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THERMAL AND OPTICAL PROPERTIES OF LIQUID CRYSTAL FORMATION IN AN EQUIMOLAR MIXTURE OF POLY(A) AND POLY(U) BY SIMULTANEOUS MEASUREMENT OF DIFFERENTIAL THERMAL ANALYSIS AND LASER TRANSMITTANCE

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ABSTRACT

A differential thermal analysis apparatus equipped with a laser was designed to allow simultaneous measurement of the thermal and optical properties of biopolymer solutions. The phase states of a concentrated solution of an equimolar poly(A)–poly(U) mixture were studied using this apparatus and a polarization microscope. This mixture shows an isotropic phase at concentrations below 3.0wt.%, a quasi-isotropic phase for the concentration range 4.0–6.0wt.%, a spherical liquid crystal phase for the concentration range 7.0–9.0wt.%, and a layer crystal phase for concentrations above 10.0wt.%. The thermal and optical changes for phase states estimated with the new apparatus correspond well with phase state changes observed with the polarization microscope.

INTRODUCTION

The relationships between the structure and properties of biopolymers are of fundamental importance in biology, but such relationships are difficult to study because of the complex structure of biopolymers. One approach which has been tried is to study the stability of structures accompanying helix–coil and/or coil–helix transitions. Most such studies have been done in dilute solutions by various methods such as thermal and optical measurements [1,2].

For a more realistic understanding, concentrated biopolymer solutions should be studied, because, in vivo, DNA exists as solutions of 70% concentration. It is well known that concentrated biopolymer solutions contain an anisotropic phase, such as lyotropic and cholesteric liquid crystals, and many studies on one-component liquid crystal biopolymer systems, such

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as bilayer lipid membranes, have been carried out [3,4]. Recently, it was reported [5] that concentrated DNA solutions contain liquid crystals of various types, depending on the concentration. No information, however, has been obtained about the mechanism of formation of liquid crystals in biopolymer solutions.

In this paper the mechanism of formation of liquid crystals in neutral biopolymer solutions containing an equimolar poly(A)–poly(U) mixture was studied with a newly constructed differential thermal analysis (DTA) apparatus equipped with a laser, and with a polarization microscope. The mixture studied was prepared from poly(riboadenylic acid) (poly(A)), having the single-stranded helical structure [6] and poly(ribouridylic acid) (poly(U)), having the random-coiled structure [7].

The changes in molecular conformation accompanying formation of liquid crystal phases in this mixture will be discussed.

MATERIALS

Poly(riboadenylic acid) (poly(A)) and poly(ribouridylic acid) (poly(U)) were from Yamasa Shoyu Co. Ltd. (Japan). The buffer solution used was 0.1 M tris-HCl [(tris-hydroxymethyl aminomethane hydrochloride)] of pH 7.60. Water used to prepare the buffer solution was purified by reverse osmosis, then distilled and deionized by ion-exchange. The concentrations of polynucleotides were determined by the phosphorous analysis method [8].

APPARATUS AND PROCEDURE

The differential thermal analysis apparatus used in this study was designed to allow simultaneous measurement of the thermal and optical properties of biopolymer and synthetic polymer solutions. Its construction is shown in Fig. 1(a,b). The furnace (a) is of stainless steel and heater block (c) is of aluminium, heated by a nichrome wire coil (d) (30 Ω). Both inner and outer lids (g) are of stainless steel. The aluminium board (h) was installed under the inner lid to maintain adiabatic conditions. The cell holder(thickness, 0.4mm) (e) was made of an alloy of nickel and chromium. A three-junction chromel–alumel thermocouple (f) is used to monitor the temperature.

The 0.5 ml cell (i) is of glass with flat top and bottom sections, while the part marked with an arrow in Fig. 1(a) was sealed tightly with a small flame to prevent concentration changes caused by solvent evaporation with an increase of temperature.

