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Compatibility of mixtures of deoxyribonucleic acid with hydroxypropylcellulose in concentrated aqueous solutions

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Abstract

In order to obtain information about the compatibility between biopolymeric substances and water-soluble polymers, the phase states of mixtures of DNA with hydroxypropylcellulose (HPC) in concentrated aqueous solutions were studied by simultaneous measurement of differential thermal analysis (DTA) and laser transmittance (He–Ne gas laser; $\lambda_0 = 633$ nm), and by means of a polarization microscope and a refractometer. From the results obtained, the phase states in mixture I exhibit an isotropic phase. Mixture II exhibits an anisotropic phase with a slight birefringence from the measurement of the refractive index at 298 K, suggesting that an anisotropic phase in this region corresponds to a quasi-liquid crystal similar to that found previously in the concentration range 3.00–5.00 wt.% of DNA solutions.

The phase states in mixture III exhibit birefringence from the measurement of refractive index at 298 K, suggesting that these phase states form stable liquid crystals which are essentially different from an already known liquid crystal of HPC or DNA in concentrated solutions. It is suggested that these liquid crystals are formed by the interaction between DNA and HPC.

In order to obtain information about the enthalpy change associated with the liquid crystal formation, the phase states of mixtures I, II and III were also studied using a DTA instrument equipped with a laser, under the same experimental conditions as observed with the polarization microscope. From the thermal and optical properties obtained, the degree of formation α of liquid crystal phases of the mixture formed by the interaction between DNA and HPC is estimated, making use of the degree of precipitation accompanying the phase separation of HPC corresponding to the difference in intensity ΔI of the transmitted laser light before and after the change in the laser with increasing temperature. From the α value, the net heat of fusion of liquid crystal phases of the mixture formed by the interaction of DNA with HPC is estimated to be about 2.4 kJ per mol of nucleotide.

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INTRODUCTION

It is well known that deoxyribonucleic acid (DNA) is very important in the functional development of living cells. DNA is present in living cells at very high concentrations, up to 70 vol.% [1]. For a more realistic understanding of the processes involved, information on the behaviour of DNA in concentrated solutions is, therefore, very important.

In a previous paper [2], the phase states of DNA in concentrated aqueous solutions were studied over the concentration range 1.00–10.0 wt.% of DNA by simultaneous measurements of differential thermal analysis (DTA) and laser transmittance, and using a polarization microscope. We reported that the phase states of DNA exhibit cholesteric liquid crystals, depending on the DNA concentrations. The repulsive forces between the phosphate groups of the main chain of DNA play an important role in the formation of the liquid crystal, and the net enthalpy change of fusion of the liquid crystal phases of DNA is estimated to be about 3 kJ per mole of nucleotide [3]. It is very interesting that hydroxypropylcellulose, a cellulose derivative, has hydrophilic and hydrophobic groups [4] which result in the consolute temperature, a lower critical solution temperature, in dilute aqueous solutions with increasing temperature [5]. In addition, in concentrated solutions, hydroxypropylcellulose forms lyotropic and cholesteric liquid crystals, depending on the concentration [6–8].

It is worth studying the compatibility of DNA with hydroxypropylcellulose, which is a typical water-soluble polymer.

In this paper, in order to obtain information about the compatibility of biopolymers with water-soluble polymers, the phase states of mixtures in concentrated solutions with various DNA concentrations at a given concentration of hydroxypropylcellulose were studied by means of a DTA instrument equipped with a laser, a polarization microscope and a refractometer. We will discuss the phase states of the mixture from the point of view of the interaction between DNA and hydroxypropylcellulose, making use of the phase separation of hydroxypropylcellulose in mixtures of aqueous solutions with increasing temperature.

EXPERIMENTAL

Materials

The samples used in this study were salmon testes deoxyribonucleic acid (DNA) with a guanine–cytosine base pair of 42%, and hydroxypropylcellulose (HPC) with a molecular weight of 6.0×10^4. They were purchased from Sigma Chemical Co. Ltd., and from Scientific Polymer Co. Ltd., USA, respectively. Samples were prepared by mixing various concentrations of

DNA with 30.0 wt.% of HPC and were used about three weeks later, after sufficient stirring to obtain a homogeneous solution.

