

ORIGINAL ARTICLE

Proton conduction of DNA–imidazole composite material under anhydrous condition

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Materials with high anhydrous proton conduction at intermediate temperatures (100–200 °C) have attracted remarkable interest for applications in a polymer electrolyte membrane fuel cell (PEMFC). Especially, for the development of PEMFC technology, an anhydrous proton-conductive material, which is low cost and benign for the environment, has been desired. In this study, an anhydrous proton conductor was prepared by mixing double-stranded DNA (dsDNA), the most important genetic material of living organisms, and the imidazole (Im) molecule, a heterocyclic molecule. This DNA–Im composite material showed a thermal stability owing to electrostatic interaction between the phosphate group of DNA and –N= group of the Im molecule. In addition, DNA–Im showed an anhydrous proton conduction of $5.2 \times 10^{-3} \text{ S cm}^{-1}$ at intermediate temperature. On the other hand, the single-stranded DNA–Im composite material did not show high anhydrous proton conduction. Therefore, the high anhydrous proton conduction in dsDNA–Im composite material was due to the arrangement of phosphate groups along the one-dimensional molecular chain. These results suggested that the DNA–Im composite material possesses two proton-conducting pathways in the composite material.

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INTRODUCTION

Double-stranded DNA (dsDNA), the most important genetic material of living organisms, can be easily purified from either salmon milts or shellfish gonads, which are generally discarded as industrial waste around the world.^{1,2} In addition, DNA is a natural product and safe for humans and the environment. Furthermore, DNA has highly specific functions, such as the accumulation of compounds into its helical structure, the complementary binding of nucleic acid bases, and the arrangement of phosphate groups along the molecular chain.^{1–4} Therefore, the utilization of DNA as a functional material has various advantages; for example, an adsorption material of harmful compounds,^{5,6} biomedical materials,⁷ optical materials⁸ or nanotechnology material.^{9,10} However, the utilization of phosphate groups along the molecular chain for use as a source of energy has not been reported. The arrangement of phosphate groups are expected to have a role in the proton conductive pathway in an anhydrous (water-free) condition. Here, we demonstrate the utilization of dsDNA as a novel anhydrous proton conductor consisting of bio-components. Furthermore, we discuss the proton conducting mechanism in dsDNA by comparison with the single-stranded DNA (ssDNA).

Anhydrous proton conductor is attractive for the electrolyte of polymer electrolyte membrane fuel cells (PEMFC) that operate at intermediate temperatures (100–200 °C).^{11–15} However, since the proton conduction of the customary perfluorinated sulfonic acid

membranes, such as the humidified Nafion membrane, is based on the presence of mobile water molecules in the membrane, the proton conductivity above the boiling point (>100 °C) abruptly decreases because of the evaporation of water.^{16,17} In addition, the production cost is extremely high. Therefore, the anhydrous proton conductor consisting of a non-expensive material is necessary for advanced PEMFC technology. The proton transport under anhydrous or low humidity conditions is a non-vehicular mechanism, in which only the protons are mobile from site to site without diffusible water molecules, such as H_3O^+ or H_5O_2^+ .^{18,19} This is called the Grotthuss mechanism. This activation energy (E_a) of proton transport depends on the distance between the hopping sites. This type of mechanism has been reported for fullerene derivatives^{20,21} or heterocycles, such as Im and benzimidazole.^{22,23}

The proton transport of an acid–base composite material, such as phosphoric acid–polybenzimidazole^{24–26} and monododecyl phosphate–undecylimidazole²⁶ composite materials, under anhydrous or low humidity conditions, is supposed to occur by the Grotthuss mechanism. In this case, the acidic and basic molecules (polymers) function as proton donors and acceptors during the proton transfer reactions, respectively. However, since these anhydrous proton conducting materials require artificial polymers or molecules, these materials are high cost and not benign for the environment. Therefore, we investigated the DNA–basic molecule composite material as the

anhydrous proton conductor. dsDNA has many phosphate groups, arranged in one direction, in its polymer and these phosphate groups have a role in the proton-conductive pathway under anhydrous (water-free) condition. Furthermore, the proton conduction of DNA under anhydrous conditions is very interesting and very important for the basic property of DNA.