As shown in Fig. 1(b), a light beam from a He–Ne gas laser ($\lambda_0 = 633$nm) (b), situated just under the furnace (a), was bisected using a half mirror, passed through the optical fibre cable (g), and then caught by a photodiode(c) after passage through the sample (S) and reference (R) cells. The intensity of transmitted light (the difference in intensity between sample and reference cells) was registered by a photodiode, amplified by the photosensitive amplifier (d) and then recorded. The e.m.f. representing the temperature difference between sample and reference cells was amplified by d.c. amplifier (k) and then recorded. The heating rate was about 1.0 K $\min^{-1}$, controlled by the temperature program controller (l) (Seiko I & E SSC/580 thermal controller, Seiko Electronics Co. Ltd., Japan).

Light scattering measurements were carried out simultaneously in the angular range 80–150° at 298 K, using a light scattering photometer

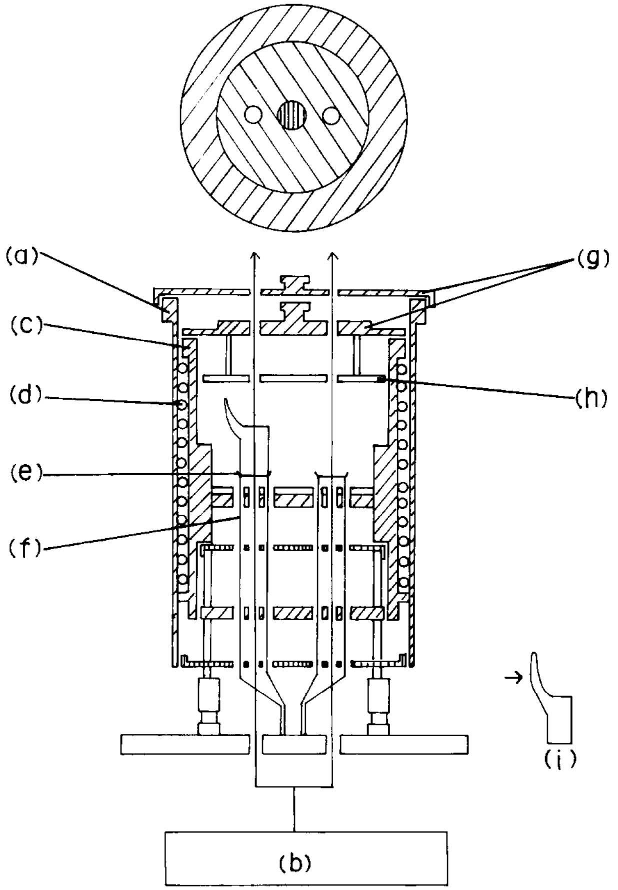

Fig. 1(a). The new DTA instrument equipped with a laser for simultaneous measurement of thermal and optical changes: (a), furnace; (b), source of He–Ne gas laser; (c), heater block; (d), heater; (e), cell holder; (f), thermocouple; (g), stainless steel lids; (h), aluminium board; (i), glass cell.

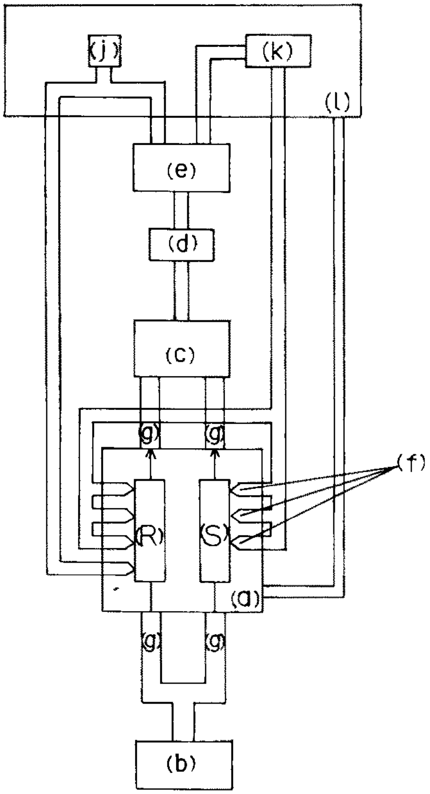

Fig. 1(b). Block diagram of the instrument: (a), furnace; (b), source of He–Ne gas laser; (c), photodiode; (d), photosensitive amplifier; (e), recorder; (f), thermocouple; (g), optical fibre cable; (l), temperature program controller; (k), d.c. amplifier; (j), 0 K point.