The water used to prepare the samples was treated by reverse osmotic pressure, distilled and finally deionized by an ion-exchange resin.

Mixtures of DNA and HPC used in this paper were abbreviated as: mixture I contains 1.00 wt.% of DNA, mixture II contains 2.00–6.00 wt.% of DNA, and mixture III contains greater than 6.00 wt.% of DNA.

Apparatus and procedure

The apparatus used in this study was a differential thermal analysis (DTA) instrument equipped with a laser, which allows the simultaneous measurement of thermal change by DTA and of changes in properties by spectroscopy, in which the radiation of a He–Ne gas laser was used as a light source (633 nm and 1 mW) (Nikon Kagaku Engineering Co. Ltd., Japan), as reported previously [9–10]. The DTA heating rate employed was about 1 K min^{-1}. Temperature calibration of this apparatus was carried out using standard samples such as benzil, benzophenone and naphthalene, and the expected results were obtained within the experimental error (accuracy, ± 0.5 K). The estimation of the enthalpy changes from the areas of the DTA curves was determined using the standard samples mentioned above.

The microscope used in this study was a polarization microscope (XTP-11, Nikon Co. Ltd., Japan), equipped with a hot stage and heating program controller (Sinku-Riko Co. Ltd., Japan): the heating rate employed was about 1 K min^{-1}.

The refractive index of the phase states of the mixtures was measured at 298 K using a refractometer (2T, Atago Co. Ltd., Japan).

RESULTS AND DISCUSSION

Polarization microscopy

The phase states for mixtures in concentrated aqueous solutions with various concentrations of DNA and 30.0 wt.% of hydroxypropylcellulose (HPC), were observed at room temperature using a polarization microscope. The results obtained are shown in Fig. 1(a), 1(b) and 1(c), respectively. As seen in Fig. 1(a), mixture I exhibits an isotropic phase similar to that of DNA alone in the concentration range of 1.00–2.00 wt.% of DNA, as reported previously [2], demonstrating that the interaction of DNA with HPC may be negligibly small. The phase state of mixture II exhibits an anisotropic phase like a liquid crystal, as shown in Fig. 1(b), and finally, as seen in Fig. 1(c), the phase state of mixture III forms a liquid crystal which

