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DNA molecular recognition of intercalators affects aggregation of thermoresponsive polymer

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Binding of intercalators to dsDNA switches the aggregation phenomena of DNA–PNIPAM because the binding decreases the dsDNA conformational entropy and/or the shielding of dsDNA charges. Thus, dsDNA–PNIPAM was dramatically aggregated in response to molecular recognition of the DNA–intercalator.

Smart polymers have potential applications in various areas because of their unique structural changes and transitions in response to their environment. Polymer materials have the significant advantage that sensor and functional probes can be incorporated *via* synthetic processes, and this versatility of molecular design allows applications of smart polymers to chemical and physical sensors, bioseparation and tissue engineering.[1–4]

Smart polymers usually have two components: one is a sensor probe that detects or recognizes a target stimulus; and the other is an actuator part that amplifies the signal detected by the sensor probe by polymer functions. Poly(*N*-isopropylacrylamide) (PNIPAM),[5] a well-known actuator, aggregates in response to temperature, and it has been combined with various sensor probes to respond to various kinds of molecules. For example, NIPAM copolymer with crown ether can respond to cations, and the capture of cations by the crown ether reduces the aggregation intensity of the copolymers.[6–10] In addition, NIPAM copolymers with boronic acid,[11,12] cyclodextrin[13–16] and antibody[17,18] can respond to glucose, some aromatic molecules and antigens, respectively. Furthermore, a porous membrane with a grafted PNIPAM–crown ether copolymer that we developed in our previous studies can amplify the target signal more because the PNIPAM action in response to the ion in its nanosize membrane pores can open and close the pores with large permeability changes.[7–10] In addition, another membrane with grafted PNIPAM–biotin copolymer responded to the presence of avidin and amplified the signal using methodology of cross-linking of the grafted PNIPAM in the pores.[19] Further, the response of PNIPAM in the pores has been converted into color using gold nanoparticles.[20] Using such PNIPAM–probe composites, the PNIPAM action can be specifically induced in response to the presence of target molecules.

Another candidate for such a sensor probe is DNA. DNA shows transformation between single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) with sequence specificity. The DNA transformation between ssDNA and dsDNA changes the physicochemical states of DNA strands: the negative charges of the DNA and DNA conformational restriction.[21–24]

PNIPAM–DNA composites affecting aggregation have been reported. Maeda *et al.*[25–27] found that PNIPAM with a grafted fully matched dsDNA (dsDNA–PNIPAM) aggregated to a greater extent than did PNIPAM with a grafted ssDNA (ssDNA–PNIPAM). The phenomenon is driven by an increase in the conformational restriction of the DNA strand *via* the formation of dsDNA from ssDNA.[26,27] This increase in conformational restriction implies a decrease in the variation of conformation. ssDNA, which exhibits a wide conformational variation, avoids aggregation because aggregation reduces the conformational entropy of ssDNA. Thus, there is entropic repulsion between the ssDNA chain.[28] The decrease in conformational variation induced by dsDNA formation weakens the entropic repulsion, followed by aggregation. In contrast, in our previous study,[29] ssDNA–PNIPAM aggregated to a greater extent than did dsDNA–PNIPAM. This phenomenon is driven by an increase in DNA charges induced by dsDNA formation. This strengthens DNA electrostatic repulsion, which is related to an enthalpic effect; the electrostatic force is driven by the decrease in electrostatic free energy.[30,31] Furthermore, our recent study demonstrated that these two opposing aggregation phenomena of DNA–PNIPAM are controlled by the balance of DNA charges and conformational restriction, and that the balance can be shifted by the salt concentration of a polymer solution and DNA fraction in a polymer chain.[32]

In addition to the transformation between ssDNA and dsDNA, which affects DNA states, DNA itself binds to ions, small molecules, polycations, and proteins, which also affect DNA charges and conformational restriction.[31,33–36] Intercalators, such as small aromatic dye molecules, can bind dsDNA *via* a stacking interaction with DNA nucleobase pairs.[37] Intercalation into DNA can lead to a curled or crumpled dsDNA conformation.[38] Furthermore, the positive charge of the intercalator can counteract dsDNA negative charges.[39] Therefore, it is possible that intercalators affect both DNA electrostatic and DNA entropic repulsion, resulting in DNA–PNIPAM aggregation. However, such effects of intercalator binding on the aggregation of DNA–PNIPAM have not been clarified.