(LS-601, Otsuka Electronics Co. Ltd., Japan). The polarization microscope used was an XTP-11 (Nikon Co. Ltd., Japan), equipped with a hot stage.

RESULTS AND DISCUSSION

Use of the new DTA instrument

As a test, the new instrument was first used to study the helix–coil and/or the coil–helix transitions known to occur in DNA and/or poly(L-lysine) solutions.

Typical DTA and laser transmittance curves for a DNA solution are shown in Fig. 2, together with the DSC curve which corresponds to the helix–coil transition of DNA solution obtained using an adiabatic differential scanning calorimeter (DASM-4, U.S.S.R.).

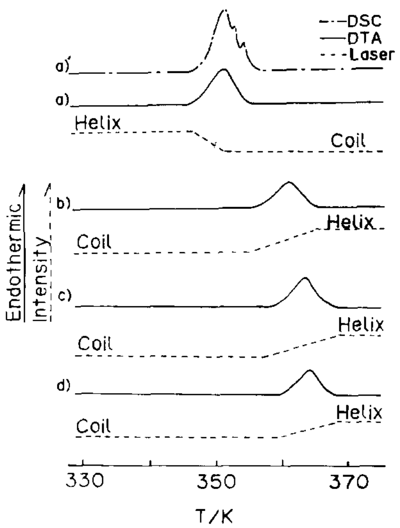

Fig. 2. Typical DTA and laser transmittance curves obtained from the new instrument and DSC curve obtained from a commercial instrument. The solid and dotted lines represent DTA and laser transmittance curves for (a) and (a)′, DNA systems; (b), poly(L-lysine)–(dioxan/water) system; (c), poly(L-lysine)–(DMF/water) system; (d), poly(L-lysine)–(ethanol/water) system.

As seen in curve (a) of Fig. 2, the laser transmittance curve decreases at first and then reaches a constant value with further increases in temperature. The DTA curve shows an endothermic peak, just where the laser transmittance curve starts to fall, suggesting that the endothermic peak and the decrease in intensity of transmitted light may correspond to the helix–coil transition. The DSC curve also shows an endothermic peak. Typical DTA thermogram and laser transmittance curves obtained for poly(L-lysine)–(dioxan/water), –(DMF/water) and –(ethanol/water) mixtures, known to show reversible transition from coiled to helical conformation, are also shown in Fig. 2. As seen in curve (b) of Fig. 2, the intensity of laser transmittance of the poly(L-lysine)–(dioxan/water) mixture increases at 358 K and then reaches a constant value with further increases in temperature, while the DTA curve shows an endothermic peak corresponding to the onset of the increase in transmitted light intensity. The behaviour of the poly(L-lysine)–(DMF/water) and –(ethanol/water) systems is similar.

It may be suggested that the endothermic peak of the DTA curve and the increase in the intensity of transmitted light correspond to the transition of poly(L-lysine) from a coiled to a helical conformation in these mixed solvents [2]. This is the well known phenomenon of the reversible transition.

The dramatic changes in laser transmittance which occur in all solutions indicate a greater intensity of transmitted light for the helical conformation than for the coiled one.

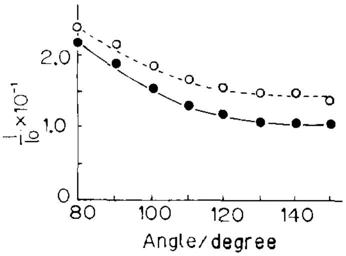

Fig. 3. Plots of intensity of scattered light against angle for DNA. I, Intensity of scattered light; I_0, intensity of incident light. ●, helical conformation; ○, coiled conformation.

In order to confirm these results, the intensities of light scattered from DNA, having the double-stranded helical structure at neutral pH and coiled conformation in solution at pH 3 [9], were measured at 298 K using a He–Ne gas laser. The results obtained are shown in Fig. 3, where the intensities for both DNA conformations are plotted as a function of angle over the range 80–150 °.

