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In Vitro Selection of the RNA Aptamer against the Sialyl Lewis X and Its Inhibition of the Cell Adhesion

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Sialyl Lewis X (sLe^X) is a tetra-saccharide glycoconjugate of membrane proteins. It acts as a ligand for the selectin proteins during cell adhesion of inflammatory process. Aberrant overexpression of sLe^X is also a characteristic of various cancer cells, especially for highly malignant ones. In this paper, the sLe^X-specific RNA aptamer was selected using a random RNA library and its affinity and specificity were measured by Surface Plasmon Resonance technique. Affinity of the selected RNA was increased about 1000-fold as compared with the original RNA pool. RNA aptamer bound more specifically to its cognate sugar than to any other similar sugars. Inhibition of the cell adhesion was also shown by *in vitro* static assay of sLe^X-expressing HL60 cells to the E- and P-selectins. It suggests that the high affinity carbohydrate specific RNA aptamer could be used as an alternative to the antibody. © 2001 Academic Press

Key Words: **RNA combinatorial library; *in vitro* selection; RNA aptamer; Sialyl Lewis X; E-selectin; P-selectin; cell adhesion.**

Carbohydrates are important mediators in the specific recognition and adhesion between cells (1). Sialyl-Lewis X (sLe^X) is one of the glycans on the cell surface and is also known as a ligand for the selectin proteins (L-, E-, and P-selectins) (2–4). Selectin-sLe^X interaction seems to play a critical role in the initial stage of the inflammation and the cancer cell metastasis (5–9). Considering roles of sLe^X in these processes, a blocker for sLe^X-selectin interaction would be useful as an anti-inflammatory or as an anti-metastasis drug (10–13). Mimicry of sLe^X has been developed by several groups of researcher as potential therapeutics (14). However, chemical synthesis of sLe^X mimetics has limited its use as a practical method not only by the low yield of synthesis but also by the low binding affinity and poor specificity of small molecules. Various antibodies also have been developed as molecules recognizing sLe^X and blocking the sLe^X-selectin interaction (15, 16). However, broad specificity and moderate binding affinities of antibodies might hinder their uses as specific and tight binding molecules (17, 18).

Combinatorial RNA library and affinity selection might have been an alternative method for generating RNA molecules with high affinity and specificity (named as "aptamer"; 19, 20). Previous attempt to select L-selectin binding aptamer resulted in specific L-selectin binding antagonists, which exhibits inhibition of the L-selectin mediated cell adhesion (21, 22). It suggested that the SELEX (Systematic Evolution of Ligand by Exponential enrichment) procedure was extremely useful method for developing specific aptamers for the cell surface proteins. However, no one ever reported a selection of aptamers for the ligand of the selectin, sLe^X carbohydrate. Considering high concentration of sLe^X glycoconjugates on leukocytes and cancer cells, the cell surface carbohydrate could have been a more effective target for prohibiting sLe^X-selectin mediated cell adhesion during inflammation and metastasis.

In this report, we employed the RNA combinatorial library and *in vitro* selection method to develop the sLe^X binding RNA aptamers. Affinities of selected RNA aptamer were subnanomolar to nanomolar range with high specificity to the sLe^X. The RNA aptamer inhibited the adhesion of sLe^X rich HL60 cells to E- and P-selectin, suggesting that it could be used as a potential cell adhesion blocker. Our results in this report suggest that RNA aptamer against a carbohydrate could be used as a useful lectin, especially as a plausible substitute for antibody against the carbohydrates.

MATERIALS AND METHODS

Cells and other reagents. HL60 cells were maintained at 37°C in RPMI1640 (GIBCO BRL) supplemented with 10% Fetal Bovine Serum and 1% penicillin/streptomycin. Normal mouse IgM was obtained from Sigma and the monoclonal anti-sLe^X antibody (KM93)

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was purchased from Seikagaku Co. sLe^X and sLe^X-BSA were from Oxford Glycosciences, BSA and recombinant soluble E- and P-selectins were from Calbiochem. All other chemicals were purchased from Sigma or Aldrich and used without further purification.

