molecular obital (MO) calculations.

The net charges for each nucleotide were calculated and the results obtained are shown in Fig. 7(a)-(d), in which the net charges presented as open and shaded circles are positive and negative, respectively, and also the areas of the circles represent the magnitude of the net charges.

The net charges of adenine (Ade) and cytosine (Cyt) are shown in Fig. 7(a). As seen in Fig. 7(a), the three possible hydrogen bonds between N_7 of Ade and N_9H_{10} of Cyt, between $N_{11}H_{12}$ of Ade and C_2O_8 of Cyt, and between $N_{11}H_{13}$ of Ade and N_3 of Cyt may be considered.

However, simultaneous hydrogen bonds between $N_{11}H_{12}$ of Ade and C_2O_8 of Cyt, and between $N_{11}H_{13}$ of Ade and N_3 of Cyt would seem to be impossible. Therefore, interaction between the non-complementary bases of Mixture 1 must be very weak if the hydrogen bond between N_7 of Ade and N_9H_{10} of Cyt is assumed to exist. These results are in reasonable agreement with those obtained from the spectral and calorimetric measurements above.

Figure 7(b) shows the net charges for adenine (Ade) and hypoxanthine (Hyp). As seen in Fig. 7(b), at first the hydrogen bonds between N_1 of Ade and N_1H_{10} of Hyp, and between $N_{11}H_{12}$ of Ade and C_6O_{12} of Hyp (the Watson-Crick interaction), are formed, and, then hydrogen bonds between N_7 of Ade and N_1H_{10} of Hyp, and between $N_{11}H_{13}$ of Ade and C_6O_{12} of Hyp (the Hoogsteen interaction) are formed. Thus, it can be suggested that Mixture 2 forms a poly(A) · 2poly(I) triplex with a triple-stranded helical structure in which the Watson–Crick and Hoogsteen interactions take place at the same time. This is in good agreement with the results of the absorbance mixing curve obtained by UV spectral measurement.

Figure 7(c) shows the net charges of cytosine (Cyt) and uracil (Ura). Hydrogen bonds between N_3 of Cyt and N_3H_9 of Ura, and between N_9H_{10} of Cyt and C_4O_{10}

