Single stranded DNA-poly(*N*-isopropylacrylamide) conjugate for affinity precipitation separation of oligonucleotides

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The conjugate between single-stranded DNA and the temperature-responsive polymer poly(*N*-isopropylacrylamide) was synthesized, and was demonstrated to distinguish its target sequence from mismatch DNAs and separate it from aqueous solution when heated.

Single stranded (ss) DNA has been widely used as an affinity ligand for detection[1] and separation[2] of DNA and RNA having specific sequences because of its high precision in molecular recognition. For affinity separation of a specific sequence, ss DNA-carrying materials such as silica gel,[3] cellulose,[4] and latex particles[5] have been widely applied in the field of molecular and cellular biology. Especially, affinity columns immobilized with poly(dT) or poly(U) are invariably used for the purification of mRNAs from the crude cell extracts.[6] However, the affinity interaction between target sequences and ligand DNA immobilized on the solid materials has been indicated to be weaker and slower than that in homogeneous conditions.[7] Here we describe an alternative system using a conjugate between oligonucleotides and poly(*N*-isopropylacrylamide) (polyNIPAAm) which is known to undergo temperature-directed phase-transition between soluble (< 31 °C) and insoluble (> 31 °C) forms.[8] This conjugate hybridizes with the target sequence in homogeneous solution, and precipitates with the target by the slight change in solution temperature (Fig. 1). The concept of 'thermally-induced affinity precipitation separation' was first proposed by Chen and Hoffman for the separation of IgG using a polyNIPAAm–protein A conjugate.[9]

The vinyl derivative of $(dT)_8$ (**1**) was synthesized by the coupling of 5′-amino-terminated $(dT)_8$ with methacryloyloxy succinimide according to the previous report.[10] **1** (75 μM) and NIPAAm (150 mM) were copolymerized in buffer solution (10 mM Tris–HCl, pH 7.4) at 20 °C using ammonium persulfate (1.3 mM) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (86 mM) as a redox initiator couple in nitrogen atmosphere to give a polyNIPAAm-$(dT)_8$ conjugate with the structure illustrated in Scheme 1. By monitoring the peak area of the monomers on a reversed phase-HPLC, conversions of **1** and NIPAAm were determined to be 71 and 75%, respectively, indicating that the amount of $(dT)_8$ incorporated in the resulting copolymer is almost the same as the feed ratio of **1** to NIPAAm (0.05 mol%) at the polymerization step. The reaction mixture was dialyzed against 10 l of deionized water for a day, followed by lyophilization. The white powder obtained was dissolved in water to 1.0 m/v %. The aqueous solution was then centrifuged at 40 °C. After removal of the supernatant, the precipitated fraction was re-dissolved in water to give a stock solution which was stored at 4 °C. Removal of the unpolymerized constituents (**1** and NIPAAm) was confirmed by disappearance of their peaks on HPLC.

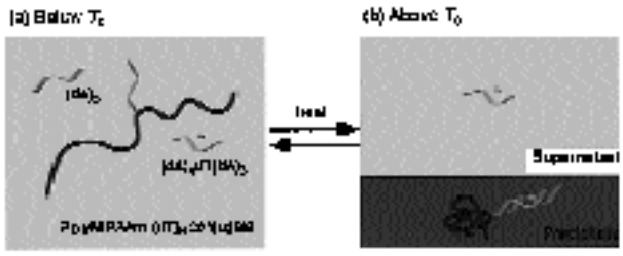
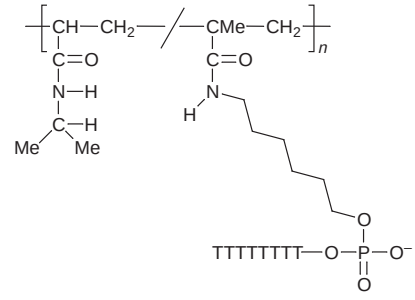


Fig. 1 Schematic illustration of the temperature-directed precipitation of oligonucleotides using polyNIPAAm–$(dT)_8$ conjugate. (*a*) At the temperature below the phase transition point (T_c) of polyNIPAAm, the conjugate is soluble in water and captures its complementary sequence in homogeneous condition. (*b*) At temperatures above T_c, the target sequence is separated to the precipitate with the conjugate. This process is fully reversible.