In this study, we switched the aggregation phenomena of DNA–PNIPAM using the binding of intercalators to dsDNA and showed

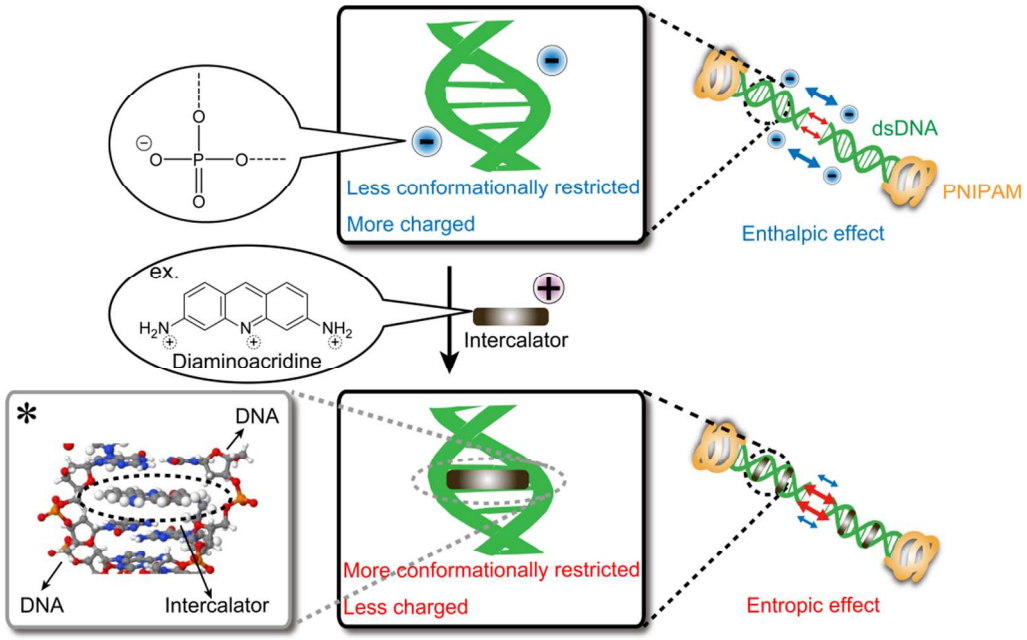

Fig. 1 Illustration of intercalation into a dsDNA base-pair pocket and its effect on the dsDNA–PNIPAM aggregation phenomena. The 3D structure (*) represents the intercalation of *N*-(2-(dimethylamino)ethyl)acridine-4-carboxamide into dsDNA, which was obtained from the Protein Data Bank (PDB code: 452D).[40] Carbon, hydrogen, nitrogen, oxygen, and phosphorus are shown in white, blue, red, and orange, respectively.

the potential of this process for the development of PNIPAM materials that can respond to intercalators. To examine the influence of intercalator binding, the positively charged 3,6-diaminoacridine hydrochloride (DAA) molecule was used. Fig. 1 provides a schematic representation of intercalation into dsDNA and its effect on the dsDNA–PNIPAM aggregation phenomena. DAA intercalates into base-pair pockets *via* a stacking interaction between this molecule and nucleobases. Furthermore, two other types of intercalators were used and compared with DAA: ethidium bromide (EtBr), which has a permanent positive charge, and 9-hydroxy-4-methoxyacridine (HMA), which is less ionized. The difference in the binding affinity of DAA and HMA was observed by fluorescence anisotropy measurement, and the relationship between the variation in PNIPAM aggregation and the binding affinity of the intercalator is discussed. This fundamental investigation of this polymeric phenomenon is the first step in the development of sensing tools using DNA–PNIPAM.

