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Design of successive ion conduction paths in DNA films with ionic liquids

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Ion conductive DNA films were prepared by two methods. A DNA with salt containing PEO mixture gave a flexible film, and that showed ionic conductivity of about 10^{-6} S cm^{-1} at room temperature. The second method gave successive ion conduction paths in DNA films with ionic liquids. When cytosine and adenine, as low molecular weight model compounds, were neutralized with acids, their melting points (T_m) were considerably decreased. These results encouraged us to prepare ionic liquid domains in DNA by the neutralization of the bases. However, the neutralization of DNA with fluoroboric acid gave a powder and it showed a low ionic conductivity. Although all adenine and cytosine moieties, about 50% of total bases in the DNA, were expected to yield ionic liquids, this was not sufficient to form a continuous ion conductive domain in the film. When ethylimidazolium tetrafluoroborate (EtImBF$_4$) was added to the DNA neutralized with HBF$_4$, a film was obtained even after adding 93 wt% of EtImBF$_4$ and, correlatively, very high ionic conductivity of 5.05×10^{-3} S cm^{-1} was observed at 50 °C.

Introduction

DNA, a very well known biopolymer, can be used as a polyelectrolyte for fabricating new and attractive materials mixed with other components. DNA is classified as a rigid-rod polymer having heteroaromatic rings and continuously stacked base pairs. Utilizing these characteristics, DNA has been receiving keen interest as a functional material for electronics, optics, *etc.* Especially, it is known that conductive materials can be prepared using $\pi-\pi$ stacking of base pairs. In 1993, Barton *et al.* measured DNA-mediated electron transfer from a photoexcited Ru complex,[1] and many scientists since have investigated such electron transfer. There have been many discussions on electron conductivity through DNA, but no concrete conclusion has yet been proposed.[2–6] As well as in classical applications such as biotechnology, it should also be possible for DNA to be applied in nonaqueous systems. Okahata *et al.* have reported the preparation of hydrophobic DNA soluble in organic media.[7] Also, DNA has been used as a component in the preparation of films.[8,9]

On the other hand, in the field of polymer electrolytes, most research has been based on poly(ethylene oxide) (PEO) salt mixtures.[10] PEO is known to dissociate salts into ions and stabilize them with a relatively large dipole moment on the ether oxygens. Ions can migrate rapidly in a PEO matrix because of its very low glass transition temperature. However, the PEO/salt mixture is a wax-like solid, and cannot be obtained as a film at room temperature. We proposed using a composite of DNA and PEO, and this was revealed to have quite excellent ionic conductivity.[11] Suitable mixing ratios of DNA and PEO can lead to highly ion conductive DNA film. When 30 mol% $NaClO_4$ was added to the DNA/PEO composite film, an ionic conductivity of 6.43×10^{-5} S cm^{-1} was observed at 50 °C. DNA was shown to be an excellent component material to prepare ion conductive films.

In recent years, molten salts at ambient temperature, so-called ionic liquids, have been proposed as novel ion conductive materials.[12–18] Ionic liquids are composed only of ions and exist in liquid form over a wide temperature range with no vapor pressure. Since they can provide less viscous liquids without any solvent, they can be used in the same way as common organic solvents.[19–21] Recently, highly ion conductive materials have been prepared from heteroaromatic rings such as imidazole, pyridine and so on by neutralization with acids.[21] We thus expected high affinity between DNA and ionic liquids, because DNA has many heteroaromatic rings as bases. Accordingly, we prepared DNA films containing ionic liquid. They were revealed to show high ionic conductivity.[22]

In this paper, a few methods are compared for the preparation of DNA containing flexible films having excellent ionic conductivity.

Experimental section

Materials

DNA sodium salt isolated from salmon milt was a gift from Daiwa Kasei Co. The average molar mass was about 60 000 to 300 000. For DNA/PEO/salt mixture, 10 mg DNA was dissolved in 1 ml pure water, and PEO (molar mass 1000) was added to this aqueous DNA solution. Then, $NaClO_4$ was added and the molar ratio of Na^+ to ether oxygen unit was varied from 15 to 45 mol%.

Adenine (**A**), cytosine (**C**), guanine (**G**) and thymine (**T**) were purchased from Sigma Chemical Co. (**A** and **T**), Tokyo Kasei Kogyo Co. Ltd. (**C**) and Aldrich Chemical Company Inc. (**G**), respectively. Lithium bis(trifloromethanesulfonyl)imide (LiTFSI) was a gift from Sumitomo 3M corporation. Bis(trifloromethanesulfonyl)imide (HTFSI) was obtained from LiTFSI by cation exchange with Amberlite IR-120B H AG.[23] All other reagents were purchased from Wako Pure Chemical Industries Ltd., and used as received unless otherwise stated.

