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DNA-based polymer hybrids
Part 1. Compatibility and physical properties of poly(vinyl alcohol)/DNA sodium salt blend

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Abstract

Transparent blend films of poly(vinyl alcohol) (PVA) and deoxyribonucleic acid (DNA) sodium salt from salmon testes were prepared by the solvent cast method from a homogeneous aqueous solution; as a new class of biopolymer-based hybrid materials. Differential scanning calorimetric (DSC), dynamic mechanical, thermogravimetric, and scanning electron microscopic analyses indicated that PVA and DNA are compatible in a wide range of compositions. As the DNA content increases, a melting peak of PVA reduces in intensity with a lower temperature shift, appearing at 203°C in the PVA/DNA (50 wt%) blend by DSC. Physical properties of the blend films were evaluated by tensile strength and contact angle measurements. The tensile strength values of PVA/DNA (10 wt%) and PVA/DNA (30 wt%) blend films were 56 and 48 MPa, respectively. The surface free energy of PVA/DNA (30 wt%) blend film was 46 dyn/cm, which is identical to that of PVA, while the pure DNA film was revealed to show hydrophobicity (surface free energy 32 dyn/cm; water contact angle 104°). © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Polymer blends; Poly(vinyl alcohol); Deoxyribonucleic acid (DNA)

1. Introduction

Biopolymers including polysaccharides, polypeptides, and nucleic acids are renewable resources with molecular information and diverse functions. Blends of synthetic polymers and biopolymers are attractive and important, because the hybrids are potentially applicable as biomedical, biochemical, and biodegradable materials. During the past two decades, some blends of commodity polymers with polysaccarides [1–9] or polypeptides [10–13] have been investigated. We have already reported the miscibility of the blends of commodity polymers with a chitin derivative [3] and with biodegradable polypeptides, i.e. poly(vinyl chloride) (PVC)/chitin-*graft*-poly(2-methyl-2-oxazoline) [4,5], poly(vinyl alcohol) (PVA)/chitin-*graft*-poly(2-alkyl-2-oxazoline) [6–8], and PVA/poly(sodium α,β-D,L-aspartate) blends [10].

Besides being a molecular repository of genetic information, deoxyribonucleic acid (DNA) is regarded as a molecular material that shows a double helical structure with π-electron rich base-pair stacking. It has been reported that fast electron transfer between two intercalated metal complexes can be mediated by DNA as an electronic molecular wire [14]. From an ecological standpoint of global environmental preservation, nucleic acids are biodegradable polymers and can be supplied at relatively low cost. Although the DNA–alginate complex [15], conjugation of DNA with vinyl polymers having an intercalator [16], and the conformational study of DNA in a polymer matrix [17,18] have been published, to the best of our knowledge no blend study has been reported concerning systems composed of a synthetic polymer and a nucleic acid. In a wide sense, some synthetic polymer/DNA/water systems have been investigated [19,20].

In the present paper, we describe the compatibility of blends of PVA and DNA sodium salt, investigated by differential scanning calorimetry (DSC), dynamic mechanical analysis, and scanning electron microscopy (SEM). In this work, the term "compatibility" does not refer to miscibility on a molecular scale, but to a micro-separated structure with improved properties. The characterization of the blend films was carried out by contact angle, tensile strength, and thermogravimetric (TG) measurements. The intercalation behavior of ethidium bromide and the circular dichroism of the blend films are also discussed. PVA is well known