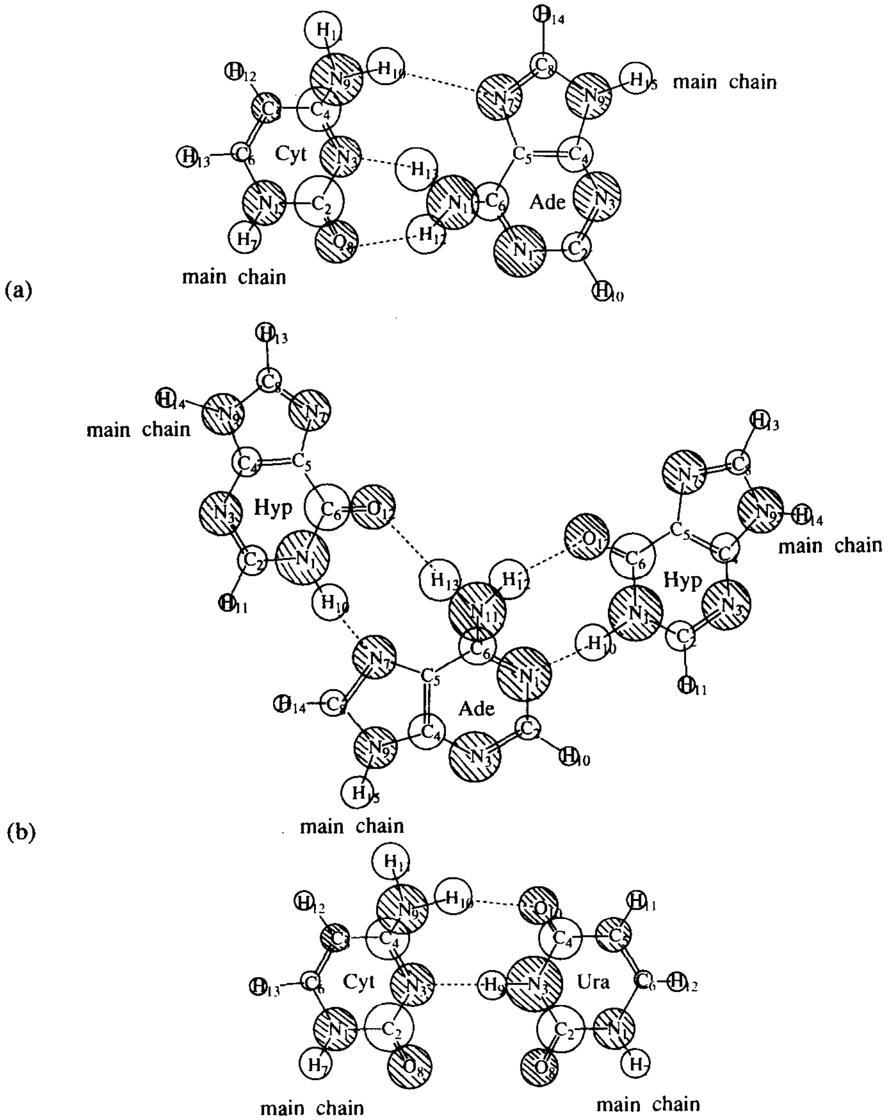

Fig. 7. (a)–(c)

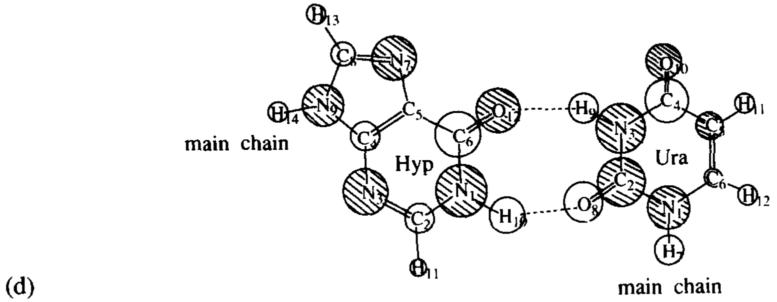

Fig. 7. Schematic diagrams of possible hydrogen-bonding modes based on non-complementary interaction between base pairs of polynucleotides. The open and shaded circles represent the magnitude of the positive and negative net charges, respectively, for each base. (a) Adenine (Ade)–cytosine (Cyt) system. (b) Hypoxanthine (Hyp)–adenine (Ade)–hypoxanthine (Hyp) system. (c) Cytosine (Cyt)–uracil (Ura) system. (d) Hypoxanthine (Hyp)–uracil (Ura) system.

of Ura are possible. However, the calorimetric and spectral results indicate that Mixture 3 does not form a complex by non-complementary interaction because of the contribution of the repulsion between C_2O_8 of Cyt and C_2O_8 of Ura.

Figure 7(d) shows the net charges of hypoxanthine (Hyp) and uracil (Ura). There are possible hydrogen bonds between N_1N_{10} of Hyp and C_2O_8 of Ura, and between C_6O_{12} of Hyp and N_3H_9 of Ura. From the results above, Mixture 4 forms a poly(I) · poly(U) duplex with a double-stranded helical structure. However, as pointed out in the preceding sections, the reason that Mixture 4 does not form a complex below concentrations of 5.0 wt% is very difficult to explain at present.

Further work is in progress to solve this problem.

Acknowledgments

The authors thank the Computer Center, Institute for Molecular Science, Okazaki National Research Institutes for use of a HITAC M-680H computer, the Library Program IMSPACK written by K. Morokuma, S. Kato, K. Kitaura, I. Omone, S. Saki and S. Obara (IMS) and the Library Program GAUSSIAN 86 written by M.J. Frisch, J.S. Binkley, H.B. Schlegel, K. Raghauachari, C.F. Melius, R.L. Martin, J.J.P. Stewart, F.W. Bobrowicz, C.M. Rohlfing, L.R. Kahn, D.J. Defrees, R.A. Whiteside, F.J. Fox, E.M. Flude, S. Topiol and J.A. Pople, registered by N. Koga, S. Yabushita, K. Sawabe, and K. Morokuma (Institute for Molecular Science).

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