A·BF$_4$. Adenine neutralized with tetrafluoroboric acid: Adenine 0.20 g (1.48×10^{-3} mol) was dissolved in 50 ml pure water. An equimolar amount of HBF$_4$ (0.3 ml) was added dropwise to the solution, and the mixture was stirred at 0 °C for

24 h. The solution was evaporated to remove water. After washing twice with methanol, the sample was dried *in vacuo* at 60 °C for 4 days. The composition was confirmed by ^{1}H-NMR (D_2O, 500 MHz) δ 8.37(s,1H), δ 8.43(s,1H).

C·BF_4. Cytosine 0.20 g (1.8×10^{-3} mol) was dissolved in 50 ml pure water. An equimolar amount of HBF_4 (0.3 ml) was added dropwise to the solution, and the mixture was stirred at 0 °C for 24 h. The mixture was evaporated to dryness. After washing twice with methanol, the sample was dried *in vacuo* at 60 °C for 4 days. The structure of this sample was confirmed by ^{1}H-NMR(D_2O, 500 MHz) δ 5.87(d,1H, J_{12} = 9 Hz), δ 7.71(d,1H, J_{12} = 7.5 Hz), δ 11.95(s,1H).

A·TFSI. Adenine 0.20 g (1.48×10^{-3} mol) was dissolved in 50 ml pure water. An equimolar amount of HTFSI (0.41 g) was added dropwise to the solution, and the mixture was stirred at 0 °C for 24 h. The solution was evaporated to dryness. After washing twice with methanol, the sample was dried *in vacuo* at 60 °C for 4 days. The structure was confirmed by ^{1}H-NMR (D_2O, 500 MHz) δ 8.37(s,1H), δ 8.43(s,1H).

C·TFSI. Cytosine 0.20 g (1.8×10^{-3} mol) was dissolved in 50 ml pure water. An equimolar amount of HTFSI (0.5 g) was added dropwise to the solution, and the mixture was stirred at 0 °C for 24 h. The mixture was evaporated to dryness. After washing twice with methanol, the sample was dried *in vacuo* at 60 °C for 4 days. The structure of this sample was confirmed by ^{1}H-NMR (D_2O, 500 MHz) δ 5.87(d,1H, J_{12} = 6.5 Hz), δ 7.72(d,1H, J_{12} = 7.5Hz), δ 11.94(s,1H).

C·CF_3SO_3. Cytosine 0.20 g (1.8×10^{-3} mol) was dissolved in 50 ml pure water. An equimolar amount of CF_3SO_3H (0.27 g) was added dropwise to the solution, and stirred at 0 °C for 24 h. The mixture was evaporated to dryness. After washing twice with methanol, the sample was dried *in vacuo* at 60 °C for 4 days. The structure was confirmed by ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 5.88(d,1H, J_{12}= 9 Hz), δ 7.73(d,1H, J_{12} = 7 Hz), δ 11.96(s,1H).

C·Br. Cytosine 0.20 g (1.8×10^{-3} mol) was dissolved in 50 ml pure water. An equimolar amount of HBr (0.77 ml) was added dropwise to the solution, and the mixture was stirred at 0 °C for 24 h. The mixture was evaporated to dryness. After washing twice with methanol, the sample was dried *in vacuo* at 60 °C for 4 days. The structure of this sample also was confirmed by ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 5.92(d,1H, J_{12}= 7.5 Hz), δ 7.74(d,1H, J_{12} = 6.5 Hz), δ 11.98(s, 1H).

C·ClO_4. Cytosine 0.20 g (1.8×10^{-3} mol) was dissolved in 50 ml pure water. An equimolar amount of $HClO_4$ (0.8 ml) was added dropwise to the solution, and the mixture was stirred at 0 °C for 24 h. The mixture was evaporated to dryness. After washing twice with methanol, the sample was dried *in vacuo* at 60 °C for 4 days. The structure of this sample also was confirmed by ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 5.87(d,1H, J_{12} = 8 Hz), δ 7.72(d,1H, J_{12} = 7.5 Hz), δ 11.95(s, 1H).

C·I. Cytosine 0.20 g (1.8×10^{-3} mol) was dissolved in 50 ml pure water. An equimolar amount of HI (1.1 ml) was added dropwise to the solution, and the mixture was stirred at 0 °C for 24 h. The mixture was evaporated to dryness. After washing twice with methanol, the sample was dried *in vacuo* at 60 °C for 4 days. The structure of this sample also was confirmed by ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 5.88(d,1H, J_{12} = 8 Hz), δ 7.73(d,1H, J_{12} = 7 Hz), δ 11.95(s,1H).

C·Cl. Cytosine 0.20 g (1.8×10^{-3} mol) was dissolved in 50 ml pure water. An equimolar amount of HCl (0.8 ml) was added dropwise to the solution, and the mixture was stirred at 0 °C for 24 h. The mixture was evaporated to dryness. After washing twice with methanol, the sample was dried *in vacuo* at 60 °C for 4 days. The structure of this sample also was confirmed by ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 5.95(d,1H, J_{12} = 7.5 Hz), δ 7.72(d,1H, J_{12} = 7 Hz), δ 12.02(s,1H).