ssDNA–PNIPAM and dsDNA–PNIPAM with 11 base and 11 base-pair lengths of DNA were synthesized using a modified scheme based on our previous study.[29] The DNA fraction, which was defined as the molar ratio of DNA strands to the NIPAM monomer unit, of DNA–PNIPAM was 0.015 mol%. The aggregation behaviors of DNA–PNIPAM in the presence or absence of an intercalator were observed by absorbance measurements at 650 nm with a temperature increase. DNA–PNIPAM was mixed with DAA, EtBr, or HMA in 10 mM Tris-HCl (pH 7.4) buffer containing 400 mM NaCl. The final polymer concentrations of the solutions were adjusted to 0.025 or 0.050 w/v%, and the final intercalators concentrations were adjusted to one- and tenfold mole of DNA strand in the solution. Fluorescence anisotropy measurements of the intercalators in the presence of dsDNA were performed using a fluorescence spectrophotometer and two polarizers. Mixtures of 20 nM of each intercalator and 0–400 μM of free dsDNA were prepared in the same buffer, and measurements were performed at 25 °C. Details of materials and procedures of experiments are given in the ESI.

Fig. 2a shows the change in the aggregation of dsDNA–PNIPAM induced by DAA binding. An increase in absorbance means the presence of large aggregates of PNIPAM. Although PNIPAM chains shrunk above 29 °C, dsDNA–PNIPAM exhibited slight aggregation in the absence of DAA because the electrostatic repulsion between the negative charges on the dsDNA strand inhibited intense aggregation of PNIPAM. In this case, the aggregation was controlled by an enthalpic effect.[28,31] In contrast, dsDNA–PNIPAM aggregated dramatically in the presence of DAA, in a DAA concentration-dependent manner. An increase in the amount of DAA added caused a shift in the temperature at which the intense aggregation started: it occurred at 34.3 ± 0.6 °C and 33.0 ± 0.0 °C when the amounts of DAA were 1 and 10 eq, respectively. The values represent the average temperatures with standard deviation of three experiments. The addition of just 1 eq, which corresponds to 1.0 μM, of positively charged DAA was sufficient to induce a switch of the control factor. Our previous results showed that ~500 mM Na^+ was necessary to switch the control factor by electrostatic shielding of the salts.[29] In the case of the intercalator, a concentration that was a few hundred thousandfold lower than that of Na^+ switched the control factor. Fig. 2b represents the change in the aggregation of ssDNA–PNIPAM induced by the addition of DAA. The change in the aggregation of ssDNA–PNIPAM in the presence or absence of DAA was much smaller than that of dsDNA–PNIPAM. The binding affinity of ssDNA to the intercalator is much lower than that of dsDNA to the intercalator.[41] Thus, our results indicate that the variation in the aggregation phenomena of DNA–PNIPAM in the presence of DAA was induced by the binding of DAA to dsDNA.

According to Fig. 2a, it is possible that dsDNA–PNIPAM mixed with another intercalator with lower binding affinity exhibits intense aggregation at a higher temperature. To examine the effect of the level of intercalator binding, a less-charged and less-protonated intercalator, HMA, was compared with DAA. The difference in the binding affinity of the two intercalators was evaluated by fluorescence anisotropy measurement, which can monitor the