In this study, we prepared the anhydrous proton conductor by mixing the dsDNA and the basic molecule Im, which is a heterocyclic molecule. This DNA–Im composite material showed a thermal stability due to its electrostatic interaction between the phosphate group of DNA and $-N=$ group of Im. In addition, DNA–Im showed an anhydrous proton conduction of $5.2 \times 10^{-3} \text{ S cm}^{-1}$ at intermediate temperature. Furthermore, the proton conduction in the DNA–Im composite material showed two activation energies at the mixing ratio of the Im molecule. These results suggested that the DNA–Im composite material possessed two proton-conducting pathways in the composite material.

EXPERIMENTAL SECTION

Materials

dsDNA (sodium salt from salmon milt, molecular weight; $>5 \times 10^6$) and ssDNA (sodium salt from salmon milt, molecular weight; $<2 \times 10^5$) were obtained from Biochem Ltd., Saitama, Japan and Nissan Chemical Industries, Ltd., Tokyo, Japan, respectively. Im was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Ion-exchange resin Amberlite IR 120(H) was obtained from Supelco Inc., Bellefonte, PA, USA. Solvents used were of analytical grade in all the experiments described. Ultra-pure water (Millipore Corporation, Billerica, MA, USA) was used in this research.

Preparation of DNA–Im composite materials

dsDNA was changed from Na^+ form to H^+ form by the ion-exchange column with ion-exchange resin Amberlite IR 120(H). Ion exchange of DNA was confirmed by the pH measurement. DNA H^+ and Im were dissolved in ultra-pure water and mixed in aqueous solution. The molar ratio (R) of DNA–Im composite material was determined by Equation (1),

$$R = \frac{[\text{Im}]}{[\text{DNA}(\text{nucleotide})]} \quad (1)$$

where [DNA (nucleotide)] and [Im] of Equation (1) are the molar concentration of nucleotide in DNA and Im molecule, respectively. The aqueous DNA–Im solution (100 μl) was cast onto a Teflon plate and dried at room temperature for 1 to 3 days or at 80 °C for 6 h. The dried membrane of the DNA–Im composite material was stripped from the Teflon plate. The aqueous ssDNA–Im solution was prepared by a similar procedure, cast onto Teflon plate, and then dried at 80 °C for 6 h.

Characterization of DNA–Im composite material

Pure DNA, DNA–Im composite, and pure Im materials were dried at room temperature overnight. The infrared (IR) absorption spectra of these materials were measured by the attenuated total reflection method using a FT-IR 8400 Fourier transform infrared spectrometer (Shimadzu Corp., Kyoto, Japan). The IR spectrum was measured with the resolution of 4 cm^{-1} . The thermal stability of the DNA–Im composite material was analyzed by thermogravimetric-differential thermal analysis (TG-DTA) (DTG-60, Shimadzu Corp.). The TG-DTA measurement was carried out at a heating rate of 10 °C min^{-1} under a dry-nitrogen flow. Sample weights of TG-DTA measurements were normalized at 1 mg.

Proton conductive measurement of DNA–Im composite material

Proton conductivity of DNA–Im composite material was demonstrated by the a.c. impedance method in the frequency range from 4 Hz to 1 MHz using a chemical impedance analyzer 35320-80 (Hioki Co., Nagano, Japan) in a stainless-steel vessel from room temperature to 160 °C under dry-nitrogen flow. In this study, as the resistance of composite material is higher than the

contact resistance, the proton conductive measurements were demonstrated by the two electrode system.^{27–30} The DNA–Im composite material was sandwiched between two platinum electrodes (diameter: 6 mm) with the Teflon spacer.^{28,30} The direction of conductive measurement is perpendicular to the composite material. The thickness of the DNA–Im composite material was measured after the proton conductive measurement. Conductivities of DNA–Im composite materials were determined from a typical impedance response (Cole–Cole plots). The resistances of the composite materials were obtained by the extrapolation to the real axis.