DNA 1.00 g was dissolved in 50 ml pure water, and slowly stirred. Acids (HBF_4, HTFSI, CF_3SO_3H, HBr, $HClO_4$, HI or HCl), 50 mol% relative to the total amount of the bases, were added dropwise to the solution, and the solutions were stirred at 0 °C for 24 h. The samples were phase-separated and collected by filtration. After washing with pure water, they were dried *in vacuo* for 4 days.

DNA·BF_4 10 mg and 5 to 80 wt% C·TFSI in 0.5 ml were mixed in pure water to obtain an homogeneous mixture. All samples were stirred for 24 h at room temperature. The solutions were then cast on a stainless steel electrode for conductivity measurement and an aluminium pan for DSC measurement, and dried under air and then *in vacuo* for 4 days.

DNA·BF_4 10 mg was dissolved in 1 ml pure water, and 5 to 96 wt% $EtImBF_4$ was added to the solution. The same procedures as above were used to prepare samples for conductivity and thermal response measurements.

Methods

Ionic conductivity measurement

The cell was obtained by casting the sample solution on a stainless steel electrode, 1.0 × 1.5 cm covered with an 80 μm thick insulator Teflon® film having a hole (diameter of 6.0 mm), and dried at room temperature. The cell was completed by covering with another stainless steel electrode, and it was dried *in vacuo* for 4 days. Ionic conductivity was measured using a complex-impedance gain-phase analyzer (Solartron model 1260; Schlumberger) in the frequency range 10 Hz to 1M Hz. The conductivity was measured dynamically from 60 °C to 10 °C with a cooling rate of 2.5 °C min^{-1}.

Differential scanning calorimetry (DSC) measurement

Melting point and glass transition temperature were determined by DSC measurement (DSC-120; SEIKO Instrument Inc.). The sample was cast in an aluminium pan and dried sufficiently before DSC measurement. After holding at 100 °C, the sample was frozen to −150 °C and then heated from −150 to +300 °C (at a rate of 5 °C min^{-1}).[24,25]

Results and discussion

DNA/PEO mixed system

DNA has Na ions as counter cations of phosphate, and it shows a fairly low ionic conductivity of less than 1×10^{-9} S cm^{-1} in the absence of solvent (Fig. 1; ◊). However, DNA showed ten times higher ionic conductivity after mixing with poly(ethylene oxide) (PEO), molar mass 1000 with a mixing ratio [base pair (bp)]/[OE unit (–O–)] of 1/22 (Fig. 1; ⧫). PEO very probably improves the ability to dissociate phosphate and to solvate Na^+ ions because of the large dipole moment on the ether oxygens.[11,26] Under this mixing ratio of DNA to PEO, the molar ratio of Na^+ to oxyethylene (OE) was estimated to be 9 mol%. This is not so small for the ordinary PEO/salt mixture. However, not all of the Na^+ in the DNA complexed with PEO. The total amount of carrier ions was considered to be insufficient. When $NaClO_4$ 30 mol% to OE unit was mixed with this DNA/PEO_{1000} mixture, the ionic conductivity of the film attained 6.43×10^{-5} S cm^{-1} at 50 °C (Fig. 1; ●). Since DNA has Na^+ ions as counter ions. For most ionic devices, such as batteries, Li^+ is used as the carrier ion. But the analysis of the system containing both Na^+ and Li^+ is not so simple, because the migration manner of Li^+ is not the same as that of Na^+.

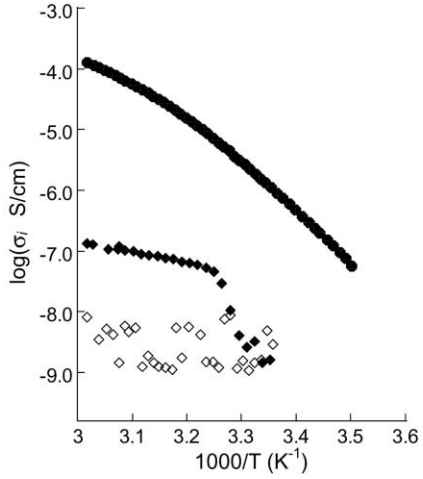

Fig. 1 Temperature dependence of the ionic conductivity of DNA (◊), DNA/PEO_{1000} ([bp]/[EO unit] = 1/22 (⧫), and $DNA/PEO_{1000}/NaClO_4$ ([bp]/[EO unit]/[$NaClO_4$] = 1/22/6.67) (●).