As seen in Fig. 3, the intensity of light scattered from the coiled conformation is greater than that from the helical structure for all angles, demonstrating that the decrease in the intensity of laser transmittance in DNA solutions depends on the change in light scattering which accompanies the helix to coil transition. The behaviour of poly(L-lysine) in mixed solvents is similarly rationalized, with the increase of transmitted light intensity corresponding to a coil to helix transition, which occurs as the temperature is increased (the so-called reversible transitions). This is because in the coil conformation more light is scattered and hence the transmitted light intensity must be smaller. In the helical conformation, the opposite is the case.

From these results, we suggest that the new apparatus will be useful for the qualitative determination of changes of conformational energy and molecular conformations, such as helical and coiled conformations.

The phase states of poly(A)–poly(U) mixture

The phase states over the concentration range 0.05–10.0wt.% for an equimolar poly(A)–poly(U) mixture were observed using a polarization microscope at room temperature. The three typical phase states were observed and are shown as polarizing microphotographs in Fig. 4(a–c). In the concentration range 0.05–6.0wt.%, the mixture shows an isotropic phase. In the concentration range 7.0–9.0wt.%, the mixture exists as a spherical liquid crystal surrounding an isotropic phase, which is essentially different from a general layer state, and the amount of the spherical liquid crystal phase increases with an increase in the concentration of the poly(A)–poly(U) mixture (Fig. 4(b)). At concentrations above 10.0wt.%, a layer liquid crystal phase (aggregation of the spherical liquid crystal phase) develops.

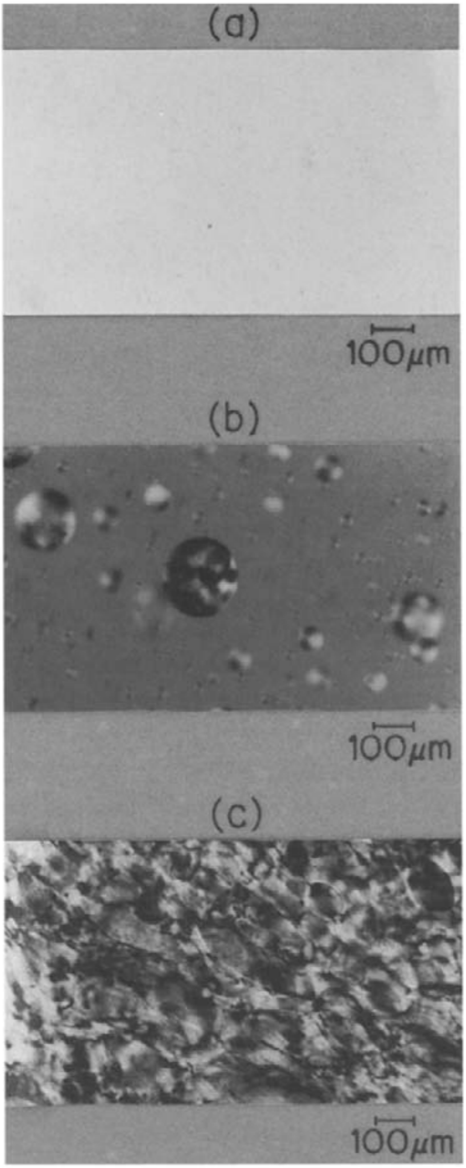

Fig. 4. Typical polarizing microphotographs of poly(A)–poly(U) mixtures. (a), an isotropic phase, the concentration below 6.0wt.% at 298K; (b), spherical liquid crystal, concentration range 7.0–9.0wt.% at 298 K; (c), layer liquid crystal, concentration above 10.0wt.% at 298 K.