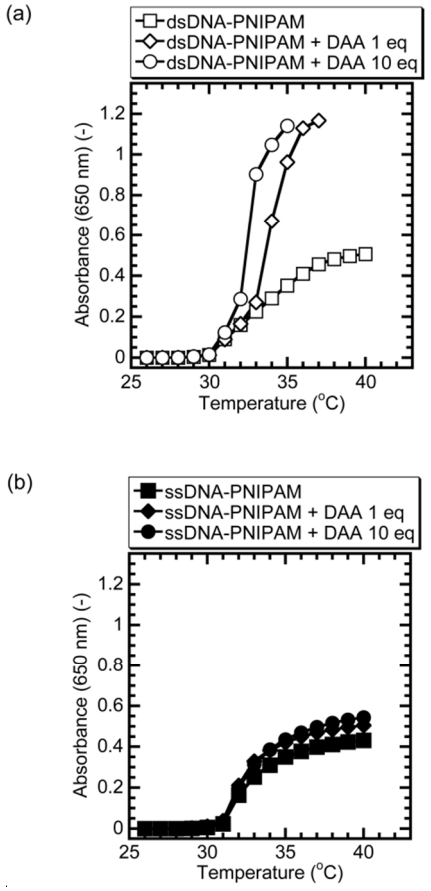

Fig. 2 Aggregation of DNA–PNIPAM in the presence of different DAA amounts: (a) 0.050 w/v% dsDNA–PNIPAM and (b) 0.025 w/v% ssDNA–PNIPAM, in 10 mM Tris-HCl (pH 7.4) buffer containing 400 mM NaCl; the plots represent the average values of three experiments, except for the plots of dsDNA–PNIPAM without DAA, which represent two experiments.

decrease in rotation speed of molecules, *i.e.*, the increase in molecular weight that occurs after binding to another molecule using polarized excitation light, and is used for the analysis of the binding of intercalators to dsDNA.[42] The results of fluorescence anisotropy measurements of DAA and HMA are shown in Fig. 3a and b, respectively. The graphs showed clearly that the fluorescence anisotropy of DAA increased depending on the concentration of dsDNA more dramatically than on that of HMA. Therefore, the binding affinity of DAA to dsDNA was higher than that of HMA; this difference may have been caused by the different numbers of positive charges in the DAA and HMA molecules.[37]

Fig. 4 shows the comparison of the aggregation of dsDNA–PNIPAM in the presence of DAA, EtBr, and HMA. EtBr is charged to the same extent as DAA and has approximately the same dsDNA-binding affinity as DAA.[43] The average temperatures at which intense aggregation started were 33.0 ± 0.0 °C when EtBr was added and 34.7 ± 1.2 °C for HMA. In the presence of EtBr, dsDNA–PNIPAM showed the same behavior as in the presence of DAA, because of their similar binding affinities and charge states. In contrast, intense aggregation of dsDNA–PNIPAM occurred at a higher temperature in the presence of HMA than it did in the presence of DAA and EtBr.

These results showed that the aggregation phenomenon of DNA–PNIPAM controlled by an enthalpic effect was switched to another phenomenon controlled by an entropic effect after binding to

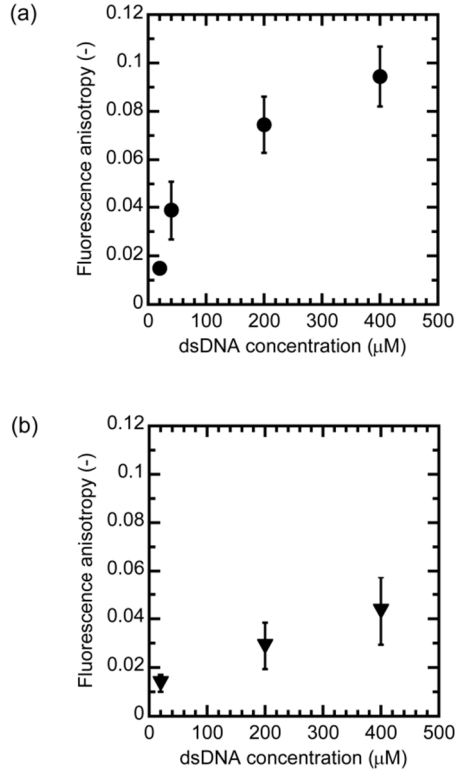

Fig. 3 Fluorescence anisotropy of (a) DAA and (b) HMA as a function of dsDNA concentration, in 10 mM Tris-HCl (pH 7.4) buffer containing 400 mM NaCl; error bars show standard deviations of three experiments.