In the present study, we used Na^+ ions as carriers. For making a flexible film, PEO with molar mass of less than 600 was used. DNA was obtained as a flexible film when [bp]/[OE unit] was 1/4–1/10. Against this, for PEO with molar mass more than 1000, [bp]/[OE unit] = 1/11–1/22 was essential to obtain the film. The film became brittle with a lower PEO_{1000} content, and a wax-like solid was obtained by adding excess PEO_{1000}. For obtaining a higher ionic conductivity at various mixing ratios, the optimum PEO concentration was found to be 5 mol% ([bp]/[OE unit] = 1/20). For a flexible film, only a small amount of PEO with low molar mass could be added to the DNA. When low molar mass PEO was used, it therefore gave a higher cation concentration. The ionic conductivity remained low at this composition. Because of these limitations, PEO with molar mass 1000 was used in subsequent studies.

$NaClO_4$ was added to a DNA/PEO_{1000} mixture ([bp]/[OE unit] = 1/22) to analyze the effect of added salt concentrations on the ionic conductivity. The ionic conductivity increased with increasing salt concentration as shown in Fig. 2 (●). The highest ionic conductivity was seen at the salt concentration ratio of 30 mol% relative to OE units. The ionic conductivity of $PEO_{1000}/NaClO_4$ simple mixture is also shown for reference (Fig. 2; ▲). Although $PEO_{1000}/NaClO_4$ simple mixture showed high ionic conductivity, it never gave a film and always remained as a wax-like solid. Although the $DNA/PEO_{1000}/NaClO_4$ system gave a little lower ionic conductivity than $PEO_{1000}/NaClO_4$, this system could yield flexible films.

In order to determine the optimum conditions for both the formation of film and excellent ionic conductivity the mixing ratio DNA to PEO_{1000} was varied from 1/11 to 1/110 by [bp]/[OE unit], and different concentrations of $NaClO_4$ were added to the mixtures. Fig. 3 shows the ionic conductivity of each sample at 50 °C. All mixtures showed the highest ionic conductivity at salt concentrations around 20 mol% (relatively to the OE unit). Further addition of salt caused a lowering of the ionic conductivity, as for simple PEO/salt mixture.

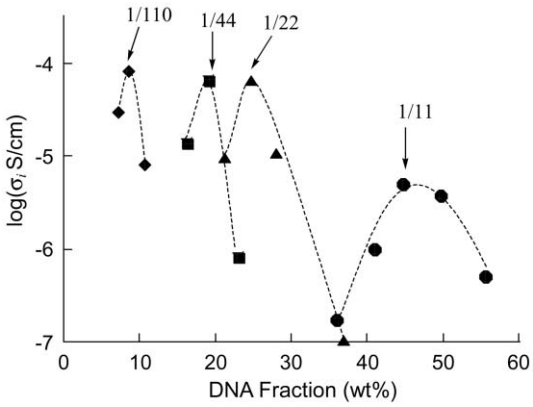

Fig. 3 Relationship between ionic conductivity of $DNA/PEO_{1000}/NaClO_4$ at 50 °C and their composition. Numbers at the peak of indicate [bp]/[EO unit]: ● 1/11; ▲ 1/22; ■ 1/44; ⧫ 1/110.

Since the segmental motion of PEO was decreased by the interaction with rigid DNA, ionic conductivity was considerably influenced by the DNA concentration. For example, addition of 10.7 to 37.5 wt% DNA increased the T_g of PEO from −60 to −52 °C. Through this experiment, the concentration of added $NaClO_4$ was kept constant at 5 mol% relative to the OE units. The ionic conductivity dropped abruptly when more than 35 wt% DNA was added (Fig. 4). This apparent threshold, 35 wt% DNA, corresponds to about [bp]/[OE unit] = 1/22. PEO could not allow fast ion migration because of restricted segmental motion when more than 35 wt% DNA was homogeneously mixed with PEO. On the other hand, flexible DNA/PEO_{1000} mixture film requires more than 38 wt% DNA. Accordingly, it is impossible to prepare flexible and highly ion conductive DNA film by mixing it with PEO_{1000}.

Preparation of ionic liquid from the bases

We had already prepared flexible DNA film with high ionic conductivity by mixing it with ionic liquid.[22] However, DNA/ionic liquid film had a drawback concerning long time stability. We therefore tried to improve the affinity of the ionic liquid for DNA by using the corresponding bases in the ionic liquid.

Before preparing ionic liquid from DNA, four kinds of bases were examined as models. There are two methods for preparing ionic liquids: (1) quaternization of the tertiary amines with

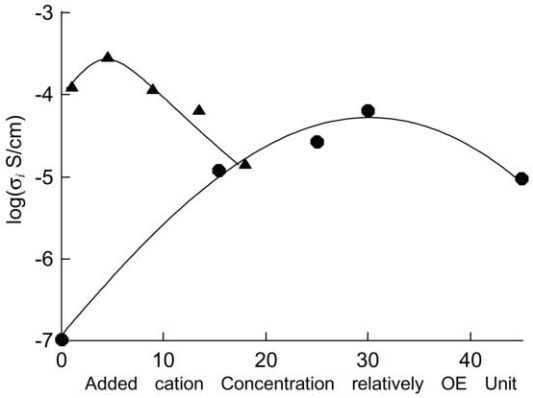

Fig. 2 Effect of cation concentration on the ionic conductivity for $PEO_{1000}/NaClO_4$(▲) and $DNA/PEO_{1000}/NaClO_4$(●) ([bp]/[EO unit] = 1/22) at 50 °C.