Thermal and optical properties of poly(A)–poly(U) mixture

It is of interest to determine the molecular conformation of the poly(A)–poly(U) mixture required for formation of liquid crystals. In order to establish whether or not an equimolar mixture of poly(A) and poly(U), in

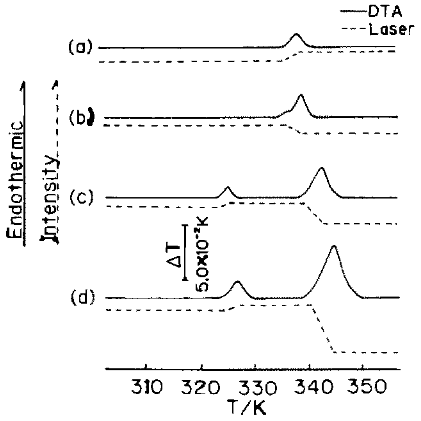

Fig. 5. Typical DTA (solid lines) and laser transmittance (dotted lines) curves of poly(A)–poly(U) solutions of various concentrations. (a), concentration below 2.0wt.%; (b), concentration 3.0wt.%; (c), concentration 4.0–6.0wt.%; (d), concentration above 7.0wt.%.

concentrated solution, keeps the double-stranded helical structure (similar to the poly(A) · poly(U) duplex formed by an equimolar mixture of poly(A) and poly(U) at neutral pH in dilute solution), the thermal and optical properties of such a mixture were studied over the concentration range 1.0–10.0wt.%, using the new DTA–laser instrument. Typical DTA thermograms and laser transmittance curves obtained are shown in Fig. 5(a–d).

As seen in Fig. 5, in the concentration range below 2.0wt.% (Fig. 5(a)), the DTA curve has an endothermic peak at 338 K and the laser transmittance curve shows an increase in intensity of transmitted light corresponding to the start of thermal change leading to the endothermic peak in the DTA curve. This increase in the transmitted light intensity suggests that the poly(A)–poly(U) mixture may undergo a transition from coiled to helical conformation (the so-called reversible transition) with an increase in temperature, as already discussed for the poly(L-lysine) solutions. In other words, the equimolar poly(A)–poly(U) mixture does not form the double-stranded helical structure at room temperature and neutral pH, unlike the case for dilute solutions. The endothermic DTA peak at 338 K corresponds to the heat of transition from coiled to helical conformation.

At a concentration of 3.0wt.%, the DTA curve shows an endothermic peak, having a shoulder at lower temperature, and the laser transmittance curve shows a decrease in intensity, corresponding to the start of this endothermic peak. On the other hand, in the concentration range 4.0–6.0wt.%, the DTA curves show the two endothermic peaks, at 326 and 342 K, and the transmitted light intensity increases slightly, corresponding to the lower temperature peak, and then decreases still further, corresponding to the higher temperature peak. This indicates that the molecular conformation is concentration dependent with a change occurring at about 3.0wt.%. Figure

5 shows that the first peak temperature does not change, but the second peak temperature does increase slightly with increasing concentrations of poly(A)–poly(U) mixture. Also the intensity of transmitted light shows a dramatic decrease at higher temperature, in comparison with that at lower temperature, with increasing concentration of poly(A)–poly(U) mixture. It is concluded from these results that at above 3wt.% concentration, different phase states must exist, although they show the same isotropic phase at a concentration less than 3.0wt.% (see Fig. 4). This demonstrates that 3wt.% is the critical concentration for development of different phase states such as an isotropic phase and a quasi-isotropic phase (intermediate between isotropic and liquid crystal phases).

At a concentration above 4.0wt.%, it is suggested that the first endothermic DTA peak and the increase in intensity of transmitted light at lower temperature may correspond to the change from coiled to helical conformation, while the second DTA peak seems to correspond to melting of a quasi-isotropic phase. The associated decrease in intensity of transmitted light is the result of increased light scattering which accompanies the transition to the coiled conformation of poly(A) · poly(U) duplex.

In the concentration range 7.0–9.0wt.%, the DTA curve has two endothermic peaks at 320 and 340 K, similar to those in the concentration range 4.0–6.0wt.%, and the corresponding laser transmittance curves are also similar to those described above. The first DTA peak, and the slight increase in transmitted light intensity at lower temperature, can be interpreted as before. However, the second DTA peak and the remarkable decrease in the transmitted light intensity are quite different from those observed at a concentration below 6.0wt.%. Since it is known (polarization microscope) that the amount of spherical liquid crystal becomes progressively greater as the concentration of the poly(A)–poly(U) mixture increases, it is concluded that the second DTA peak corresponds to the heat of fusion of the liquid crystal phase of the poly(A)–poly(U) mixture.