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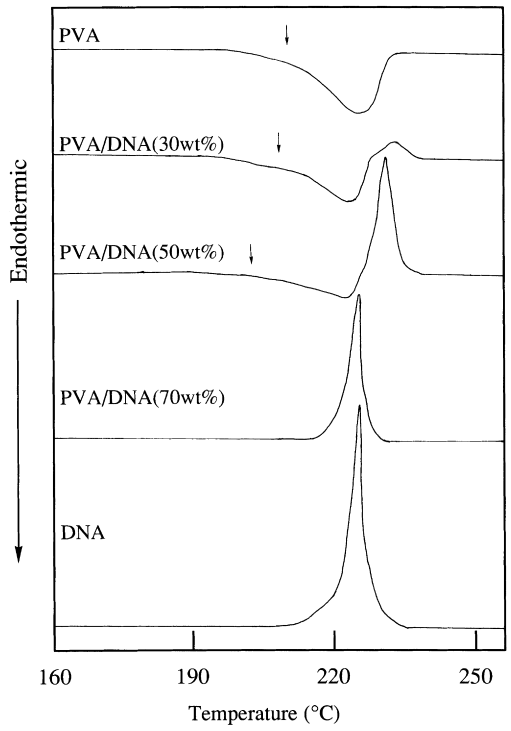

Fig. 1. DSC curves of PVA/DNA blend films. Heating rate: 5°C/min. The arrow marks the position of T_m.

as a biologically friendly commodity polymer, although its biodegradation rate is not high. Pairing PVA and DNA has a purpose in that it is a hybridized material of an industrially supplied biodegradable synthetic polymer and a pure natural polymer. Investigation of the phase behavior of this blend system and some physical properties will provide useful information to design new functional hybrid materials composed of DNA and synthetic polymers.

2. Experimental section

2.1. Materials

Deoxyribonucleic acid (DNA) sodium salt from salmon testes was purchased from Sigma Chemical Co, St. Louis, and used without further purification. The molecular size of DNA was estimated to be 10.7 kbp by electrophoresis on an agarose gel. Commercial poly(vinyl alcohol) (PVA, Nacalai Tesque, Inc, Kyoto, $M_n = 88,000$, saponification value 99–100%) was employed for the preparation of the films. The M_w/M_n value of PVA was 1.97 by size exclusion chromatography calibrated with poly(ethylene oxide) standards in 0.05 M K_2HPO_4 (aq.) at 27°C. The syndio-, iso-, and heterotacticities of PVA were 30, 19, and 51%, respectively, by ^{1}H NMR in dimethyl sulfoxide-d_6 [21]. Ethidium bromide was obtained from Wako Pure Chemical Co, Osaka. Water used for the preparation of films by the solvent cast method was purified by distillation.

2.2. Instrument and measurements

The percentage transmittance in blend films (thickness: 20 μm) was measured, at a wavelength of 500 nm, with a JASCO Ubest-30 UV–vis spectrophotometer. ^{1}H NMR spectra were taken with a Bruker ARX 400 spectrometer operating at 400 MHz. DSC measurements of the dried films (9–13 mg) were carried out with a Perkin–Elmer DSC-2 and a Seiko DSC-100 (calibration: indium and tin, heating rate: 5°C/min). In order to provide the same thermal history for all samples, the thermal data for the blends were obtained after one heating cycle to 110°C followed by quenching to 40°C in the DSC. The melting temperature (T_m) and the decomposition temperature (T_d) were measured at the onset and the maximum of the peaks of DSC traces, respectively. The glass transition temperature (T_g) was taken as the inflection point of the specific heat increment. The dynamic mechanical measurements were conducted at a frequency of 10 Hz with a Seiko DMS 210 visco-elastometer. The temperature was raised at a rate of 2.0°C/min in the range from −140 to 200°C. The samples (ca. $40 \times 10 \times 0.04$ mm^3) were dried at 50°C for 3 days under reduced pressure prior to the measurement. A Seiko TG/DTA 6200 was used for the thermogravimetric (TG) measurement. The measurement of the films (ca. 5 mg) was carried out from room temperature to 800°C at a heating rate of 10°C/min under a nitrogen atmosphere flowing at 100 cm^3/min. The fractured surfaces of the films were observed by a Hitachi S-4500 scanning electron microscope (SEM). To prepare the SEM samples, the blend films were embedded in epoxy resin (Ouken Co., Ltd. EPON 812) at 35–60°C for 5 days. The embedded blends were cut and sputter-coated with platinum–palladium (Pt–Pd (8:2)). The tensile strength of the blend film ($40 \times 5 \times 0.04$ mm^3) was measured with a Shinkoh TCM-500 testing machine with extension rate of 20 mm/min at room temperature (relative humidity: 50%). The tensile strength values reported herein are the means of 5–9 samples. The contact angle measurements were carried out at room temperature with a CAD type goniometer (Kyowa Co. CA-DT). The contact angles of each film were measured in at least 15 places and the values were averaged. The intercalation behavior of ethidium bromide in PVA/DNA blend films was investigated by an electronic UV transilluminator (FAS II, Toyobo, Co.). The dye-intercalated films (thickness: 40 μm) were prepared by immersion in ethidium bromide ethanol solution (25 μM) for 2 days. The circular dichroism (CD) spectra of PVA/DNA cast films (thickness: 2–3 μm) on a quartz plate were recorded by a JASCO J-720 spectrometer at room temperature (relative humidity: 44%). The CD data are presented as the intensity per mole of a nucleotide unit.