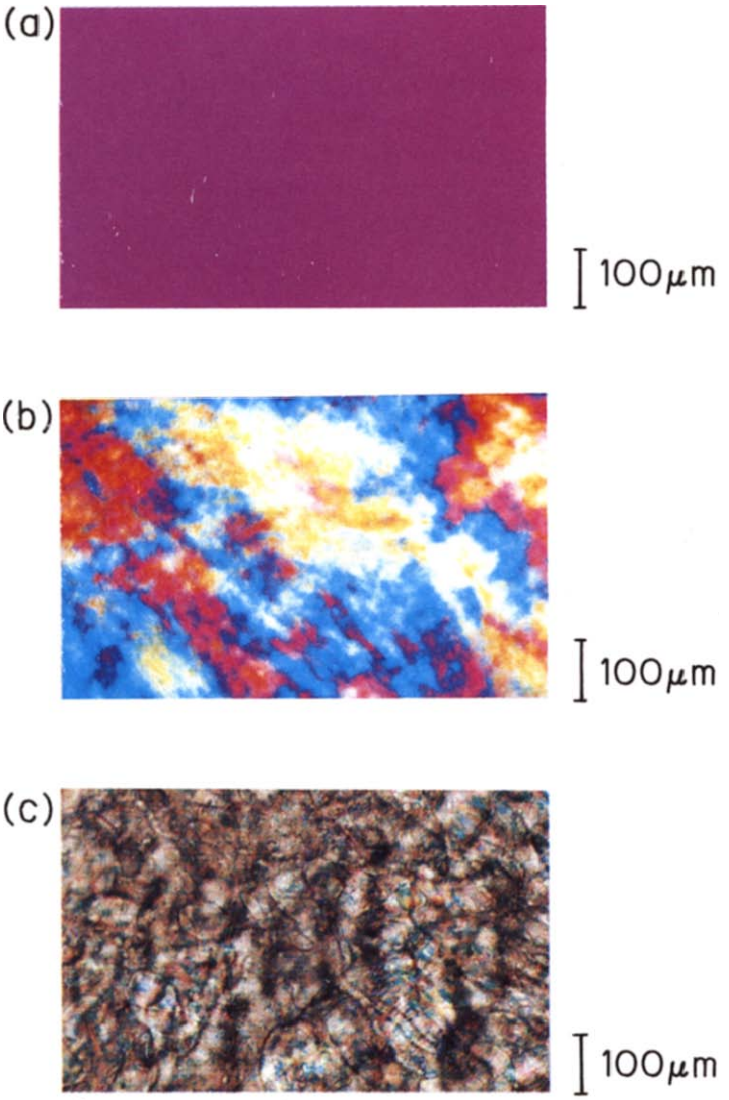

Fig. 1. Polarization microphotographs for (a) mixture I, (b) mixture II and (c) mixture III (see text).

is essentially different from the stable liquid crystal of DNA alone, as reported previously [2].

Refractive index of the mixture

In order to obtain information about the various phase states of mixtures I, II and III, their refractive indices were measured at 298 K using a refractometer; the results obtained are shown in Fig. 2. Figure 2(a) shows

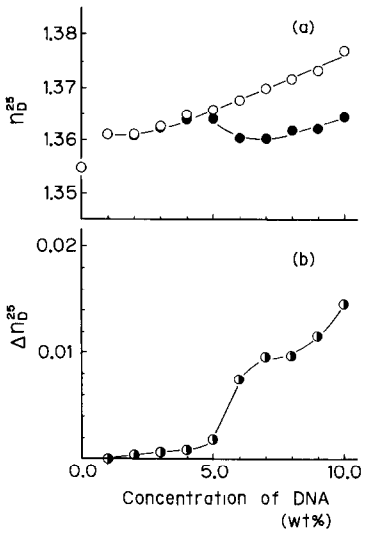

Fig. 2. Plots of (a) refractive index n_D^{25} and (b) birefringence Δn_D^{25} against DNA concentration: ○, refractive index parallel to the prism plane; ●, refractive index perpendicular to the prism plane; and (◑), the difference between refractive indices parallel and perpendicular to the prism plane.

the refractive index n_D^{25} for various DNA concentrations: deviations in refractive indices, both parallel and perpendicular to the prism plane, appear from the concentration of 2.00 wt.% of DNA; from 6.00 wt.% of DNA, pronounced changes take place, demonstrating that mixtures II and III contain liquid crystal phase states formed by the interaction between DNA and HPC. Figure 2(b) shows the birefringence Δn_D^{25} (the difference between the refractive indices parallel and perpendicular to the prism plane) plotted against each DNA concentration: mixture II seems to form a quasi-liquid crystal phase state and mixture III forms a stable liquid crystal phase by the interaction of DNA with HPC (Fig. 2(b)).

Thermal and optical behaviour of the mixtures

In order to obtain further information about the thermal and optical behaviour of the liquid crystal of mixtures II and III, the thermal and optical properties of the mixtures were studied by means of a DTA instrument equipped with a laser. The results obtained are shown in Fig. 3(a)–(c), together with those of HPC (Fig. 3(d)) and DNA (Fig. 3(e)) solutions. As seen in Fig. 3(a), the DTA curve of mixture I exhibits two endothermic peaks at 317 and 348 K. It is suggested that the first endothermic peak at 317 K corresponds to the precipitation temperature accompanying the phase separation of HPC; this is called the lower critical solution temperature. Also, the peak at 317 K seems to relate to the enthalpy