At concentrations above 10.0wt.%, the thermal and optical properties from the DTA and laser transmittance curves are similar to those in the concentration range 7.0–9.0wt.%, although the transition temperature and the heat of phase transition are somewhat higher. This implies that the thermal change recorded by the DTA curve for the layer liquid crystal phase (formed by aggregation of the spherical liquid crystal phase) may correspond to the enthalpy change and transition temperature accompanying the fusion of the liquid crystal.

These results are shown in Fig. 6(a,b). As seen in Fig. 6, 3.0, 6.0 and 10.0wt.% (the dotted lines) seem to be the critical concentrations corresponding to the different phase states, viz. isotropic phase, spherical and layer liquid crystals, respectively, as shown in the polarizing microphotographs of Fig. 4, indicating concentration-dependent phase behaviour. Figure 6(b) shows the difference (ΔI) for the transmitted light intensity, before and

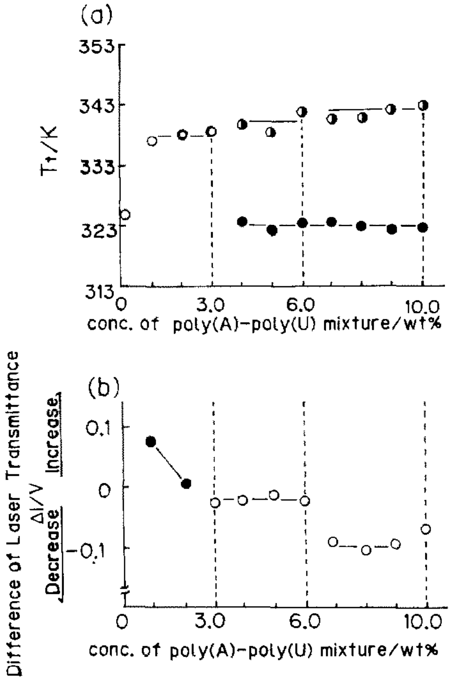

Fig. 6. (a) Plots of transition temperature (Tt) against the concentration of poly(A)–poly(U) mixtures. ● and ○ represent the lower and higher transition temperatures from the DTA curve. (b) Plots of the difference (ΔI) of the intensity of transmitted light before and after conformation change (except for the intensity of transmitted light corresponding to the first peak of the DTA curve for a concentration above 4.0wt.%) against the concentration of poly(A)–poly(U) mixture. ● and ○ represent positive and negative values of ΔI, respectively.

after phase change, from a base-line with increasing temperature, except for the increase in transmitted light intensity corresponding to the first DTA peak at a concentration above 4.0wt.%. As seen in Fig. 6(b), ΔI at a concentration below 3.0wt.% is positive, whereas above 3.0wt.% it is negative. As pointed out in Figs. 4 and 6, the concentration dependence of ΔI seems to reflect the molecular conformation of the poly(A)–poly(U) mixture.

In addition, in order to confirm the nature of the thermal changes in the various forms of the liquid crystals with increasing temperature, the spherical liquid crystal formed in the 9.0wt.% poly(A)–poly(U) mixture was also observed using a polarization microscope equipped with a hot stage. The results obtained are shown in Fig. 7 which shows polarizing microphotographs corresponding to the transition temperature for each phase state as indicated by the endothermic DTA peaks and by the base-lines for before and after thermal change. As seen in Fig. 7(a), the phase state corresponding to the base-line of the DTA curve at 297 K shows the spherical liquid crystal. With increasing temperature, the amount of this phase becomes