2.3. Preparation of blend films by the solvent cast method

A typical example is as follows. PVA (50 mg) was

Table 1
Thermal properties of PVA/DNA blend films (prepared by the solvent cast method from an aqueous solution) by DSC

	T_g[a] (°C)	T_m[b] (°C)	T_d[c] (°C)
PVA	68	212	–[d]
PVA/DNA (30 wt%)	68	209	234
PVA/DNA (50 wt%)	–[d]	203	231
PVA/DNA (70 wt%)	–[d]	–[d]	226
DNA	–[d]	–[d]	226

[a] Glass transition temperature.
[b] Melting temperature.
[c] Decomposition temperature.
[d] Not detected.

dissolved in 10 ml of water in an autoclave at 120°C. After cooling at room temperature, 50 mg of DNA was added to the solution. The mixture was stirred at 27°C for 24 h. The solution was placed on a Teflon laboratory dish (50 mm diameter) at 50°C to remove the solvent. After drying further in vacuo at 50°C for 3 days, removal of water was confirmed by the TG measurement. While thickness values of films for testing transparency and for CD measurement were 20 and 2–3 μm, respectively, that of all other films examined was 40 μm. The films were stored over P_2O_5 in a desiccator.

3. Results and discussion

3.1. Phase behavior of PVA and deoxyribonucleic acid sodium salt

The binary blend films of PVA and deoxyribonucleic acid (DNA) sodium salt, obtained by the solvent cast method from an aqueous solution, were optically clear regardless of the blend composition. Transmittance values at 500 nm of PVA/DNA blend films with 10–90 wt% DNA content were 81–88%, while those of the single component films of PVA and DNA were 93 and 81%, respectively. This suggested that no phase separation occurred at any higher level above micron size.

The DSC measurement was carried out for PVA/DNA blend samples (Fig. 1). The pure PVA showed a melting endotherm peak (T_m) at 212°C, and pure DNA gave a relatively sharp decomposition exothermic peak (T_d) at 226°C. As the DNA content increases up to 50 wt%, the melting peak of PVA reduces in intensity with a lower temperature shift, appearing at 203°C in the PVA/DNA (50 wt%) blend. For polymer blends in which one of the blend components is a semicrystalline polymer, a depression in the melting point indicates that the two components interact to some extent [22]. Additionally, T_d of DNA shifted towards higher temperature with increasing PVA content, which indicated that DNA molecules were stabilized by PVA. Nishioka et al. [23] have correlated the difference in thermal stability of blends containing cellulose with that in compatibility. In a physical mixture of PVA powder and DNA fiber (50/50, wt/wt), the T_m of PVA and T_d of DNA were overlapped, because T_m of PVA and T_d of DNA did not shift. This result seems to reflect the compatibility of PVA/DNA blends.