Preparation of affinity matrix. Two hundred milligrams of cyanogen bromide (CNBr) activated agarose beads was washed with 2.5 ml of 10 mM diethylpyrocarbonate-treated NaOAc (pH 4.5) to remove lactose stabilizer. Activated beads were washed 5 times with 2.5 ml of 100 mM Hepes buffer (pH 8.0). To prepare sLe^X-conjugated column, the beads were allowed to mix with 2.5 ml of 10 μM sLe^X solution in Hepes buffer for 2 h at room temperature with stirring. Control bead was also prepared by the same method, except omitting sLe^X in the binding buffer. To block unbound activated groups, 0.25 ml of 1 M *n*-buthylamine was added to a resulting suspension and stirred for 2 h at room temperature. Beads were then washed again with 2.5 ml of Hepes buffer and stored at 4°C. To be used as affinity matrices, prepared beads were washed and pre-equilibrated with RNA binding buffer [150 mM NaCl, 20 mM Hepes (pH 7.4), 1 mM $CaCl_2$, 1 mM $MgCl_2$] just before use.

Preparation of the RNA library. Random DNA library was synthesized by the Midland Company (Midland Certified Reagent Co.). Random region was composed of 70 nucleotides flanked by 5′ and 3′ primer regions. At the primer regions, T7 promoter binding sequences and a few restriction sites were included for the *in vitro* transcription and the cloning process, respectively. Therefore, 5′ and 3′ primer for PCR and *in vitro* transcription was 5′-CCGTAATACGACTCACTATAGGGGAG-CTCGGTACCGAATTC-3′ and 5′-AAGGATCCTCTGCAAAGCTT-3′, respectively. Ten tubes of polymerase chain reactions (PCRs) were performed with 57.5 μM of 110-mer DNA random library, 200 μM each of 5′- and 3′-primers, 1.6 mM $MgCl_2$, 2.5 mM each of dNTPs, 1 U/μl of Taq DNA polymerase. DNA library (3.5×10^{14} molecules) was converted to the RNA library by *in vitro* transcription reaction in 50 mM Tris (pH 7.5), 7.5 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 100 mM DTT, 0.5 mM each of rNTPs, 1 U/μl of RNase inhibitor, 50 U of T7 RNA polymerase for 2 h in 37°C. To make the original RNA pool, PCR was limited to ten cycles, not to amplify skewed population of random RNA library. Template DNA was removed by the DNaseI digestion and the resulting RNA was purified by phenol:chloroform extraction and ethanol precipitation. The RNA band of expected 110 nucleotides was cut from the 7 M urea/6% polyacrylamide gel and eluted from the gel by incubating in 0.5 M ammonium acetate, 0.1% SDS, 1 mM EDTA for 3 h in 37°C. Purified RNA library was quantitated by using an UV spectrophotometer.

In vitro selection procedure. One milligram of the original RNA library was used in the initial selection cycles. Following a brief incubation at 75°C in the RNA binding buffer [150 mM NaCl, 20 mM Hepes (pH 7.4), 1 mM $CaCl_2$, 1 mM $MgCl_2$], RNA was cooled to room temperature to make most stable structures before loading onto the column. To prevent enrichment of the nonspecific RNA with an agarose bead binding activity, counter-selection with the agarose precolumn was performed in each cycle. Flow-through fraction of was collected and equilibrated with the sLe^X-conjugated agarose bead for 30 min in room temperature. After washing with 50 column volume of binding buffer, bound RNA was affinity eluted with 10 mM sLe^X in early selection rounds; after 9 cycles, 4 M NaCl and 100 mM EDTA was used sequentially to elute bound RNA. Eluted RNA was extracted and concentrated either by ethanol precipitation or by Sephadex G50 chromatography. RNA was reverse transcribed by 50 units of AMV reverse transcriptase (Promega) and amplified by 20 cycles of PCR. After confirming the PCR products by 2% agarose gel, DNA was transcribed to RNA by *in vitro* transcription as described above. The RNA band of right size was purified by eluting from 6% polyacrylamide/7 M urea gel and used for the next round of selection. After 17 rounds of the selection, RNA was converted to DNA and amplified by primers containing recognition sites for *Sac*I (in 5′ primer) and *Bam*HI (in 3′ primer). Using these restriction sites, DNA sequences were inserted into pBluescriptII-KS and the recombinant DNA was transformed into *Escherichia coli* DH5α. Plasmid DNA were prepared from 20 different colonies, and sequenced both with silver staining sequencing kit (GIBCO BRL) and with automatic sequencer (ABI prism).