Before the measurements of proton conduction, the composite materials were dried at 160 °C in a stainless steel vessel for 3 h to evaporate the water from proton conductive samples. Furthermore, all the experimental procedures in proton conduction were carried out under dry-nitrogen flow. Therefore, the measured impedance response indicates an anhydrous proton conduction of membranes.^{22,23} On the other hand, the proton conduction of pure ssDNA material and ssDNA–Im composite material were measured by similar methods.

RESULTS AND DISCUSSION

Preparation and characterization of DNA–Im composite material

Generally, the DNA has existed as Na salts in nature. Therefore, in this study, the DNA was changed from the Na^+ form to H^+ form using an ion-exchange column. The ion exchange of DNA was confirmed by the pH measurement. The aqueous DNA–Im solution was cast onto a Teflon plate and dried at room temperature. This DNA–Im composite material was carefully stripped from the Teflon plate. Figure 1 shows a photograph of the DNA–Im composite material. The thickness of the membrane is ca. 20 μm . The free-standing membrane is transparent, flexible and homogeneous. Figure 2 shows the IR spectra of (a) pure DNA, (b) $R=0.1$, (c) $R=1$, (d) $R=3$, (e) $R=8$, (f) $R=10$ and (g) pure Im materials. The absorption band at 1205 cm^{-1} , the antisymmetric vibration of the phosphate group,^{31,32} shifted to a higher wavenumber, ca 20 cm^{-1} , with an increase in the concentration of the Im molecule. In addition, the absorption band at 1055 cm^{-1} , which can be attributed to the P–O stretching in the ionic phosphate group,³³ increased in comparison to the spectrum of the pure Im molecule by addition of Im. These results suggested that the phosphate group of DNA deprotonates by the addition of Im and forms the P–O[−] group. On the other hand, the absorption band at ca. 3100 cm^{-1} in the composite materials, the stretching band of the N–H group,^{31,34}

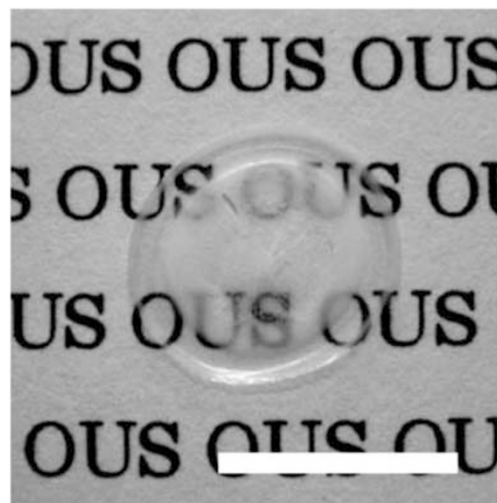


Figure 1 Photograph of DNA–Im composite material $R=10$. The free-standing membrane is transparent, flexible and homogeneous. The thickness of membranes is ca. 20 μm . Scale bar is 10 mm.