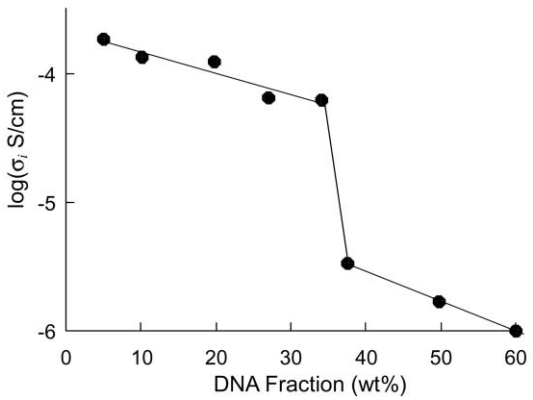

Fig. 4 Effect of DNA fraction of $DNA/PEO_{1000}/NaClO_4$ mixture on the ionic conductivity at 50 °C [$NaClO_4$]/[EO unit] = 1/20.

Table 1 T_m of **A**, **C**, **G** and **T** before and after reaction with different acids

	T_d (°C)	+HBF_4		+HTFSI	
		T_m (°C)	Appearance	T_m (°C)	Appearance
Adenine	360–365	243.5	White powder	—	Glass
Cytosine	360	185.9	White powder	32.3	Liquid
Guanine	320–325	251.3	White powder	50.6	White powder
Thymine	335–337	317.9	White powder	220.6	White powder

alkyl halide followed by the anion exchange,[12] and (2) neutralization of bases with acids in pure water.[21] Since DNA is soluble in an aqueous medium, ionic liquids of DNA can be prepared by the neutralization method in pure water. Table 1 summarizes melting point (T_m) and glass transition temperature (T_g) of the salts obtained from the neutralization of four kinds of bases (adenine **A**, guanine **G**, cytosine **C** and thymine **T**) with HBF_4 or HTFSI. $TFSI^-$ is an excellent anion for polymer electrolyte preparation,[27] and it also gave ionic liquids.[28] All these bases before neutralization are white powders, and they decompose above 320 °C without melting.[29] Following neutralization with HBF_4 the salts were still obtained as white powders, but they showed T_m 317.9 ~ 185.9 °C depending on the base species, as shown in Table 1. Adenine neutralized with bis(trifluoromethanesulfonyl)imide (HTFSI) had no melting point but showed T_g at −13.2 °C. Furthermore **C**·TFSI salt obtained by the neutralization of cytosine with HTFSI became an ionic liquid with T_m at 32.3 °C. They are classified as a new kind of ionic liquid prepared from the corresponding bases of DNA. **A**·TFSI and **C**·TFSI were only slightly soluble in water due to hydrophobicity of the TFSI anion.

Since the pK_b of **A**, **C**, **T** and **G** are 9.8, 9.4, 4.1 and 3.2, respectively,[29,30] both **A** and **C** can be neutralized with acid. No reaction occurred when thymine was mixed with acid. Guanine was degraded by the acid added yielding a product having a lower T_m at 50 °C. IR and ^{1}H-NMR measurements suggested the cleavage of purine rings. From these results, adenine and cytosine were shown to form ionic liquids.

The temperature dependence of the ionic conductivity of the corresponding bases after neutralization with HBF_4 or HTFSI is shown in Fig. 5. The salts neutralized with HBF_4 showed low ionic conductivity around 10^{-9} S cm^{-1} (Fig. 5; ○ and □). On the other hand, **A**·TFSI and **C**·TFSI showed relatively high ionic conductivity, especially **C**·TFSI which yielded 6.85×10^{-5} S cm^{-1} at 50 °C (Fig. 5; ■). The high ionic conductivity was probably due to the low T_g, *i.e.*, **A**·TFSI and **C**·TFSI had T_g at −13.2 and −30.8 °C, respectively. These

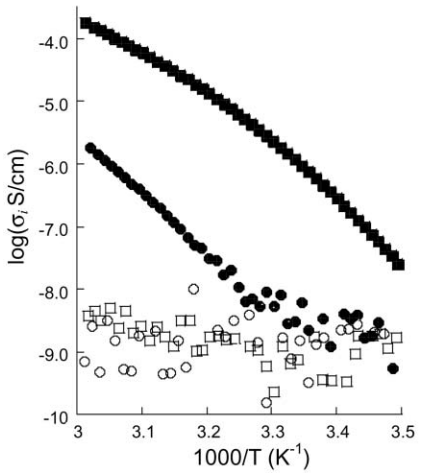

Fig. 5 Temperature dependence of the ionic conductivity of neutralized bases. ● **A**·TFSI; ■ **C**·TFSI; ○ **A**·BF_4; □ **C**·BF_4.