Radiolabeled RNA binding assay. Selected RNA was radiolabeled with ^{32}P to test enrichment of high affinity RNA to sLe^X. Approximately 0.5–1 μg of DNA was incubated in transcription buffer [50 mM Tris (pH 7.5), 7.5 mM $MgCl_2$, 2 mM spermidine, 10 mM NaCl, 100 mM DTT, 500 μM each of rAGCmix, 10 μM rUTP] with 30 μCi [α-^{32}P]UTP (800 Ci/mmol), 50 unit of T7 RNA polymerase, 20 unit of RNase inhibitor (Promega) at 37°C for 2 h. Reactions were then terminated by incubating with 4 units of the RQ1 DNase at 37°C for 15 min. After extracting with phenol/chloroform/isoamylalcohol, RNA was precipitated with ethanol, resuspended and electrophoresed on a 6% polyacrylamide/7 M urea gel. Correct size RNA was isolated from the gel and the radioactivity was measured by the scintillation counter. About 10^4–10^5 cpm of labeled RNA was incubated either with agarose bead or with sLe^X-conjugated agarose bead in RNA binding buffer for 30 min in room temperature as above. Unbound RNA was removed by washing the beads 3–4 times; bound RNA was eluted from the bead by phenol extraction, loaded to 6% PAGE/7 M urea gel. The gel was dried and the bound RNA was directly visualized by autoradiography.

Biosensor assay. Biacore 2000 was used for the surface plasmon resonance experiments. To attach sLe^X ligand to the CM5 sensor chip, surface of the chip was preequilibrated with Hepes and activated with 0.05 M of *N*-hydroxysuccinimide (NHS) and 0.2 M of *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide (EDC) by modifying carboxymethyl group. After preactivation, sLe^X-BSA (12.6 mol sLe^X/mol BSA) solution was injected to one of the flowcell and BSA to the other. After immobilization of the ligand, the chip surface was deactivated with 1 M ethanolamine hydrochloride, pH 8.5. After stabilizing the base line, various concentrations (500, 250, 125, 62, and 31 nM) of the original RNA pool, SELEX cycle 17 RNA and cloned RNA were injected to measure K_D values of these RNA. After each analyte injection, 10 mM EGTA was used to regenerate the ligand surface.

To test the specificity of selected RNA to sLe^X, RNA was immobilized to sensor chip, and various carbohydrates were analyzed for their binding to RNA. First, 5′-ends of original RNA and the selected clone 5 RNA were biotinylated with oligonucleotide biotin labeling kit (Amersham Life Science). Biotinylated RNA samples were then immobilized to two different flowcells of the streptavidin (SA) sensor chip. sLe^X-specific KM-93 monoclonal antibody and control normal mouse IgM were also immobilized to the other flowcells for comparison. Many different types of carbohydrates, including sialyl Lewis X, Lewis X, sialyl Lewis A, and Lewis A were injected as analytes. At least five different concentrations of each carbohydrate were injected to measure binding constants.

Cell adhesion assay. Recombinant soluble E-selectin or P-selectin (Calbiochem) was added to the microtiter plate (250 ng/well) in 0.05 M $NaHCO_3$ at pH 9.2 (10 μg/ml) and incubated overnight at 4°C. TNF-α activated (10 ng/ml for 20 h) HL60 human promyelocytic leukemia cells were harvested and suspended to a density of 1×10^6 cells/ml in RNA binding buffer [150 mM NaCl, 10 mM Hepes (pH 7.4), 1 mM $CaCl_2$, 1 mM $MgCl_2$]. Fifty microliters of cell suspension was pipetted into the wells and incubated for 15–30 min at room temperature. Cells were washed with washing solution (DEPC-treated 1× PBS, 1 mM $CaCl_2$, 1 mM $MgCl_2$) until no floating cells were detected. BSA was also coated to the plate to measure nonspecific bindings. To measure the inhibition of the cell adhesion by RNA molecules, cells were preincubated with various amounts of the original RNA, the clone 5 RNA and the yeast tRNA for 30 min at room temperature and the numbers of adhered cells were measured as described above. Adhered cells were counted and averaged from 10 different microscopic fields, excluding the highest and the lowest numbers from each set of data. For each data, duplicate reactions were performed.