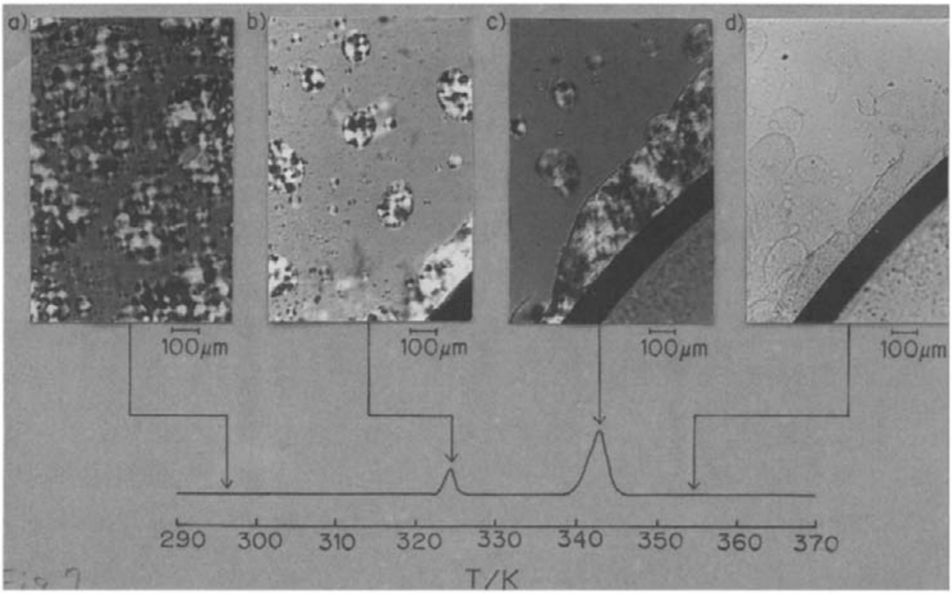

Fig. 7. Temperature dependence of the phase states, corresponding to the endothermic peaks of the DTA curve, in the 9.0wt.% poly(A)–poly(U) solution.

relatively small (Fig. 7(b)), corresponding to the appearance of the first endothermic DTA peak at 322 K, this peak being due to the conformational change from coil to helical conformation. In the phase state corresponding to the second endothermic peak at 342 K, almost all of the spherical liquid crystal phase has disappeared and with further increase in temperature, its disappeared completely (Fig. 7(d)). It is confirmed from Fig. 7 that the first and second endothermic DTA peaks correspond to a conformation change (coil–helix transition) and to the fusion of the spherical liquid crystals.

Phase diagram of poly(A)–poly(U) mixture

The phase states of the poly(A)–poly(U) mixture have been discussed in the two preceding sections and the phase diagram based on these results is presented in Fig. 8, together with polarizing microphotographs corresponding to each critical concentration of the poly(A)–poly(U) mixture. As seen in Fig. 8, the phase diagram can be divided into five regions. The transition temperature at 324 K in dilute solution (0.1wt.%) corresponds to a general helix–coil transition of the equimolar poly(A) · poly(U) duplex. However, with increasing concentration, the poly(A)–poly(U) mixture undergoes various phase changes, with critical concentrations of 1.0, 3.0, 6.0 and 10.0wt.% corresponding to the different phase states, as shown by the dotted lines in Fig. 8 (and discussed in the preceding section). In the concentration range 1.0–3.0wt.%, the increase in the intensity of transmitted light indicates that