The thermal properties of the PVA/DNA blends as characterized by DSC are listed in Table 1. One of the most commonly used methods to investigate blend miscibility has been the determination of T_g of the blend compared with the T_gs of the components in the blend. T_g of PVA was observed at 68°C and that of DNA was not observed in the range of 40–200°C. Denaturation of DNA was not detected in the first run of the DSC scan. The T_g value of the PVA/DNA (30 wt%) blend was 68°C, indicating microphase separation, although the PVA/DNA (30 wt%) blend film was transparent. However, the T_g values of PVA films

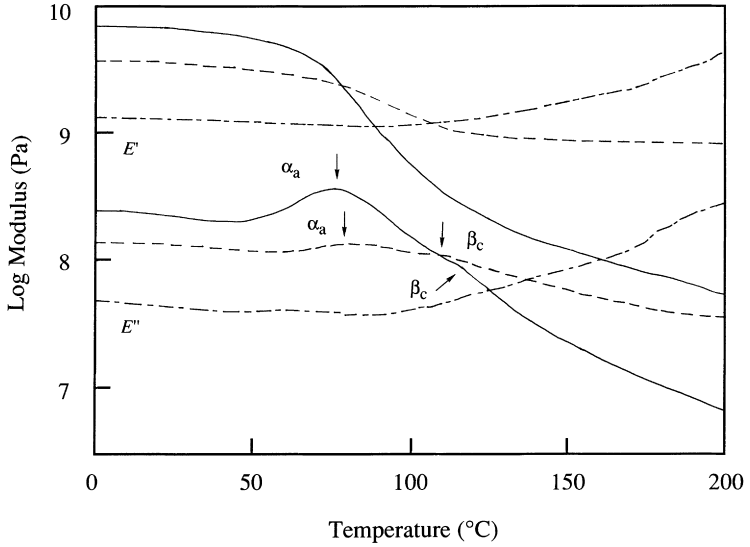

Fig. 2. Temperature dependence of the dynamic storage modulus (E') and loss modulus (E'') for sample films: (—) PVA; (- - -) PVA/DNA (50 wt%) blend; (- · -) DNA.

Table 2
Dynamic mechanical characterization of PVA/DNA blend films (prepared by the solvent cast method from an aqueous solution on a Teflon plate with a film thickness of 40 μm)

	Maxima of α_a dispersion (°C)		Maxima of β_c dispersion (°C)	
	E''	tan δ	E''	tan δ
PVA	77	86	115	116
PVA/DNA (10 wt%)	75	88	111	116
PVA/DNA (30 wt%)	79	86	110	113
PVA/DNA (50 wt%)	79	87	110	113
PVA/DNA (70 wt%)	80	88	106	108
PVA/DNA (90 wt%)	–[a]	–[a]	–[a]	–[a]
DNA	–[a]	–[a]	–[a]	–[a]

[a] Not detected.

with higher DNA content (>30 wt%) could not be detected because DNA did not show a well-defined clear glass transition.

In many miscibility studies on polymer blends, dynamic mechanical analysis (DMA) has proven to be more sensitive than calorimetric measurements for the detection of T_g. Accordingly, we measured the dynamic mechanical relaxation spectra of the blends, hoping to find the transition of the blends with more than 30 wt% DNA. The results of the dynamic mechanical measurements for the water-cast PVA, PVA/DNA(50 wt%), and pure DNA films are given in Fig. 2. The figure shows the temperature dependence of dynamic storage modulus (E') and loss modulus (E''). The dynamic mechanical properties of PVA have been previously investigated in detail mainly on a water-cast film by Nagai and Takayanagi [24]. Each dispersion in the DMA curves could be assigned on the basis of their report. In the PVA sample prepared in this work, two main mechanical dispersions appeared at 77 and 115°C in the E'' versus temperature curve (Fig. 2). The former is the primary dispersion α_a corresponding to the glass transition of PVA and the latter is β_c dispersion due to the crystalline relaxation of PVA. On the contrary, the DNA film had no mechanical dispersion under the present experimental conditions. Dynamic mechanical study on DNA films has not been published previously, to our knowledge. The α_a and β_c dispersion maxima in E'' and mechanical loss tangent (tan δ) of the PVA/DNA blends are summarized in Table 2. As the DNA content increases up to 70 wt%, β_c shoulders in tan δ and E'' shift to the lower temperature side. The β_c shift is ascribed to the decrease in PVA crystallinity [24]. However, the α_a peaks in tan δ and E'' were nearly constant within the experimental error. The dynamic mechanical characterization as well as DSC analysis suggested that PVA and DNA were compatible in a wide range of compositions.