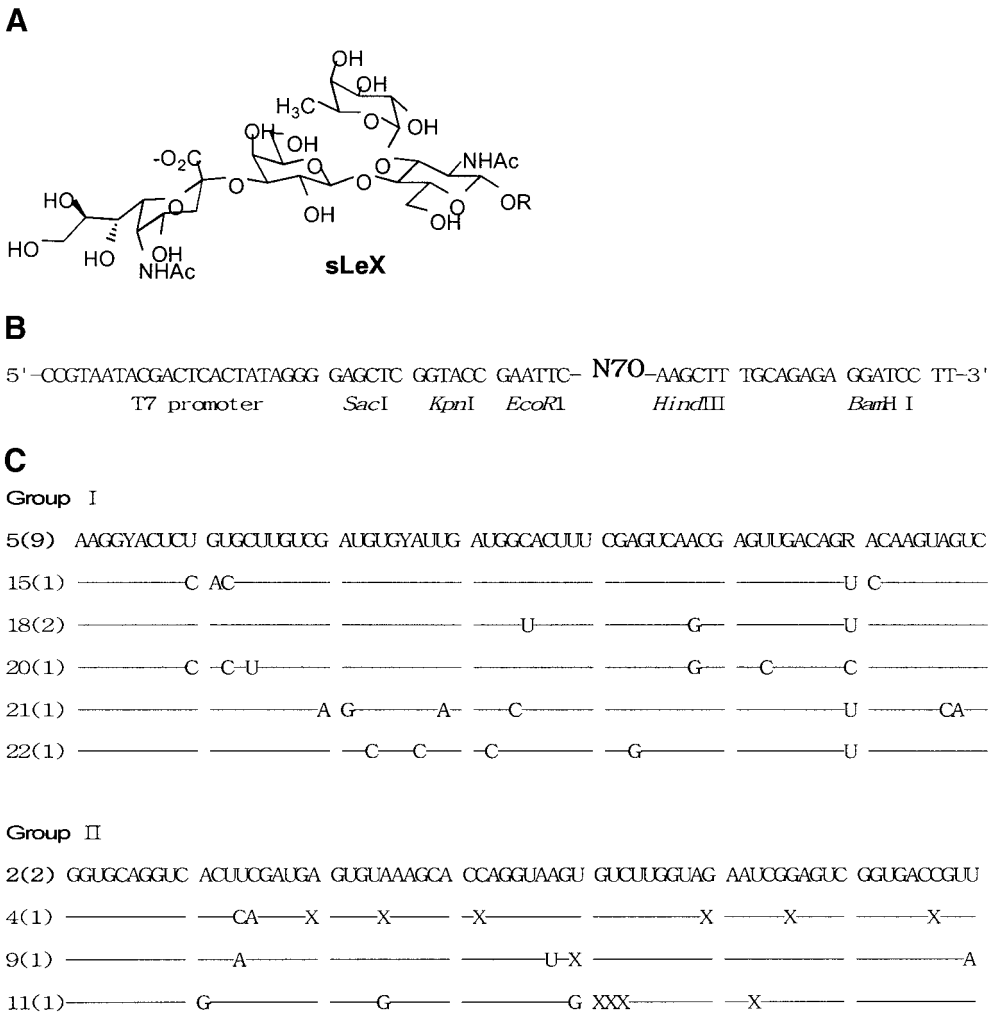

FIG. 1. Sequences and structures of the RNA library and the selected RNA aptamers. (A) Structures of sialyl Lewis X (sLeX) sugar molecule. (B) Sequences of DNA for the Original RNA Library. Random 70 nucleotides are flanked by the defined sequences at the 5′- and the 3′-ends that could be used as the PCR primers and the cloning restriction sites. The 5′ sequences include the T7 RNA promoter for the transcription of random RNA. (C) Sequences of the cloned RNA aptamers. Among 15 clones of Group I, highly homologous sequences, as shown for clone 5, are found in 9 independent clones. In Group II, a couple of clones were found to have deletions (shown as X). Y (pyrimidine), R (purine) and N (A or C). Other clones with minor mutations are also shown. Numbers in parentheses represent the number of clones found for each sequence.