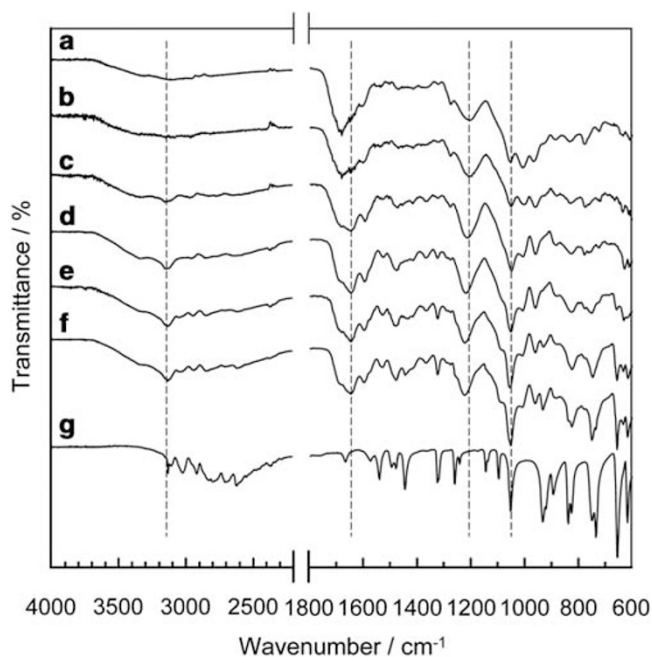


Figure 2 IR spectra of DNA-Im composite material. (a) pure DNA; (b) $R=0.1$; (c) $R=1$; (d) $R=3$; (e) $R=8$; (f) $R=10$; (g) pure Im materials. The IR spectrum was measured at the resolution of 4 cm^{-1} .

moderately increased when compared with the spectrum of pure Im. In addition, the absorption band at 1649 cm^{-1} , related to the aromatic C=N of the heterocycle, increased with the addition of DNA. The increases in absorbance were due to the construction of the N-H group by the protonation to the $-N=$ group of the Im group and the disappearance of the aromatic C=N group by the protonated Im molecule. Similar phenomena have been reported for the acid-base composite material with the heterocyclic molecule.^{22,23} These results suggest that the P-OH group of DNA deprotonates by the addition of Im and forms the P-O⁻ group. This free proton strongly interacts with the non-protonated $-N=$ group of the Im molecule. Therefore, the acidic polymer DNA and basic heterocycle Im produces an acid-base composite salt. Similar composite materials have been reported in the monododecyl phosphate-benzimidazole composite materials.^{22,26}

Characterization of DNA-Im composite material

Figures 3a and b show the TG-DTA of (1) pure DNA, (2) $R=1$, (3) $R=5$, (4) $R=10$, (5) pure Im materials, respectively, with the heating rate of 10 °C min^{-1} up to 300 °C under dry-nitrogen flow. Pure DNA material without the addition of Im had a TG weight loss of approximately 10% at 150 °C (line (1) in Figure 2a). This weight loss is due to the evaporation of water from the DNA material. The DTA analysis of the pure DNA material indicated an exothermic peak at 166.7 °C by thermal decomposition. This temperature of the exothermic peak was lower than that of the DNA with the Na salts.^{8,35} This is due to the influence of the ion exchange from the Na⁺ form to H⁺ form. This exothermic peak disappeared by the addition of the Im molecule (see lines (2)–(4) in Figure 2b). In contrast, the pure Im molecule showed two endothermic peaks due to its melting and boiling, respectively. These endothermic peaks decreased with an increase in the addition of DNA. These results suggested that the DNA-Im composite material formed an acid-base complex through the electrostatic interaction in the composite mem-

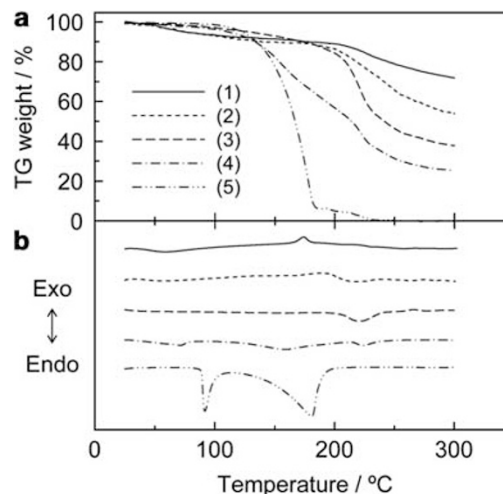


Figure 3 TG (a) and DTA (b) curves of DNA-Im composite materials with the heating rate of 10 °C min^{-1} under dry-nitrogen flow. (1) Pure DNA; (2) $R=3$; (3) $R=5$; (4) $R=10$; (5) pure Im materials.

brane, and as a result, becomes more stable than the pure DNA or Im materials. Furthermore, these results indicated that the DNA-Im composite material does not produce a diffusible ion by the melting of the samples and is stable at intermediate temperature ($<160\text{ °C}$). Therefore, the proton conductive samples were prepared by drying at 160 °C for 3 h under dry-nitrogen flow condition to evaporate the water.