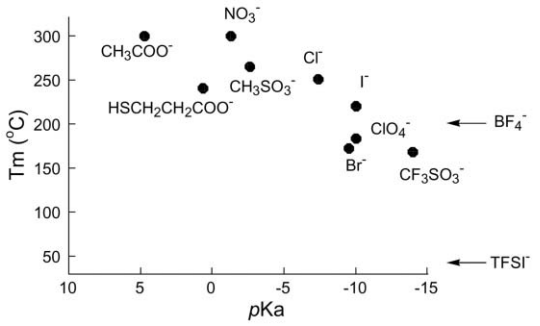

Fig. 6 T_m of cytosine after neutralization as a function of the pK_a of the acids used.

remarkable characteristics of corresponding bases were the result of ionic liquid formation though neutralization with HTFSI.

In order to select a suitable acid for the formation of ionic liquid, cytosine was neutralized with various acids (HBF_4, HTFSI, CF_3SO_3H, HBr, $HClO_4$, HI, HCl CH_3SO_3H, HNO_3, CH_3COOH or $HSCH_2CH_2COOH$). The products were studied with ^{1}H-NMR. The chemical shifts of protons at the C–H(5) and C–H(6) positions were shifted towards lower magnetic field and were used to evaluate salt formation. Though pure cytosine decomposed above 360 °C, cytosine salts showed T_m values which depended on the acid species used, as seen in Fig. 6. Salt **C**·TFSI had the lowest T_m at 32.3 °C. No other acid was found to be more effective to make ionic liquid. Fig. 6 summarizes the relation between T_m of the obtained salt and pK_a of the applied acid for neutralization. Since the pK_a values of both HBF_4 and HTFSI are unknown, the T_m values of these two salts are indicated with arrows in Fig. 6. Strong acids like $HClO_4$ and CF_3SO_3H decreased the T_m of the salts. Unexpectedly, hydrogen bromide also decreased the T_m of the salt. The dependence of the anion on the T_m was the same as that for an imidazolium type ionic liquid.[14] However, the ionic liquids from corresponding bases generally showed higher T_m values than imidazolium salts.

DNA ionic liquid

Since adenine and cytosine formed ionic liquids after neutralization, these subunits in DNA should also form ionic liquids after acid treatment. DNA has quite a lot of bases aligned on the chain. If all of these bases are converted into ionic liquids, successive ionic liquid domains should be obtained along the helix. From the results in Fig. 6, HBF_4, HTFSI, CF_3SO_3H, HBr, $HClO_4$, HI or HCl were predicted to be the most effective for that purpose. The four kinds of bases were assumed to be contained equally in the DNA. The acids 50 mol% to the total bases were mixed with DNA to neutralize all adenines and cytosines. After neutralization with the above mentioned acids individually in pure water, all the products were obtained as precipitates. In the case of the model reaction, degradation of guanine was detected after acid treatment. Guanosine-5'-phosphate disodium salt (GMP) was also neutralized with HTFSI. Since the guanine ring was confirmed to be unchanged by ^{1}H-NMR, we expected that the guanine rings in the DNA would also not be cleaved by the neutralization. The hydrogen