RESULTS

Selection of sLe^X-Specific RNA

In order to select the sLe^X-specific RNA, the affinity matrix for the sLe^X was prepared by attaching sLe^X to the agarose beads using hydroxyl functionality in the sLe^X (Fig. 1A; 23). The 110-mer DNA library was designed to contain random nucleotides in the central 70 positions flanked by the defined sequences at each end. T7 promoter was placed at the 5′ of the random sequences for *in vitro* transcription; various restriction sites were included at each end for the PCR amplification and for the cloning of selected sequences (Fig. 1B). To remove non-specific RNA binding to the agarose, RNA molecules were first loaded to the agarose precolumn and the unbound fraction was loaded to the sLe^X-immobilized column. After washing the column with excess binding buffer, sLe^X bound RNA was affinity eluted with the sLe^X solution in the early cycles or with high salt and EDTA in the later cycles. The eluted RNA was then reverse transcribed and amplified by PCR for the next round of the selection. After enriching RNA by 17 cycles of selection, RNA molecules were cloned into pBluescriptII-KS using *Sac*I and *Bam*HI sites in 5′- and 3′-ends, respectively. Specific sequences were enriched as shown in Fig. 1C.

Selected RNA Binds to sLe^X with High Affinity

We evaluated the binding affinity of the selected RNA by two methods. First, RNA was radiolabeled,

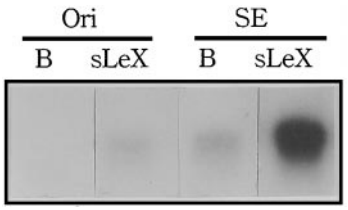

FIG. 2. Enrichment of sLe^X binding RNA following *in vitro* selection cycles. Binding assay was performed with the Original RNA library (Ori) and RNA after 17 cycles of SELEX (SE). RNA was radiolabeled with [α-^{32}P]UTP and incubated either with sLe^X-conjugated bead (sLeX lanes) or with bead alone (B lanes). After washing out nonspecific binding RNA, bound RNA was eluted from the beads, electrophoresed in 6% PAGE/7 M urea gel and directly visualized by the autoradiography. In contrast to no detectable binding of the Ori RNA pool, large amount of SE RNA bound to the sLe^X.

allowed to bind to the sLe^X conjugated agarose bead and its binding was visualized by gel electrophoresis and autoradiography (Fig. 2). RNA from 17 rounds of selection (SE) binds strongly to the sLe^X (sLeX lane), while it hardly binds to the agarose bead (B lane), suggesting that the RNA aptamer specifically binds to sLe^X but not to agarose bead itself. In contrast, almost no original RNA pool (Ori) binds either to the sLe^X or to the agarose bead.

Secondly, the surface plasmon resonance (SPR) measurement was employed to accurately assess the binding affinities of the selected RNA pool and the cloned RNA aptamers (24). After immobilizing the sLe^X-BSA and the control BSA into each flowcell of the sensorchip, RNA solution was injected to the chip. Selected RNA bound more strongly to sLe^X-BSA over to BSA, suggesting that the selected RNA specifically bound to sLe^X portion of the sLe^X-BSA, but not to BSA protein. The binding parameters of many different RNA molecules were determined by injecting six different concentrations of RNA (Table 1). Binding affinity of the selected RNA was increased 1000-fold, up to subnanomolar scale as compared to the original pool of RNA. Interestingly, all of the selected clones showed similar or even better binding affinities to sLe^X than the commercially available anti-sLe^X antibody KM-93 did. It suggests that the *in vitro* RNA selection against the carbohydrates might generate higher affinity aptamers, which could substitute for lower affinity antibodies for non-antigenic small molecules.

Specificity of the Selected RNA

Since the clone 5 RNA was found to have the maximum binding affinity, we utilized it as a model aptamer for testing the specificity. Clone 5 RNA was immobilized to the sensor chip and various carbohydrates were injected for the SPR measurement. To immobilize the RNA molecules without disturbing native structure, the 5′-end of the RNA was biotinylated and attached to the streptavidin-coated sensorchip.

TABLE 1

Binding Affinity of the Selected RNA Aptamers to sLe^X

RNA	k_a (1/M sec)	k_d (1/sec)	K_A (1/M)	K_D (M)
Original pool	2.6×10^4	3.3×10^{-3}	7.6×10^6	1.3×10^{-7}
Selected pool	2.4×10^5	1.4×10^{-3}	1.7×10^9	5.8×10^{-10}
Clone 5	1.3×10^5	1.1×10^{-5}	1.1×10^{10}	8.5×10^{-11}
Clone 2	9.8×10^5	7.3×10^{-5}	1.2×10^9	8.0×10^{-10}
Clone 15	3.5×10^5	8.1×10^{-4}	4.3×10^8	2.3×10^{-9}
Clone 18	5.1×10^5	2.0×10^{-3}	2.5×10^8	3.9×10^{-9}
Clone 4	4.1×10^5	3.1×10^{-3}	1.3×10^8	7.4×10^{-9}
Clone 9	3.5×10^5	3.1×10^{-3}	9.5×10^7	1.0×10^{-8}
Anti-sLe^X Ab	2.5×10^3	1.7×10^{-5}	1.5×10^8	6.7×10^{-9}