Anhydrous proton conductive measurements of DNA-Im composite material

The proton conductive measurements of the DNA-Im composite materials were demonstrated by the a.c. impedance method over the frequency range from 4 Hz to 1 MHz under dry-nitrogen flow. Figure 4 shows the typical impedance response (Cole-Cole plots) of the DNA-Im composite materials. Generally, the Cole-Cole plots corresponding to the lower conductivities show two well-defined regions:³⁶ a semi-circle passing through the origin in the high-frequency, which is related to the conduction process in the bulk of the sample, and a monotonically decreasing curve with increasing frequency in the low frequency that is attributed to the electrode/solid electrolyte interface. Therefore, the bulk resistance of material is obtained from the intercept of either curve (high frequencies semicircle or low frequencies tail) with the real axis.³⁶ However, in a material with high conductivity, the semicircle passing decreases with the increase of conductivity³⁶ and the bulk resistance arise at the electrode/electrolyte interface.^{16,37,38} In fact, the features of the Cole-Cole plots for the DNA-Im composite material are similar to that of a highly ionic conducting membrane, such as humidified Nafion¹⁶ an organic/inorganic hybrid membrane mixed with PWA,³⁷ or anhydrous proton conductors.^{22,23,26,38} The resistances of the composite materials were obtained from the extrapolation to the real axis. On the other hand, these DNA-Im composite materials did not indicate any electronic conductivity under DC conditions. In addition, as the composite material was dried at 160 °C for 3 h under dry-nitrogen flow to evaporate diffusible water molecules from the proton conductive samples, the diffusible ions other than the protons do not exist in these materials (see the TG-DTA in Figure 3). Therefore, the impedance responses of the DNA-Im composite material are mainly due to the anhydrous proton transfer.

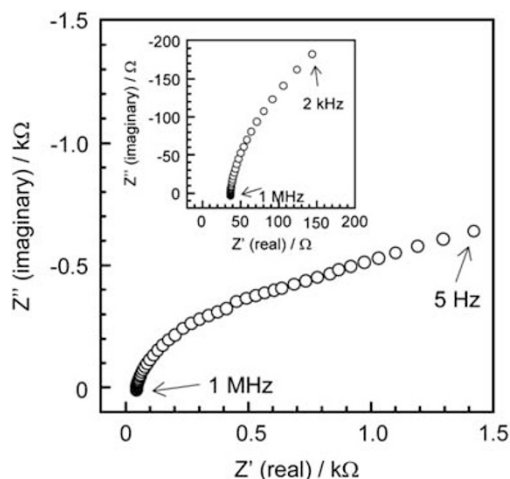


Figure 4 Typical impedance response (Cole-Cole plots) of DNA-Im composite material at 160 °C under anhydrous condition. Frequency range is from 5 Hz to 1 MHz. The inset indicates the high frequency range (2 kHz–1 MHz) of DNA-Im composite material.

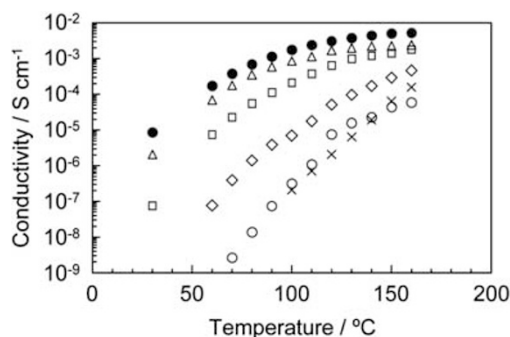


Figure 5 Proton conductivities of DNA-Im composite materials with different Im mixing ratio under anhydrous condition. These samples were dried at 160 °C for 3 h under dry-nitrogen flow. Proton conductivity was performed by the a.c. impedance method. (○), pure DNA material; (×), $R=1$; (◇), $R=3$; (□), $R=5$; (△), $R=8$; (●), $R=10$.