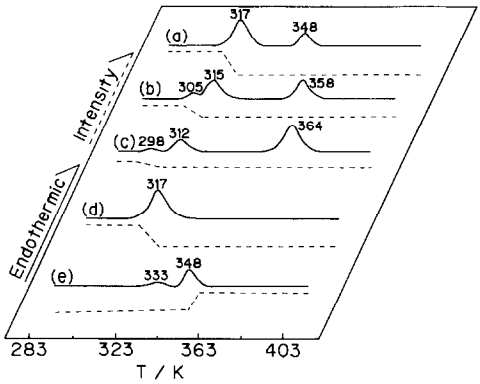

Fig. 3. Typical DTA (solid line) and laser transmittance (dotted line) curves for (a) mixture I, (b) mixture II and (c) mixture III; (d) aqueous HPC solutions (15.0 wt.%); and (e) aqueous DNA solutions (1.00–2.00 wt.%).

change accompanying the phase separation of free HPC. The intensity of the laser transmittance at the beginning of the thermal change associated with the first endothermic peak decreases with an increase in temperature, which is probably a result of the turbidity accompanying the phase separation of free HPC.

With increasing temperature, the DTA peak at 348 K appears, corresponding to the helix-coil transition of DNA solution as shown in Fig. 3(e). However, an endothermic peak at 348 K seems to correspond to the total enthalpy change of the helix-coil transition and the fusion of the liquid crystal phase formed by the interaction of DNA with HPC, although the intensity of the laser transmittance corresponding to the beginning of the thermal event at 348 K does not change, indicating that the transmitted laser light is strongly shielded owing to the turbidity associated with the phase separation of HPC, despite the fact that a change in the intensity of the laser transmittance at 348 K does exist in practice.

Furthermore, it is seen from Fig. 3(a)–(c) that the first endothermic peak for the mixtures at 317 K becomes smaller than that of free HPC, as shown in Fig. 3(d), demonstrating that a decrease in the areas of the first endothermic peaks for the mixtures seems to correspond to an increase in the interaction of DNA with HPC.

The DTA curves for mixtures II and III are shown in Fig. 3(b) and 3(c), respectively: they display complicated thermograms with a shoulder at 305 K for the first endothermic peak. The general trend for mixture III is similar to that for mixture II, although the peak temperature for the first endothermic peak with a shoulder for mixture III shifts to a lower temperature than that for mixture II. The magnitude of the area of the first endothermic peak of mixture III is smaller than that for mixture II. The thermogram of mixture III shows an independent peak at 298 K separated

from the first endothermic peak at 312 K; the thermogram of mixture II has a shoulder at 305 K on the first endothermic peak at 315 K. The results obtained above indicate that the thermal behaviour of the phase state of mixture III differs clearly from that of mixture II, demonstrating that the liquid crystal phase formed by the interaction of DNA with HPC increases in concentration with increasing DNA concentration. The thermal behaviour of the stable liquid crystal of mixture III and of the quasi-liquid crystal of mixture II is in good agreement with the results obtained with the polarization microscope and refractometer, as mentioned above.

The intensity of the laser transmittance decreases with increasing DNA concentration, which corresponds to a decrease in the precipitation of free HPC owing to an increase in the interaction of DNA with HPC.

With further increasing temperature, the DTA curves of mixtures II and III show an endothermic peak at higher temperature corresponding to the helix–coil transition of DNA, as seen in mixture I, and the endothermic peak shifts to higher temperature, demonstrating that the liquid crystal phase forms gradually with increasing DNA concentration.