Note. Binding parameters of the sLe^X to the various RNA molecules and the antibody was measured by the SPR technique. sLe^X-BSA and BSA were immobilized to different flowcells of biosensor CM5 chip and at least five different concentrations of various RNA and antibody were injected to determine kinetic parameters. After subtracting background binding to BSA, binding parameters for sLe^X-BSA were evaluated using BIA evaluation program. Only the data that meet the evaluation criteria are presented here.

The K_D value of the RNA aptamer for the sLe^X-BSA was much higher than for that of BSA (Table 2), confirming the previous result. Binding parameters of the clone 5 RNA aptamer to various carbohydrates were also presented in Table 2. The RNA aptamer tightly bound to the cognate molecule, sLe^X, followed by Le^A, sLe^A and Le^X. Even though the binding affinity to the sLe^X is only 5–10 times stronger than similar Lewis group sugars, it is 100 times higher than that of the dissimilar sugar, such as the lactose. Above binding data suggested that the selected RNA aptamer could discriminate the minor differences in carbohydrates, with the highest affinity to its cognate sugar, sLe^X.

TABLE 2

Specificity of the RNA Aptamer

Carbohydrates	k_a (1/M sec)	k_d (1/sec)	K_A (1/M)	K_D (M)
sLe^X-BSA	6.4×10^7	3.7×10^{-3}	1.7×10^{10}	5.7×10^{-11}
BSA	2.2×10^4	2.3×10^{-3}	9.9×10^6	1.0×10^{-7}
sLe^X	1.7×10^5	5.5×10^{-4}	3.0×10^8	3.3×10^{-9}
sLe^A	1.2×10^3	1.9×10^{-5}	5.9×10^7	1.7×10^{-8}
Le^X	6.7×10^2	1.6×10^{-5}	4.1×10^7	2.4×10^{-8}
Le^A	7.3×10^2	1.0×10^{-5}	7.2×10^7	1.4×10^{-8}
Lactose	6.3×10^2	1.8×10^{-4}	3.4×10^6	2.9×10^{-7}

Note. Binding parameters of the clone 5 RNA aptamer to sLe^X-related various sugars as measured by the SPR technique. Clone 5 RNA and Original RNA library were biotinylated and immobilized to the different flowcells of the streptavidin-coated biosensor chip. Various concentrations of the carbohydrates were injected and the binding parameters were determined. Specific binding to the clone 5 RNA aptamer was calculated for each sugar, after subtracting background binding to the original RNA library. Specificity of RNA aptamer was also confirmed by injecting sLe^X-BSA or BSA to the immobilized clone 5 RNA aptamer.

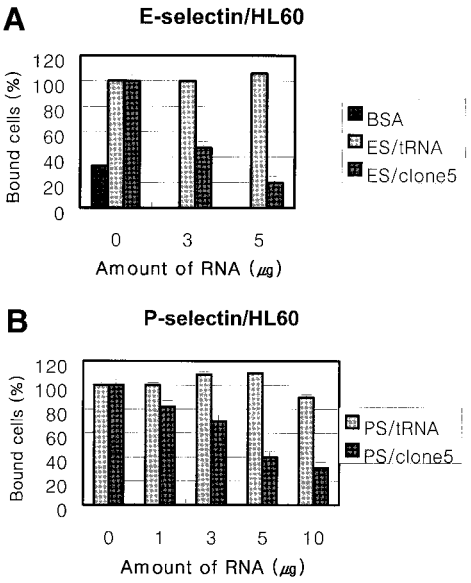

FIG. 3. Inhibition of the HL60 cell adhesion to E-selectin and P-selectin. (A) Recombinant soluble E-selectin was coated to the microtiter plates and the activated HL60 cells were incubated along with various amounts of the clone 5 RNA aptamer or with a control yeast tRNA. After washing out nonspecifically bound cells, numbers of adhered cells were counted. Percentages of the adhered cells were presented here in comparison to the cells without RNA incubation. (B) Inhibition of the HL60 cell adhesion to the P-selectin was performed as described above.