Figure 5 shows the anhydrous proton conductivity of (○) pure DNA, (×) $R=1$, (◇) $R=3$, (□) $R=5$, (△) $R=8$, (●) $R=10$ in the temperature range from room temperature to 160 °C under dry-nitrogen flow condition. The anhydrous proton conductivity of the DNA-Im composite material increased with the temperature and reached a maximum value at 160 °C. Although the pure DNA material showed a maximum proton conductivity on the order of 10^{-5} S cm^{-1} at 160 °C under anhydrous conditions, that of the DNA-Im composite material increased with the mixing ratio of the Im molecule and indicated a maximum proton conductivity of 5.2×10^{-3} S cm^{-1} at $R=10$. On the other hand, since the pure Im molecules without the mixing with DNA evaporated due to the drying process (at 160 °C for 3 h), this sample did not show any measurable proton conductivity. Furthermore, at ≥ 170 °C, the proton conductivity of DNA-Im composite material decreased.

Figure 6 shows the Arrhenius plot of the proton conductivity of the DNA-Im composite materials. The solid lines are the results of the least-squares fitting. The conductivities of the DNA-Im composite material are plotted almost on a straight line from 90–160 °C. The E_a

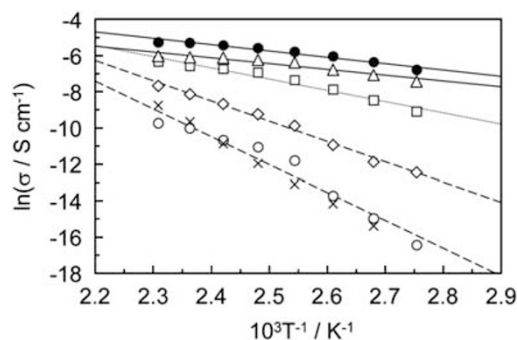


Figure 6 Arrhenius plots of the proton conductivity of DNA-Im composite materials. The solid lines are the results of the least-squares fitting. (○), pure DNA material; (×), $R=1$; (◇), $R=3$; (□), $R=5$; (△), $R=8$; (●), $R=10$.

Table 1 Activation energies and proton-conducting pathways in DNA-Im composite material at the low mixing ($5 > R$) and the high mixing ($5 < R$) ratios

R value ^a	$5 > R$	$5 < R$
Activation energy	1.0–1.8 eV	0.3 eV
Proton-conducting pathway	Phosphate group of DNA	Heterocyclic molecules

Abbreviation: Im, imidazole.

^a R values calculated from Equation (1).

of the proton conduction was estimated from the slope of the Arrhenius plot. Table 1 shows the E_a values of DNA-Im composite material at 90–160 °C. The E_a values are 1.0–1.8 eV and 0.3 eV at the low mixing ($5 > R$) and high mixing ($5 < R$) ratios, respectively. The E_a of 0.3 eV at the high mixing ratio is one order of magnitude higher than that of the customary humidified Nafion membrane^{16,18,19} and almost the same as the acid–base composite materials for anhydrous proton conductors.^{18,19} Therefore, the proton transfer in the DNA-Im composite material at the high mixing ratio is considered to be mainly based on the Grotthuss mechanism, in which only protons are mobile from site to site without the assistance of diffusible vehicle molecules, such as H_3O^+ or H_5O_2^+ . In contrast, the E_a of 1.0–1.8 eV at low mixing ratio is higher than that of the general anhydrous proton conductor.^{15,18,19} These results suggest that the DNA-Im composite material has two proton conductive mechanisms. On the other hand, since the pure DNA material undergoes a thermal decomposition at approximately 160 °C, the Arrhenius plot of the proton conductivity of the pure DNA material could not demonstrate a least-squares fitting in the range of 90–160 °C (see in Figure 5).