Fig. 3a indicates the thermogravimetric (TG) curves of the PVA/DNA blends. Thermal degradation behavior of a compatible polymer blend is often quite different from that of the blend components [23]. Recently, Sato et al. have demonstrated that the temperature-programmed analytical pyrolysis techniques are effective to evaluate miscibility in the PVC/chitin derivative blend system [5]. The weight decrease of the pure PVA film started at 258°C followed by a gentle weight decrease taking place over the temperature range 421–474°C (see also Fig. 3b). The residual weight percent at 500°C was 5%. On the contrary, the decomposition of pure DNA film started at 230°C and the residual weight percent at 500°C was 53%. The start point of degradation of DNA in the PVA/DNA blends increased with the increase in the content of PVA. For example, the start temperature of degradation of the PVA/DNA (50 wt%) blend was 234°C. It is clear that the increase in PVA content makes the DNA in the blend thermally stable, which was consistent with the result of DSC analysis (*vide supra*). The stabilization of DNA was not observed in a physical mixture of PVA and DNA by the TG measurement. The difference in the thermal decomposition behavior of these samples can be seen more clearly from the derivative thermogravimetric (DTG) curves shown in Fig. 3b. The decomposition rate of DNA has two maxima at 233 and 300°C. The DTG curve of PVA shows a large peak at 284°C and a small peak at 436°C. For the given blends, the peak at a lower temperature of DNA shifted to higher temperature and the small peak of PVA increased in intensity as the content of PVA increased. It is considered that these characteristic differences in thermal decomposition behavior reflect the segmental interaction between PVA and DNA at the interface.

The phase morphologies of the fractured sections of the PVA/DNA blends were studied by scanning electron microscopy (SEM) at a voltage of 3 keV. The blends containing 50 and 70 wt% DNA had homogeneous and continuous phases on a micron scale, while an alignment of DNA component parallel to the surface was observed on a sub-micron scale in the PVA/DNA (50 wt%) blend as shown in Fig. 4b. On the contrary, the blend film with 30 wt% DNA showed macro-phase separation at the surface of the air side (Fig. 4a).