bonding between complementary base pairs was broken after neutralization, and their hydrophobic bases turned outside the helix making whole chains more hydrophobic. Actually, CD and IR spectra strongly suggested that these DNAs no longer maintained double strand helix structure.[31] The ionic conductivity of the neutralized DNA was about 1×10^{-9} S cm^{-1} at room temperature. This can be explained by an insufficient ionic liquid fraction. The weight fraction of all the bases in solid DNA is about 40 wt%. However, if all the adenines and cytosines yielded ionic liquids, the corresponding domain would be only about 20 wt%, insufficient to form a closely packed ion conduction path. Salt C·TFSI, which showed the lowest T_m among the neutralized bases, was added to the neutralized DNA to construct a continuous ion conduction path. The neutralized DNA and **C**·TFSI need to be mixed in solvent for casting as films. Although DNA·TFSI is probably the best matrix from the viewpoint of ionic conductivity, it cannot be dissolved in any solvent. Since DNA·BF_4 can be dissolved in excess of water, DNA·BF_4 was used in further experiments. When the **C**·TFSI content in DNA·BF_4 was less than 50 wt%, the ionic conductivity of the mixture was very low, around 1×10^{-8} S cm^{-1} at 50 °C (Fig. 7; ▲). It increased when more than 70 wt% **C**·TFSI was added. The ionic conductivity reached 4.76×10^{-5} S cm^{-1} at 50 °C when 80 wt% of **C**·TFSI was added to the DNA·BF_4. However the mixture was obtained as a flexible film with 80 wt% **C**·TFSI, wherever the ionic conductivity was not high enough. Since the ionic conductivity of **C**·TFSI in the bulk was only 6.85×10^{-5} S cm^{-1}, the DNA·BF_4 and **C**·TFSI mixed film showed reasonable conductivities suggesting the formation of successive ionic liquid phases. To prepare a film showing higher ionic conductivity, DNA·BF_4 needed to be mixed with more a conductive ionic liquid. For this purpose, ethylimidazolium tetrafluoroborate (EtImBF_4)[21] was added to the DNA·BF_4 (Fig. 7; ●). Since both DNA·BF_4 and EtImBF_4 have the same BF_4^- anion and are soluble in pure water, DNA·BF_4/EtImBF_4 mixture was conveniently prepared as a homogeneous film by casting. When EtImBF_4 was mixed at up to 10 wt%, the ionic conductivity of the mixture was the same as that for pure DNA·BF_4. However, the ionic conductivity of the film containing 15 wt% EtImBF_4 was about 4.62×10^{-7} S cm^{-1}, and that of film containing 23.7 wt% EtImBF_4 was 1.74×10^{-4} S cm^{-1} at 50 °C. This excellent ionic conductivity was retained until 85 wt%. The highest ionic conductivity of 5.05×10^{-3} S cm^{-1} at 50 °C was observed when the film was prepared with 93 wt% EtImBF_4. Further addition of EtImBF_4 was also effective in maintaining high ionic conductivity, but film was no longer obtainable. It is surprising that a film can be formed with as little as 7 wt% DNA with 93 wt% ionic liquid. This is because of the high molar mass of DNA, and strong affinity of EtImBF_4

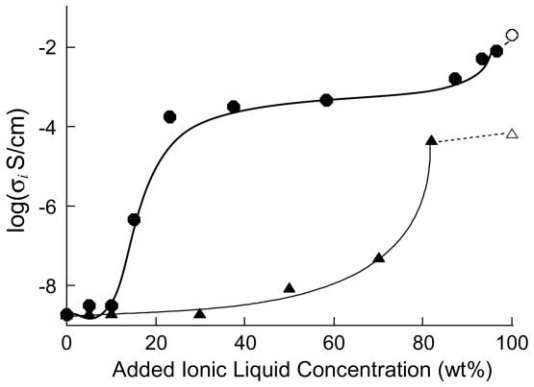

Fig. 7 Effect of added ionic liquid concentration on the ionic conductivity of DNA·BF_4/**C**·TFSI (▲), DNA·BF_4/EtImBF_4 (●) at 50 °C. The conductivity of pure **C**·TFSI (△) and pure EtImBF_4 (○) are also shown for reference.

Fig. 8 Photograph of flexible and transparent DNA film. DNA·BF_4/EtImBF_4 (40 wt%).

for DNA·BF_4. We have already reported the preparation of excellent ion conductive films from the mixture of native DNA and EtImBF_4.[23] There is no difference in the ionic conductivities between DNA·BF_4/EtImBF_4 and DNA/EtImBF_4 films at high ionic liquid content (>70 wt%). However, DNA·BF_4/EtImBF_4 film showed much higher ionic conductivity at low EtImBF_4 content. This can be explained by the formation of an effective ionic liquid pathway in the DNA matrix by the neutralization of the bases. Furthermore, the DNA·BF_4/EtImBF_4 film showed high ionic conductivity and excellent flexibility over a wide range of ionic liquid content. This is a great advance in both the ionic conductivity and flexibility of DNA films over the DNA/PEO_{1000}/$NaClO_4$ film (Fig. 2). A photograph of a flexible and transparent DNA film prepared from 40 wt% EtImBF_4 mixed with DNA·BF_4 is shown in Fig. 8.; its ionic conductivity was 1.32×10^{-3} S cm^{-1} at room temperature. In other words, flexible film having high ionic conductivity can be obtained when DNA·BF_4 is mixed with only a small amount of EtImBF_4. The experiments on stability were carried out under a dry nitrogen atmosphere at room temperature. DNA·BF_4/EtImBF_4 film showed excellent stability, and no leakage of ionic liquid was detected. This excellent stability was observed to continue over several months.

Conclusions

Ion conductive and flexible DNA film was prepared by both the direct mixing of DNA with salt containing PEO and neutralization of bases on the DNA with acid. The bases on the DNA were neutralized with several acids to convert these bases into ionic liquid. Addition of further ionic liquid (C·TFSI or EtImBF_4) allowed the preparation of highly ion conductive films. The highest ionic conductivity of 5.05×10^{-3} S cm^{-1} was found at 50 °C when 93 wt% EtImBF_4 was mixed with HBF_4 neutralized DNA. This opens a new field on the use of DNA as a biomass.