Estimation of the enthalpy change accompanying the fusion of liquid crystal phase formed by interaction between DNA and HPC

Assuming that the area of each DTA curve at lower temperature corresponds to the enthalpy change accompanying the phase transition with increasing temperature, the observed heat ΔH_{obs} can be determined for each mixture, using the standard samples as described in the experimental section. In addition, ΔH_{obs} was estimated from the total area under the complicated thermograms with shoulders, because of the difficulty of separating the curve from the shoulder for mixtures II and III, as shown in Fig. 3(b) and 3(c). The results obtained are shown in Fig. 4(a), where ΔH_{obs} is plotted against the concentration of DNA. The dotted line in Fig. 4(a) represents an imaginary heat of precipitation ΔH_{ppt} of HPC, assuming that all the HPC participates perfectly in the phase separation with increasing temperature. As seen in Fig. 4(a), ΔH_{obs} decreases, approaching a definite value and then reaches a definite value again with an increase in concentration of DNA, demonstrating that the interaction between DNA and HPC increases with increasing DNA concentration.

Therefore, the liquid crystal phase is formed by the interaction between DNA and HPC with increasing DNA concentration. Figure 4(b) shows the difference, ΔI, in the intensity of the transmitted light before and after the laser change. As shown in Fig. 4(b), the behaviour of ΔI is in good agreement with that of ΔH_{obs} as shown in Fig. 4(a), suggesting that the thermal and optical properties seem to reflect the molecular conformational change of the DNA–HPC mixture accurately.

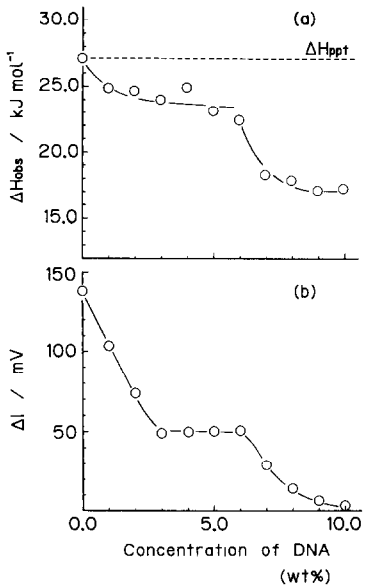

Fig. 4. (a) The observed heat ΔH_{obs} at lower temperatures and (b) the difference ΔI in the intensity of the transmitted light before and after the laser change, plotted against DNA concentration (mol indicates mole of HPC).

The degree of formation α of liquid crystal based on the interaction of DNA with HPC can be estimated from the behaviour of ΔI. Assuming that ΔI_0 is the transmittance at zero DNA concentration in Fig. 4(b) and that it reaches 100% in an isotropic phase, the ratio of ΔI to ΔI_0 for each DNA concentration corresponds to the degree of formation α of liquid crystal phase formed by the interaction of DNA with HPC. The α values estimated in this way are listed in Table 1 and shown in Fig. 5, where α is plotted against the DNA concentration. As seen in Fig. 5, α for mixture II increases considerably and reaches a definite value with increasing DNA concentration, demonstrating that the quasi-liquid crystal phase is formed in this region. With further increasing DNA concentration, α for mixture III increases and then reaches a definite value, suggesting that the liquid crystal phase forms.

The enthalpy change $\Delta H_{obs'}$, estimated from the area of the endothermic peak in the DTA curve at higher temperatures, is shown in Fig. 6, where $\Delta H_{obs'}$ is plotted against DNA concentration. As seen in Fig. 6, $\Delta H_{obs'}$ of mixture II increases gradually and that of mixture III also increases, approaching a constant value.

From the results obtained above, the enthalpy change accompanying the fusion of liquid crystal formed by the interaction between DNA and HPC can be determined as follows: $\Delta H_{obs'}$ is the sum of the heat of helix-coil transition ΔH_t of DNA and the heat of fusion $\alpha \Delta H_F$ of liquid crystal

TABLE 1

The observed heat ΔH_{obs} estimated from the peak area of the DTA curves at higher temperatures, and the heat of fusion ΔH_F between DNA and HPC, estimated by taking into consideration the degree of formation α

Conc. of DNA (wt.%)	ΔH_{obs} (kJ mol^{-1}) [a]	α	ΔH_F (kJ mol^{-1}) [a]	ΔH_t [b] (kJ mol^{-1})
1.00	12.9	0.25	0.0	12.8
2.00	12.8	0.46	0.0	
3.00	12.8	0.64	0.0	
4.00	13.0	0.62	0.3	
5.00	13.2	0.62	0.6	
6.00	13.8	0.62	1.6	
7.00	14.2	0.71	1.9	
8.00	15.0	0.90	2.4	average
9.00	15.2	0.96	2.5	2.4
10.0	15.5	0.97	2.8	

[a] Here mol indicates mole of nucleotide.