The resultant conjugate between polyNIPAAm and $(dT)_8$ was applied to the one-pot separation of its complementary sequence $(dA)_8$. The conjugate [0.45 m/v %; 20 μM of $(dT)_8$ in strand] was mixed with the target DNA [$(dA)_8$; 10 μM in strand]. Then 1.0 m/v % of polyNIPAAm was added to the mixture because we found that a certain concentration (> 1 m/v %) of homopolymer was required for the reproducible precipitation of polyNIPAAm-$(dT)_8$ conjugate. The concentration of NaCl and $MgCl_2$ was adjusted to be 1.5 and 0.1 M, respectively. The melting curve of the duplex between $(dT)_8$ and $(dA)_8$ and transmittance curve of polyNIPAAm conjugated with $(dT)_8$ under these solution conditions is shown in Fig. 2. From the curves, melting temperature of the duplex between $(dT)_8$ and $(dA)_8$ was determined to be 16.2 °C, while the transition temperature of the conjugate was *ca.* 14 °C.‡ The mixture (150 μl) was incubated at 0 °C for 6 h for hybridization and then heated to 15 °C for desolubilization of the conjugate. The resulting turbid mixture was centrifuged at the temperature, and the supernatant was collected. The amount of $(dA)_8$ in the supernatant was evaluated by the peak area on HPLC. The precipitate fractions were dissolved in 150 μl of water and analyzed similarly.

As is shown in Table 1, 84% of $(dA)_8$ in the system was concentrated into the precipitate fraction in the presence of conjugate. On the other hand, only a small amount of $(dA)_8$ (*ca.* 6%) was distributed to the precipitate when the separation experiment was performed in the absence of the conjugate. The precipitation % of $(dT)_8$ did not depend on the presence of the conjugate, being a small value of *ca.* 5% which should be ascribed to the incompleteness in the collection of the aqueous phase.



Scheme 1 Chemical structure of the polyNIPAAm–$(dT)_8$ conjugate

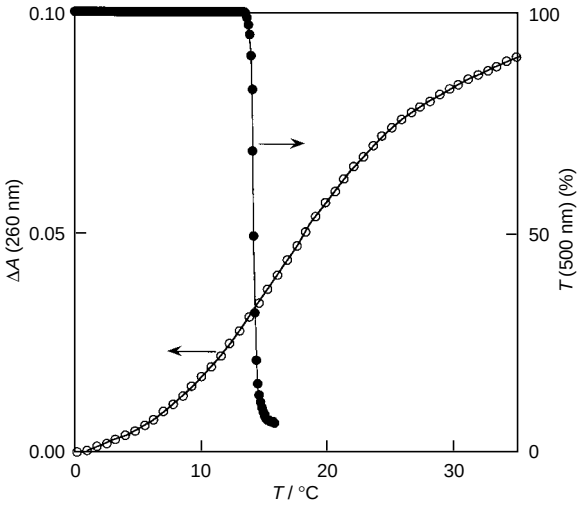


Fig. 2 Melting curve of the $(dA)_8 \cdot (dT)_8$ duplex and transmittance (at 500 nm) curve of the polyNIPAAm–$(dT)_8$ conjugate under the following solution conditions: 1.5 M NaCl, 0.1 M $MgCl_2$ and 10 mM Tris–HCl (pH 7.4). The $(dA)_8 \cdot (dT)_8$ concentration was 3 μM in the strand in the melting temperature measurement, and the concentration of polyNIPAAm–$(dT)_8$ conjugate was 0.068 m/v % [$(dT)_8$ unit, 3 μM in strand] at the transition temperature measurement. Heating rate at both measurements was 0.25 °C min^{-1}.