Inhibition of the HL60 Cell Binding to E- and P-Selectins

Since the selected RNA aptamer binds to sLe^X with high affinity and specificity, it is presumed to act as a blocking agent for the sLe^X-mediated cell adhesion. We therefore tested whether the clone 5 RNA aptamer could inhibit the cell adhesion mediated by the sLe^X and the selectin proteins. Static microtiter cell adhesion assay was performed by immobilizing recombinant E- or P-selectins on the plate, followed by incubating with sLe^X expressing cells on the protein coated wells. Promyelocytic leukemia cell line HL60 was used in this study, because the surface of the cell was rich in sLe^X, especially after TNF-α treatment (25). The number of HL60 cells that adhered to the E-selectin coated wells was much higher than that of control BSA-coated wells, as expected (Fig. 3A). To test whether the RNA aptamer could specifically inhibit the HL60 adhesion to the E-selectin by blocking the cell surface sLe^X, various amounts of the clone 5 RNA aptamer were incubated with the HL60 cells and allowed to bind to the cell surface sLe^X. As the amount of the RNA aptamer was increased, the adhered cells to the E-selectin coated plates were significantly reduced. Such inhibitory effect of the clone 5 RNA aptamer is not resulted from nonspecific binding of the RNA, because the same amount of tRNA did not reduce the cell adhesion. Control experiments were also performed to confirm the specific inhibition of the sLe^X mediated cell adhesion.

Similar results were obtained when HL60 cells were incubated with P-selectin coated plates (Fig. 3B). Clone 5 RNA aptamer inhibited cell adhesion, whereas tRNA did not reduce or even increased the adhesion of HL60 to P-selectin. These results suggest that most of the interaction between E- and P-selectin and the sLe^X on the HL60 cells are specific and could be inhibited by the sLe^X binding RNA aptamer. More studies needs to be done to demonstrate the inhibitory effect of the RNA aptamer.

DISCUSSION

In this study, we utilized the *in vitro* RNA selection method to develop novel RNA aptamers to the sLe^X sugar of the cell surface glycoproteins. Selection from a large pool of the original random RNA (3.5×10^{14} molecules) resulted in the enrichment of the specific RNA aptamers. Binding affinity was increased about 1,000-fold after 17 rounds of iterative selections. Selected RNA aptamer was found to have higher binding affinity to its cognate sugar, sLe^X and similar Lewis group sugars, but much lower affinity to dissimilar ones.

Large combinatorial pool of RNA could adopt various conformations that could bind to the target molecules with high affinity and specificity (26–28). *In vitro* selection of the RNA library has been an extremely useful method for isolating high affinity RNA aptamers against various proteins and small molecules (29–32). Sugar molecules could specifically bind to RNA, as demonstrated by the selection of aminoglycoside binding RNA aptamers (33–35). Selection of the RNA aptamers against aminoglycosides could have been relatively easily achieved due to the strong charge-charge interactions between protonated amino functionalities in aminoglycosides and negatively charged phosphate backbones in the RNA molecules. However, the interactions of RNA molecules with the carbohydrates in the cell surface were rarely studied, in spite of their potency as therapeutic targets.

Selection of the RNA aptamer that binds to other neutral sugar, such as the cellobiose unit of cellulose, has been reported (36). It suggests that carbohydrate-binding RNA aptamer could be selected, even though it has relatively low affinity (around 10^{-5} to 10^{-7} M) and broad specificity. In this study, we have shown that the K_D of sLe^X binding RNA aptamer is around 10^{-9} to 10^{-11} M and it has high specificity for the sLe^X related sugars. To our knowledge, this is the first report on the selection of RNA aptamer that has nanomolar affinity with specificity against carbohydrate antigen in cell surface glycoconjugates.

Selection of RNA and/or DNA aptamer against L-selectin has been previously shown to have an an-

tagonistic effect for the binding (21, 22). Even though the flow-cell rolling assay needs to be done to directly demonstrate the inhibition of cell adhesion, the RNA aptamer against the sLe^x sugar was likely act as an inhibitor for the cell adhesion, as shown in this study. Specific inhibitors for cell adhesion mediated by selectin and sLe^x is hoped to be an extremely useful therapeutic agents for chronic inflammation and cancer cell metastasis. High affinity sLe^x-specific RNA aptamer that we presented in this paper might shed light on the development of a lead molecule for the cell adhesion blocking anti-inflammatory therapy.

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