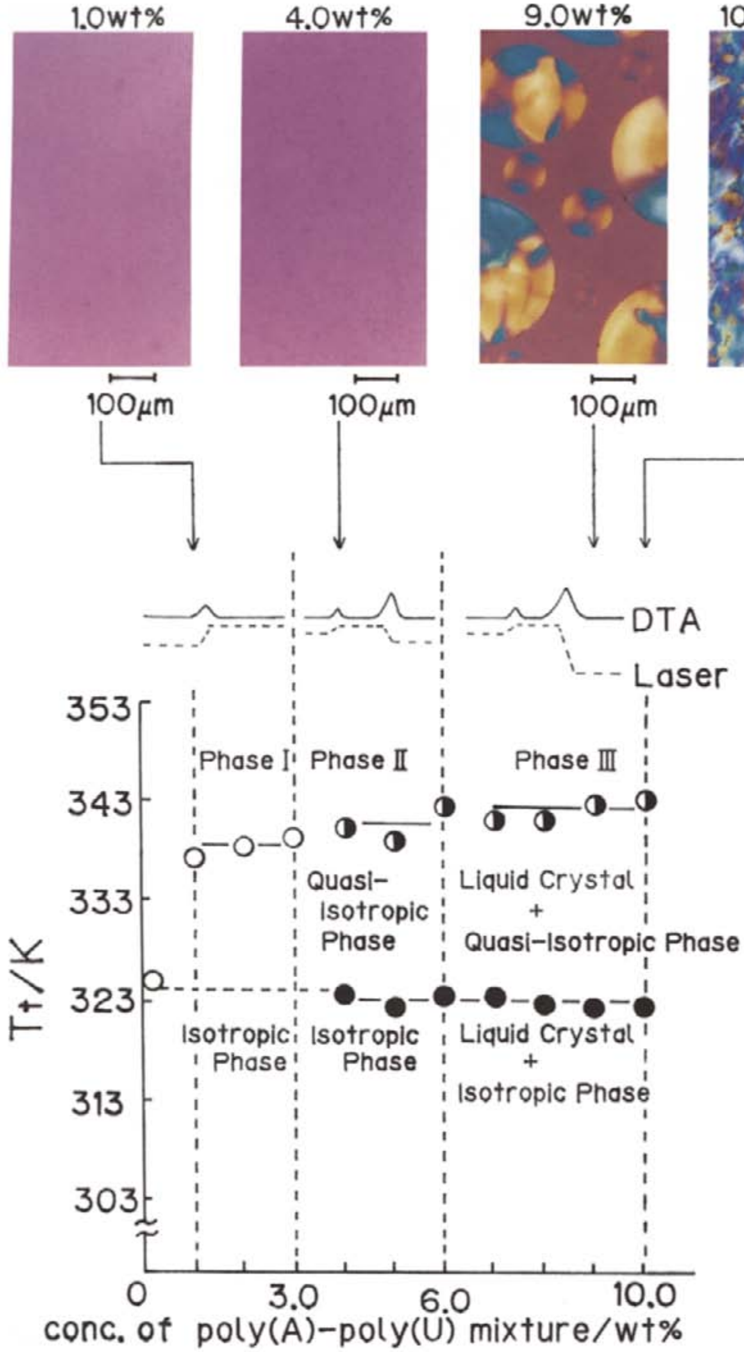

Fig. 8. Phase diagram of the poly(A)–poly(U) mixture in the concentration range 0.1–10.0wt.% and polarizing microphotographs at 298 K corresponding to the different phase states.

the thermal change at 322 K corresponds to the transition from an isotropic phase (coiled conformation) to phase I (helical conformation), the so-called reversible transition. In the concentrations range between 3.0 and 6.0wt.%, transition from an isotropic phase to a quasi-isotropic phase takes place and, with further increase in temperature, transition from the quasi-isotropic phase to phase II (coiled conformation) occurs. In the concentration range 6.0–9.0wt.%, a spherical liquid crystal phase, surrounding an isotropic phase, appears at room temperature and, with increasing temperature, the isotropic phase transforms to a quasi-isotropic phase, corresponding to appearance of the first endothermic DTA peak. The spherical liquid crystal remains unchanged at this temperature, but with further increase in temperature, it may melt to form phase III (coiled conformation), corresponding to the appearance of the second endothermic DTA peak. Finally, at concentrations above 10.0wt.%, a layer liquid crystal, formed by aggreation of the spherical liquid crystal phase, appears at room temperature. With increasing temperature, this phase behaves in much the same way as solutions of concentration 7.0–9.0wt.% (Fig. 7).

The first (lower temperature) thermal change observed in the DTA curve for solutions of concentration above 4.0wt.% appears to correspond to the transition already observed for the poly(A) · poly(U) duplex in dilute solutions.

The present study has provided a lot of qualitative information about concentrated solutions of an equimolar mixture of poly(A) and poly(U), but a quantitative treatment is difficult without data on conformational change, hydration, dehydration and so on. Work is in progress to obtain more information about liquid crystal formation in equimolar poly(A)–poly(U) mixtures.

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