Anhydrous proton conduction of ssDNA-Im composite material

dsDNA has the arrangement of phosphate groups along the one-dimensional molecular chain. As ssDNA possesses a flexible structure, ssDNA does not have the arrangement of one dimensional phosphate groups along the molecular chain. Therefore, we demonstrated the proton conduction of pure ssDNA material and ssDNA-Im composite material under anhydrous conditions. Figure 7 shows the anhydrous proton conductivity of (○) pure ssDNA, (△) $R=3$ composite, and (□) $R=10$ composite materials in the temperature range from room temperature to 160 °C under dry-nitrogen flow conditions. The anhydrous proton conduction of ssDNA-Im composite material increased with the temperature and reached a maximum value at

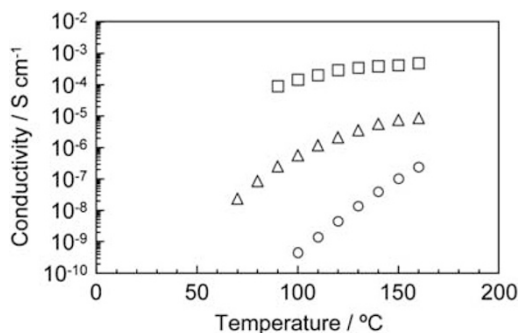


Figure 7 Proton conductivities of ssDNA-Im composite materials with different Im mixing ratio under anhydrous condition. These samples dried at 160 °C for 3 h under dry-nitrogen flow. Proton conductivity was performed by the a.c. impedance method. (○), pure ssDNA material; (△), $R=3$; (□), $R=10$.

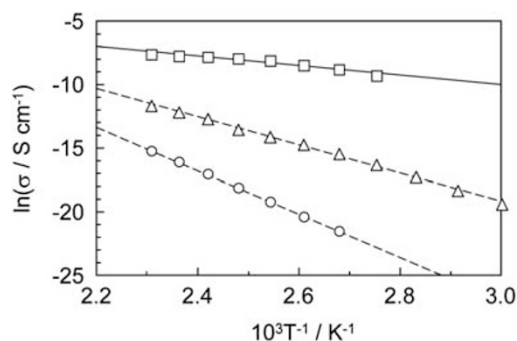


Figure 8 Arrhenius plots of the proton conductivity of ssDNA-Im composite materials. The solid lines are the results of the least-squares fitting. (○), pure ssDNA material; (△), $R=3$; (□), $R=10$.

160 °C. The maximum proton conduction of pure ssDNA material was in the order of $10^{-7} \text{ S cm}^{-1}$ and lower than that of pure dsDNA material (see ○ in Figure 5). In addition, although the proton conduction of composite material increased with the mixing ratio of Im and indicated a maximum value of $4.9 \times 10^{-4} \text{ S cm}^{-1}$ at $R=10$, this proton conduction was one order of magnitude lower than that of dsDNA-Im ($R=10$) composite material (see ● in Figure 5). Figure 8 shows the Arrhenius plot of the proton conductivity of (○) pure ssDNA, (△) $R=3$ composite, and (□) $R=10$ composite materials. The E_a of the proton conduction was estimated from the slope of the Arrhenius plot. The E_a values of pure ssDNA material, $R=3$ composite material, and $R=10$ composite material were 1.5 eV, 1.0 eV, and 0.3 eV, respectively. These E_a values were the same as that of dsDNA-Im composite material with the low ($5 > R$) and high ($5 < R$) mixing ratios of Im.