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References

1 C. J. Murphy, M. R. Arkin, Y. Jenkins, N. D. Ghatlia, S. H. Bossmann, N. J. Turro and J. K. Barton, *Science*, 1993, **262**, 1025–1029.
2 D. B. Hall, R. E. Holmlin and J. K. Barton, *Nature*, 1996, **382**, 731–735.
3 Y. Okahata, T. Kobayashi, K. Tanaka and M. Shimomura, *J. Am. Chem. Soc.*, 1998, **120**, 6165–6166.
4 I. Saito, T. Nakamura, K. Nakatani, Y. Yoshioka, K. Yamaguchi and H. Sugiyama, *J. Am. Chem. Soc.*, 1998, **120**, 12686–12687.

5 Hans-Werner Fink and C. Schönenberger, *Nature*, 1999, **398**, 407–410.
6 D. Porath, A. Bezryadin, S. de Vries and C. Dekker, *Nature*, 2000, **403**, 635–638.
7 K. Tanaka and Y. Okahata, *J. Am. Chem. Soc.*, 1996, **118**, 10679–10683.
8 Yu. Lvov, G. Decher and G. Sukhorukov, *Macromolecules*, 1993, **26**, 5396–5399.
9 Yu. M. Lvov, Z. Lu, J. B. Schenkman, X. Zu and J. F. Rusling, *J. Am. Chem. Soc.*, 1998, **120**, 4073–4080.
10 D. Baril, C. Michota and M. Armand, *Solid State Ionics*, 1997, **94**, 35–47.
11 (*a*) N. Takizawa and H. Ohno, *Proceedings of the 196th Annual Meeting of The Electrochemical Society*, Hawaii, October 1999, Abstr., No. 49; (*b*) H. Ohno and N. Takizawa, *Chem. Lett.*, 2000, 642–643.
12 J. S. Wilkes and M. J. Zaworotko, *J. Chem. Soc., Chem. Commun.*, 1992, 965–967.
13 B. Vestergaard, N. J. Bjerrum, I. Petrushina, H. A. Hjuler, R. W. Berg and M. Begtrup, *J. Electrochem. Soc.*, 1993, **140**, 3108–3113.
14 P. Bonhôte, Ana-Paula Dias, N. Papageorgiou, K. Kalyanasundaram and M. Grätzel, *Inorg. Chem.*, 1996, **35**, 1168–1178.
15 A. B. McEwen, S. F. McDevitt and V. R. Koch, *J. Electrochem. Soc.*, 1997, **144**, L84–L86.
16 S. Iinou, M. Handa and Y. Sasaki, *Electrochemistry*, 1999, **67**, 22–26.
17 A. B. McEwen, H. L. Ngo, K. LeCompte and J. L. Goldman, *J. Electrochem. Soc.*, 1999, **146**, 1687–1695.
18 S. Tait and R. A. Osteryoung, *Inorg. Chem.*, 1984, **23**, 4352–4360.
19 B. Ellis, W. Keim and P. Wasswerscheid, *Chem. Commun.*, 1999, 337–338.
20 W. Chen, L. Xu, C. Chatterton and J. Xiao, *Chem. Commun.*, 1999, 1247–1248.
21 M. Hirao, H. Sugimoto and H. Ohno, *J. Electrochem. Soc.*, 2000, **147**, 4168–4172.
22 H. Ohno and N. Nishimura, *J. Electrochem. Soc.*, 2001, **148**, E168–E170.
23 A. Bakker, S. Gejji, J. Lindgren, K. Hermansson and M. M. Probst, *Polymer*, 1995, **36**, 4371–4378.
24 S. Rüdisser, A. Hallbrucker and E. Mayer, *J. Phys. Chem.*, 1996, **100**, 458–461.
25 S. Rüdisser, A. Hallbrucker, E. Mayer and G. P. Johari, *J. Phys. Chem. B*, 1997, **101**, 266–277.
26 P. V. Wright, *Br. Polym. J.*, 1975, **7**, 319–327.
27 S. Lascaud, M. Perrier, A. Vallée, S. Besner, J. Prud'homme and M. Armand, *Macromolecules*, 1994, **27**, 7469–7477.
28 V. R. Koch, C. Nanjundiah, G. Battista Appetecchi and B. Scrosati, *J. Electrochem. Soc.*, 1995, **142**, L116–L118.
29 S. Budavari, Ed., *The Merck Index*, 12th Ed., MERCK and CO.,INC., Whitehouse Station, N. J., 1996.
30 *CRC Handbook of Chemistry and Physics*, ed. D. R. Lide, CRC Press, London, 82nd edn., 2001.
31 D. Voet, W. B. Gratzer, R. A. Cox and P. Doty, *Biopolymer*, 1963, **1**, 193–208.