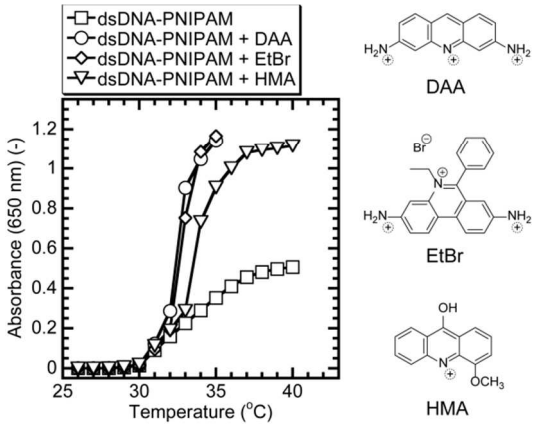

Fig. 4 The aggregation of dsDNA–PNIPAM with 10 eq DAA, EtBr, and HMA, DNA–PNIPAM concentration was 0.050 w/v% in 10 mM Tris-HCl (pH 7.4) buffer containing 400 mM NaCl, merged with the chemical structure of DAA, EtBr, and HMA; the plots represent the average values of three experiments, except for the plots of dsDNA–PNIPAM without intercalator, which represents two experiments.

intercalators. There are two possible causes for this observation. One is that intercalation into dsDNA base-pair pockets renders dsDNA more conformationally restricted, which implies a decrease in the entropy of conformation, thus favoring the aggregation of dsDNA–PNIPAM. Regarding the conformational restriction by an intercalator, the literature reports that intercalation into DNA can

yield a curled or crumpled dsDNA conformation,[38] in which strands seem more restricted conformationally. The other possible cause is that the intercalation induces shielding of the negative charges of dsDNA by the positive charge of the intercalator, thus weakening the electrostatic repulsion of dsDNA. This situation can also induce a change in the control factor from an enthalpic effect to an entropic effect. Furthermore, the entropic effect becomes stronger as the temperature increases;[44] thus, the switch of the control factor from an enthalpic effect to an entropic effect does not occur at low temperature. With the increase in the amount of intercalators binding to dsDNA, the entropic effect readily becomes a stronger controlling factor. Thus, a larger amount of intercalator or an intercalator with higher binding affinity can induce the switch in the driving force of the aggregation phenomena from an enthalpic to an entropic effect by a smaller temperature increase. The different binding affinity changes the temperature at which the switch of aggregation phenomena occurs. These results imply that membrane pores with DNA–PNIPAM as grafted polymer can be opened and closed in response to intercalators, and the membranes can be used as simple, rapid and sensitive analytical tools. Fundamental investigation in this study proved the potential of DNA–PNIPAM for future applications.

Conclusions

We demonstrated that the aggregation phenomena of DNA–PNIPAM were switched by the binding of intercalators to dsDNA and that a small amount of intercalators can affect the aggregation state of DNA–PNIPAM. The balance of enthalpic and entropic effects controls the aggregation phenomena of DNA–PNIPAM. The balance was then shifted from the case when the enthalpic effect is the main control factor to the case when the entropic effect is the main control factor. This is because the dsDNA conformational entropy was reduced by intercalation, and the positive charges of intercalators counteracted the negative charges of DNA. In other words, the change in the physico-chemical states of DNA caused by intercalator binding was amplified to dramatic aggregation of PNIPAM, which made it possible to respond to small amounts of intercalators with different binding affinities.

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Notes and references

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†Electronic Supplementary Information (ESI) available: Details of materials, procedures used for DNA–PNIPAM preparation and aggregation measurements, and fluorescence anisotropy measurement. See DOI: 10.1039/c000000x/

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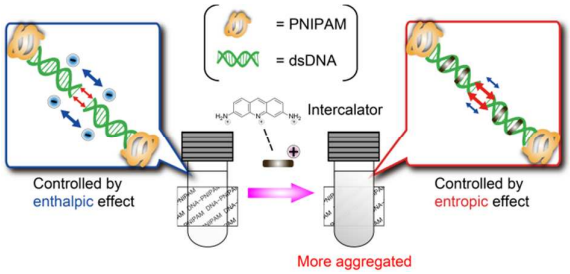

Binding of intercalators to dsDNA switches the aggregation phenomena of DNA–thermoresponsive polymer. The molecular recognition of DNA–intercalator can induce the dramatic aggregation.