Table 1 Precipitation efficiency (%) of the oligonucleotides by heating and centrifugation in the presence of the polyNIPAAm–$(dT)_8$ conjugate

	Precipitation %	
Target DNA	+ Conjugate	− Conjugate
$(dA)_8$	83.6 ± 0.8	5.5 ± 0.3
$(dT)_8$	5.3	4.6

An aqueous solution of polyNIPAAm (1 m/v %) and target DNA (10 μM in strand) was heated and centrifuged at 40 °C in the presence (+) or absence (−) of polyNIPAAm–$(dT)_8$ conjugate. Precipitation % was calculated as follows; % = 100 × [DNA found in precipitate fraction]/{[DNA in supernatant] + [DNA in precipitate fraction]}. The precipitation % of $(dA)_8$ is given as mean ± standard error (n = 3).

The applicability of this separation system was further examined for a mixture of $(dA)_8$ and $(dA)_3dT(dA)_4$ (10 μM each). A typical example is shown in Fig. 3: 84.0 ± 1.4 (n = 3) % of $(dA)_8$ was concentrated into the precipitate by the procedure, while 91.7 ± 0.8 (n = 3) % of $(dA)_3dT(dA)_4$ remained in the supernatant. This result clearly indicates that the polyNIPAAm–$(dT)_8$ conjugate distinguished $(dA)_8$ from $(dA)_3dT(dA)_4$, and isolated the complementary DNA selectively from the aqueous solution. In fact, 99% of the precipitated $(dA)_8$ was recovered when the precipitate was resuspended in deionized water and centrifuged at 40 °C.‡

Here we have proposed a simple method for the one-pot separation of ss DNAs with a unique sequence. We have described the separation of $(dA)_8$ using polyNIPAAm–$(dT)_8$ conjugate, because the conjugate having poly(dT) will be useful for the separation of polyadenylated mRNAs which are of great importance in cDNA cloning.

Wolf *et al*. reported that ss DNA attached to latex particles hybridized, in certain conditions, with its complementary oligonucleotide at a comparable rate with free DNA, while it

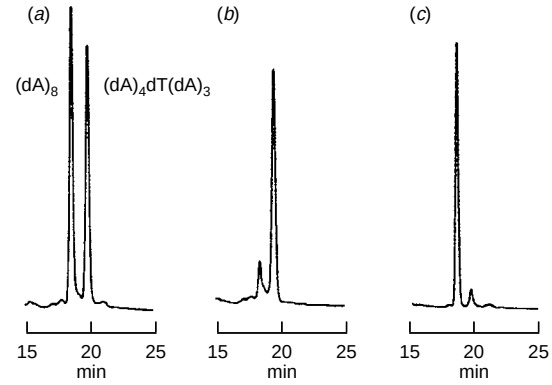


Fig. 3 HPLC chromatograms of $(dA)_8$ and $(dA)_3dT(dA)_4$ before and after the precipitation separation using polyNIPAAm–$(dT)_8$ conjugate. The equimolar mixture of $(dA)_8$ and $(dA)_3dT(dA)_4$ was heated in the presence of polyNIPAAm–$(dT)_8$ conjugate and centrifuged. (*a*) HPLC chromatogram before the process. (*b*) HPLC chromatogram of the supernatant fraction after the process. (*c*) HPLC chromatogram of the precipitated fraction after the process.

hybridized much slower with the larger DNAs,[11] probably because of the steric repulsion due to the latex surface. In this sense, the soluble conjugate described here would be advantageous especially for the larger target molecules such as mRNAs. In addition, this property would be also advantageous even in the post-separation stages. For instance, we hypothesize that cDNA synthesis of the separated mRNA on the polyNIPAAm–poly(dT) conjugate would be much more efficient than that on solid materials such as poly(dT)–silica.[12] These conjectures are under further investigation.

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Notes and References

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‡ PolyNIPAAm undergoes dehydration (and precipitation) at *ca.* 31 °C in deionized water, as was also the case for the polyNIPAAm–$(dT)_8$ conjugate. However, the addition of salt lowers the transition temperature of polyNIPAAm,[8] which was found to be 14 °C in the present solution conditions for the one-pot separation.

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