3.2. Characterization of PVA/DNA blend films

The surface at the air side of PVA/DNA blend films was

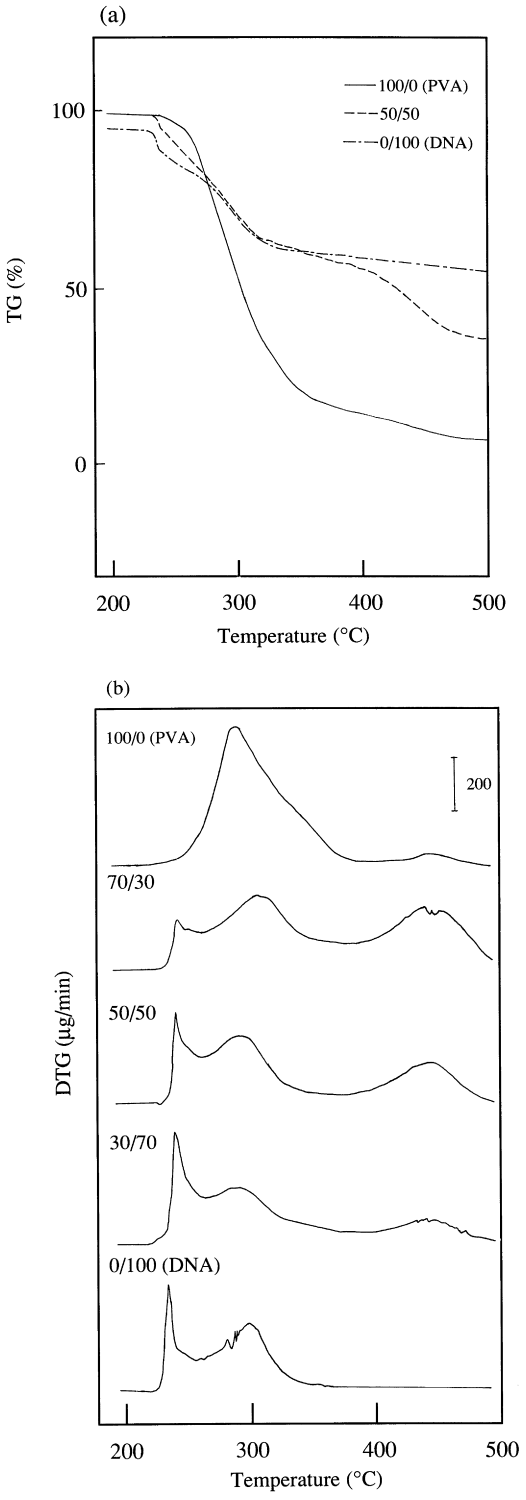

Fig. 3. Thermograms of PVA/DNA blend films: (a) thermogravimetric (TG) curves; (b) derivative thermogravimetric (DTG) curves.

characterized by measuring the contact angles of water and methylene iodide. We have reported the degradation acceleration of PVA in soil by blending with DNA [25]. The surface free energy influences the biodegradation behavior, because the adhesion of enzyme to the surface is important [26]. The surface free energy (γ_s) of the blend films, which is given as the sum of energies derived from hydrogen bonding (γ_s^h) and dispersion force (γ_s^d), was estimated from the observed contact angles by applying the Owens equation [27]. Interestingly, the surface of the pure DNA film indicated higher contact angles against water (104°) and methylene iodide (56°), and therefore a lower surface energy, compared to the PVA film (Table 3). The surface of the PVA/DNA (30 wt%) blend had almost the same surface property as that of PVA. The SEM and contact angle measurements indicated that the air side surface of the blend film was covered with the PVA component. The surface energy of the blend films decreased with increasing DNA content.

Table 3 also lists the tensile strength values of PVA/DNA blend films of different compositions. The tensile strength of PVA was 87 MPa, which was in agreement with the previously reported value (84 MPa) [28], and that of the DNA film was 13 MPa. Although the increase in the tensile strength was not found for the PVA/DNA blends without drawing, the tensile strength of these blends with lower DNA content seems to be enough for practical applications. For example, the tensile strength values of PVA/DNA (10 wt%) and PVA/DNA (30 wt %) were 56 and 48 MPa, respectively. It is considered that this is caused by the intermolecular interaction between DNA with PVA.

3.3. Conformation of DNA strands in the blend

The intercalation behavior of ethidium bromide into the PVA/DNA blends was investigated by immersing the films into an ethidium bromide ethanol solution. The stained films were excited by UV irradiation at 300 nm. The light emission was detected in PVA/DNA (30 wt%) and PVA/DNA (50 wt%) blend films, while not in a PVA film. The emission intensity of the blend films increased with the increase in the content of DNA. This result suggests that DNA forms a double-stranded conformation, which allows intercalation of ethidium bromide, even in a PVA matrix.

Fig. 5 shows the CD spectra of PVA/DNA blend films. The PVA/DNA (70 wt%) blend film gives a positive Cotton effect at 283 nm and a negative Cotton effect at 232 nm similar to native DNA in an aqueous solution, which suggests that DNA in the blend takes the B-form structure [29,30]. The positive Cotton effect shifted to a longer wavelength and the intensity decreased gradually with increasing PVA content. Similar changes in the CD spectra are observed in DNA aqueous solutions containing ethanol [31,32], ethylene glycol [33], and lithium chloride [34]; and in a DNA–lipid complex [35]. The spectral change has been ascribed to the tilting of base pair with accompanying conformational change of DNA from the B-form of the double-stranded DNA, in which water interact with oxygen of ribose and phosphate, to the tightest C-type conformation of parallel DNA threads. It is supposed that the conformation of DNA in a matrix of PVA also was altered from

