Affinity separation of messenger RNA by thermo-responsive polymer carrying oligo(dT)

Takeshi Mori, Fumi Oda, Daisuke Umeno, Masaharu Murata, and Mizuo Maeda
Department of Materials Physics and Chemistry, Graduate School of Engineering, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

ABSTRACT
The conjugate between oligo(dT)\textsubscript{16} and thermo-responsive polymer, poly(N-isopropylacrylamide), was prepared for isolation of poly(A)\textsuperscript{*} RNA from total RNA. The hybridization reaction between the conjugate and poly(A) (average length: 320 base) was equilibrated in 10 min, and all the poly(A) (16 nmol base for 24 nmol base of conjugate) was precipitated when raising the solution temperature to 35 °C. The precipitate was dissolved in water, and poly(A) was dissociated from the conjugate by heating to 65 °C. This separation system was successfully applied to the isolation of poly(A)\textsuperscript{*} RNA from total RNA.

INTRODUCTION
Isolation of poly(A)\textsuperscript{*} RNA from total RNA or cell extracts is important as a first process for cDNA cloning, and oligo(dT)- or poly(U)-immobilized column has been used for this process. Recently, the batch type separation systems using oligo(dT)- or poly(U)-immobilized materials such as latex particles (1), magnetic beads (2), and paper sheets (3) have been replacing the conventional column method. We present here a novel batch type isolation system of poly(A)\textsuperscript{*} RNA using oligo(dT)-carrying water-soluble polymer, poly(N-isopropylacrylamide) (polyNIPAAm), which is known to undergo temperature-induced phase transition between soluble (< 31 °C) and insoluble (> 31 °C) forms (4). This conjugate between polyNIPAAm and oligo(dT) hybridizes with poly(A)\textsuperscript{*} RNA in homogenous solution, and precipitates with the RNA by heating the solution to 35 °C.

RESULTS AND DISCUSSION
The conjugate between polyNIPAAm and oligo(dT)\textsubscript{16} with the structure shown in Figure 1 was synthesized according to the method reported previously (5). The (dT)\textsubscript{16}-polyNIPAAm conjugate containing 0.034 mol\% of (dT)\textsubscript{16} macromonomer unit was used in this study. At first, the conjugate was applied to the temperature-induced precipitation separation of poly(A) as a model for poly(A)\textsuperscript{*} RNA. The conjugate (0.3 wt%; containing 160 μM base of (dT)\textsubscript{16}) and poly(A) (average length: 320 base) were mixed in 10 mM Tris-HCl (pH 7.4) containing 0.5 M NaCl. Then, 1 wt% of polyNIPAAm was added as a co-precipitating agent. For the hybridization between poly(A) and conjugate, the solution (150 μl) was incubated at 10 °C for 10 min (we found that the reaction became equilibrated within 10 min). Then the solution was heated to 35 °C for 3 min to precipitate (dT)\textsubscript{16}-polyNIPAAm conjugate (at these solution conditions, phase transition temperature of the conjugate was 28 °C). The resultant turbid mixture was centrifuged for 15 min at 35 °C (17 000 g). The supernatant was collected, and the precipitate was dissolved in 150 μl of RNase free water. The obtained solution was heated to 65 °C for 5 min to dissociate poly(A) from the conjugate (we confirmed that almost all the poly(A) was dissociated by this procedure). The resultant turbid mixture was centrifuged at 40 °C for 15 min (17 000 g), and then the supernatant was collected. The precipitate
fraction was dissolved in 150 μl of RNase free water. The amount of poly(A) in each fraction was quantitated by UV absorbance at 260 nm.

As shown in Figure 2, all the loaded poly(A) was precipitated and recovered from conjugate (containing 24 nmol base of (dT)16) when the amount of loaded poly(A) was less than 16.5 nmol base. Application of larger amounts of poly(A) to the conjugate resulted in incomplete recovery of poly(A). For a control experiment, these separation procedures were carried out for poly(C) (average length: 600 base). However, poly(C) was not precipitated at all by the conjugate. These results indicate that the conjugate has binding specificity for poly(A).

We applied this separation system for isolation of poly(A)+ RNA from total RNA. The conjugate (0.3 wt%; containing 160 μM base of (dT)16 and 1.1 mM base of total RNA (mouse brain; Ambion Inc., USA) were mixed in 10 mM Tris-HCl (pH 7.4) containing 0.5 M NaCl, 1 mM EDTA and 0.1 wt% SDS (buffer A). 1 wt% of polyNIPAAm was also added. The mixture (200 μl) was incubated for hybridization and heated for precipitation according to the same procedures as described above. The washing of the resultant precipitate was carried out twice by dissolving it in 200 μl of buffer A, followed by heating to 35 °C for precipitation. For dissociation of RNA from the conjugate, the resultant solution was treated similarly as described above. The recovered RNA was precipitated with ethanol, and analyzed by denaturing agarose gel electrophoresis.

As shown in Figure 3, the ribosomal RNA bands (28S, 18S), which were clearly observed in total RNA (lane 1), disappeared and a smeared band resulting from poly(A)+ RNA was observed in the recovered fraction (lane 2). This result indicates that the conjugate selectively bound to poly(A)+ RNA and separated it from other RNAs.

We reported here a novel poly(A)+ RNA isolation system using (dT)16-polyNIPAAm conjugate. The principal advantage of this method is that hybridization reaction is conducted in a homogeneous solution. This property would be also advantageous in the following direct cDNA synthesis of the separated poly(A)+ RNA on the conjugate.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Science, Sports and Culture of Japan. Financial support by the Tokuyama Science Foundation is also acknowledged.

REFERENCES