Proton conductive mechanism of DNA-Im composite material

The proton conductivity of heterocycles, such as Im, under anhydrous conditions has been investigated.¹⁸ Previously, we also reported an anhydrous proton conductor using heterocyclic molecules, such as Im and benzimidazole.^{22,23} In these cases, the transport of the proton can occur from protonated heterocyclic molecules to a non-protonated neighbor heterocyclic molecule.²² The E_a of these composite materials is ca. 0.2 eV and this value is the same as that of the DNA-Im composite material at a high mixing ratio ($5 < R$). In our experiments,

the DNA-Im composite material formed an acid-base composite material by the electrostatic interaction between the phosphate group of DNA and $-N=$ group of the Im molecule (see IR spectra in Figure 2). Namely, the free protons from the DNA molecule have strongly interacted with the $-N=$ group of the Im molecule and constructed the protonated Im molecule. Therefore, this anhydrous proton conduction has occurred from the protonated Im molecules to the non-protonated neighbor Im molecules. On the other hand, the Im molecules have been reported to form a molecular cluster, consisting of approximately twenty molecules, through intermolecular hydrogen bonding.³⁹ In addition, the heterocyclic molecules have also been known to interact with the phosphate group or nucleic acid base of DNA through the hydrogen bonding.^{3,4} Therefore, the Im clusters arrange along the direction of the dsDNA chain and construct the proton conductive pathway and domain in the DNA-Im composite material. As a result, the DNA-Im composite material at high mixing ratio ($5 < R$) showed the high proton conductivity of $5.2 \times 10^{-3} \text{ S cm}^{-1}$ at 160 °C under anhydrous conditions. In fact, as the ssDNA-Im composite material, which does not have the one-dimensional proton-conductive pathway, did not construct the proton conductive pathway and domain in the composite material, these ssDNA composite materials did not show high anhydrous proton conduction (see □ in Figure 7).

At the low mixing ratio ($5 > R$) of Im, the E_a value was 1.0–1.8 eV, and this value was higher than that of the other reported materials.^{18,19} This proton conduction is due to the proton transfer between the phosphate groups of DNA. In fact, pure DNA material without the addition of the Im molecule, showed an E_a value of 1.8 eV at 90–130 °C. The anhydrous proton conducting mechanism was postulated as follows: the phosphate group in the DNA forms a proton defect site ($P-O^-$) by the electrostatic interaction with the Im molecule, and as a result, the neighboring proton in the DNA can transfer to the proton defect site without the assistance of diffusible vehicle molecules. The similar proton conducting mechanism, such as the proton transfer to the proton defect site of the phosphate group, has been reported for methanediphosphonic acid-chitosan³⁸ and methanediphosphonic acid-pyridinesulfonic acid⁴⁰ composite materials. Therefore, the DNA-Im composite material at the low mixing ratio ($5 > R$) showed a proton conductivity of $4.7 \times 10^{-4} \text{ S cm}^{-1}$ at 160 °C under anhydrous conditions. On the other hand, as ssDNA with the flexible structure did not have the phosphate group arranged along the one-dimensional chain, the ssDNA-Im composite material did not show the high proton conduction between the phosphate groups (see ○ and △ in Figure 7).

CONCLUSION

We prepared an anhydrous proton conductor by mixing dsDNA, a discarded polymer, and Im, which is a heterocyclic molecule. This DNA-Im composite material formed thermally stable acid-base composite salts by the electrostatic interaction between the phosphate group of DNA and $-N=$ group of the Im. In addition, this DNA-Im showed a proton conduction of $5.2 \times 10^{-3} \text{ S cm}^{-1}$ at 160 °C under anhydrous conditions. In contrast, the ssDNA-Im composite material did not show the high anhydrous proton conduction. Therefore, the high anhydrous proton conduction in dsDNA-Im composite material was due to the arrangement of phosphate groups along the one-dimensional molecular chain. The dsDNA-Im composite material may have the potential not only for its superior ion-conducting properties, in particular, under anhydrous (water-free) or extremely low humidity conditions, but also in bio-electrochemical devices, including bio-sensors and an implantable battery.

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