[b] ΔH_t is the average value of the heat of the helix-coil transition for DNA solution in the concentration range 1.00–2.00 wt.%.

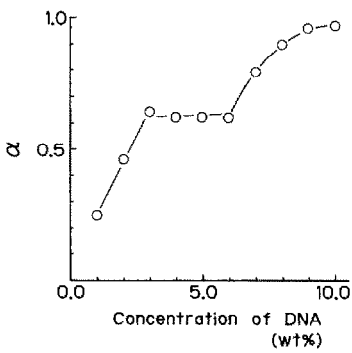

Fig. 5. The degree of formation α of liquid crystal phase formed by the interaction of DNA and HPC, plotted against DNA concentration.

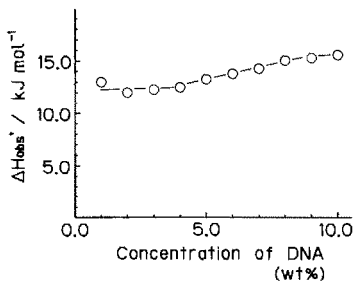

Fig. 6. The observed heat $\Delta H_{obs'}$ at higher temperatures plotted against DNA concentration (mol indicates mole of nucleotide).

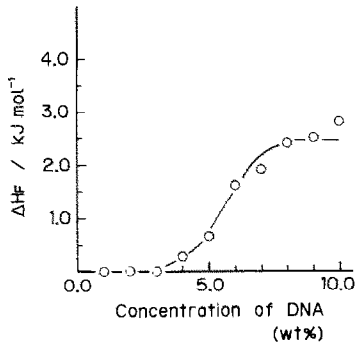

Fig. 7. The heat of fusion ΔH_F of the liquid crystal phase formed by the interaction of DNA and HPC, plotted against DNA concentration (mol indicates mole of nucleotide).

formed by the interaction of DNA with HPC, i.e. $\Delta H_{obs'} = \Delta H_t + \alpha \Delta H_F$. Using the α values listed in Table 1, the net heat of fusion ΔH_F for the liquid crystal phase can be determined from $(\Delta H_{obs'} - \Delta H_t)/\alpha$. The ΔH_F values calculated for each DNA concentration are listed in last column of Table 1 and are shown in Fig. 7, where ΔH_F is plotted versus DNA concentration. As seen in Fig. 7, ΔH_F is zero at DNA concentrations below 3.00 wt.%. However, ΔH_F increases gradually from DNA concentrations above 4.00 wt.% and reaches a constant value with increasing DNA concentration. It is suggested that this constant value corresponds to the net heat of fusion of the liquid crystal phase formed by the interaction between DNA and HPC and is estimated to be about 2.4 kJ per mol of nucleotide, the average value of ΔH_F at DNA concentrations above 7.00 wt.% listed in last column of Table 1. The net $\Delta H_F = 2.4$ kJ obtained in the present work seems to be comparable with that of the cholesteric liquid crystal phase of DNA obtained previously [3]. However, this is very difficult to determine because, at present, it is not known whether or not the conformation of the liquid crystal phase formed by the interaction of DNA with HPC forms a cholesteric liquid crystal similar to that of DNA in concentrated solutions.

In order to describe exactly the binding mode and structure of the liquid crystal formed by the interaction between DNA and HPC, the contribution to the cooperative interaction of the base of DNA with the side chain of HPC, and the conformational change of DNA caused by the hydrophobic effect of HPC in aqueous solutions, must be solved.

Further study of these various problems associated with the formation of liquid crystals of DNA with HPC is in progress.

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