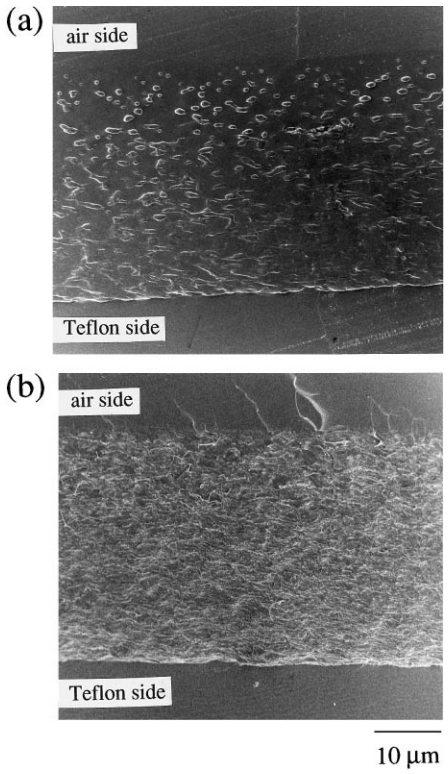

Fig. 4. SEMs of fractured surfaces of PVA/DNA blend films: (a) PVA/DNA (30 wt%); (b) PVA/DNA (50 wt%).

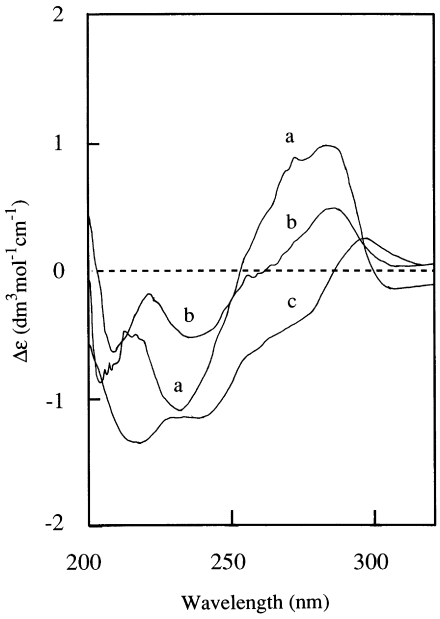

Fig. 5. CD spectra of PVA/DNA blend films (temperature, room temperature; relative humidity, 44%; film thickness, 2–3 μm): (a) PVA/DNA (70 wt%); (b) PVA/DNA (50 wt%); (c) PVA/DNA (30 wt%).

the B-type conformation to C-type conformation. Not only ethanol but also PVA, which is regarded as a polymer homolog of ethanol, seems to affect the conformational change of DNA as a "polymeric solvent".

This study revealed that PVA and DNA were compatible in a wide range of compositions to form a new class of biopolymer hybrids [7,10]. The surface free energy of the PVA film decreased by blending with DNA. It is suggested that DNA formed double helical structures even in the PVA/DNA blend films, in which the conformation of DNA changed depending on the PVA content. The control of the surface property and the alignment of DNA fiber in DNA-based polymer hybrids would be important for their applications as various functional materials including biomedical materials, biodegradable materials, and electronic devices [14,25].

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Table 3
Surface characteristics and tensile strength of PVA/DNA blend films (prepared by the solvent cast method from an aqueous solution on a Teflon plate with a film thickness of 40 μm)

	Contact angle[a] (°)		Surface free energy[b] (dyn/cm)			Tensile strength (MPa)
	Water	CH_2I_2	γ_s^d	γ_s^h	γ_s	
PVA	66	39	35	11	46	87
PVA/DNA (30 wt%)	64	41	33	13	46	48
PVA/DNA (50 wt%)	70	42	33	10	43	29
PVA/DNA (70 wt%)	96	48	32	4	36	25
DNA	104	56	32	0	32	13

[a] The surface of the air side, measured at 20°C.
[b] Calculated by the Owens equation, according to Ref. [27].

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