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Notes

Characterization of PEGylated Anti-VEGF Aptamers Using Surface Plasmon Resonance

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Introduction

Aptamers are a new class nucleic acid therapeutics with different characteristics from antisense oligodeoxy-nucleotide (ODN), artificial transcript factor and small interfering RNA (siRNA) which interrupt the synthesis of target proteins. Aptamers form unique three-dimensional structures and bind to target protein molecules with high affinity and specificity, inhibiting the function of target proteins.[1] Systemic evolution of ligands by exponential enrichment (SELEX) has been devised and used to rapidly isolate specific oligonucleotide sequences (aptamers) from the libraries of RNA, DNA, or modified nucleic acids.[2] Typically, aptamers are 10-15 kDa (30-45 nucleotides) in size and have a sub-nanomolar affinity to their target molecules.[3,4] Due to the attractive physico-chemical characteristics, there have been considerable research efforts for the development of aptamers as a promising alternative to the conventional therapeutic antibody drugs.[5] However, a short half-life of aptamer, caused by the degradation by nuclease and the rapid renal clearance,[6] hampered its clinical applications. In order to achieve a therapeutically applicable long circulation, the chemically modified nuclease-protected aptamer has been conjugated to poly(ethylene glycol) (PEG).[7,8] PEG has been used for various applications.[9-11] Macugen™ (pegaptanib) is the first FDA-approved aptamer therapeutics in 2004. It is a PEGylated vascular endothelial growth factor (VEGF) inhibitor developed by Pfizer and Eyetech Co. for the treatment of age-related macular degeneration.[12]

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In this work, we proposed a novel protocol using surface plasmon resonance (SPR) for the analysis of binding characteristics of aptamers before and after PEGylation. SPR has been used for a binding affinity analysis of various biomolecules.[13] Anti-VEGF 2'-O-methyl (2'-OMe) RNA aptamer was synthesized and used as a model for various aptamer therapeutics. The anti-VEGF aptamer consists with 27 nucleotides and binds to VEGF in a calcium-dependent manner.[12] Aptamers, which contain 2'-OMe nucleotides, are stable *in vivo*, inexpensive to synthesize, and ubiquitous in biological systems.[14] Macugen™ is the very example of 2'-OMe-containing aptamer therapeutics. This article describes the PEGylation of anti-VEGF 2'-OMe RNA aptamer and the characterization of the binding affinity of PEGylated aptamer by SPR analysis.

Experimental

Anti-VEGF 2'-OMe RNA aptamer was synthesized using a solid phase phosphoramidite chemistry with an automated oligonucleotide synthesizer. The sequence of the aptamer was 5'-AmUmGmCmAmGmUmUmUmGm AmGmAmAm-GmUmCmGmCmGmCmAmU-3'. For a PEGylation, amine group was introduced to the 5' terminal group.[14] As a reference, DNA aptamer was also synthesized with the same sequence. The anti-VEGF RNA aptamer with amine group was dissolved at a concentration of 2 mM in sodium carbonate buffer (100 mM, pH 8.5) and reacted for an hour with 5 molar excess of methoxy-polyethylene glycol succinimidyl propionic acid (mPEG-SPA, MW 20 kDa) in equal volume of acetonitrile. The resulting product was fractionated and characterized by reverse phase-high performance liquid chromatography (RP-HPLC) using a UV detector and a Vydac C18 column. The eluant was acetonitrile containing 50 mM triethyl ammonium acetate (TEAA).

In vitro binding affinity of the PEGylated anti-VEGF 2'-OMe RNA aptamer was assessed using surface plasmon resonance (SPR) analysis. The SPR analysis was carried out using a BIAcore 2000 instrument. The 20K PEGylated anti-VEGF 2'-OMe RNA aptamer, purified by RP-HPLC fractionation method, was dissolved in phosphate buffered saline (PBS, pH=7.4) at a concentration of 500 nM. A series of diluted solutions of anti-VEGF 2'-OMe RNA aptamer, PEGylated anti-VEGF 2'-OMe RNA aptamer, and anti-VEGF DNA aptamer were passed over the immobilized VEGF on the chip. The concentrations varied from *ca.* 30 to 500 nM. The adsorption of aptamers onto the VEGF resulted in the formation of aptamer-VEGF complex, which was detected by SPR. Before each injection, the surface of the chip was regenerated with 0.03% sodium dodecyl sulfate (SDS) and 50 mM sodium hydroxide (NaOH) containing 0.5 M sodium chloride (NaCl). Nonlinear regression analysis of

single sensorgram at five concentrations was used to determine the kinetic parameters of the complex formation.

Results and Discussion

Figure 1 shows the schematic representation of anti-VEGF RNA aptamer PEGylation. Anti-VEGF 2'-OMe RNA aptamer with a sequence of 5'-AmUmGmCmAm GmUmUmUm-GmAmGmAmAmGmUmCmGmCmGmCmAmU-3' was successfully synthesized using a solid phase phosphoramidite chemistry. As a reference, anti-VEGF DNA aptamer with the same sequence was also synthesized and compared in terms of binding affinity. PEGylation was carried out by the formation of the amide bond between the succinimidyl group of mPEG-SPA and the terminal amine group of anti-VEGF 2'-OMe RNA aptamer (Figure 1). The reaction chemistry is well known and has been widely used for the chemical modification of various biomolecules.

Figure 2 shows the reverse phase-high performance liquid chromatogram (RP-HPLC) of PEGylation products after the conjugation reaction for an hour. Un-reacted PEG (A), un-reacted anti-VEGF 2'-OMe RNA aptamer (B), and PEGylated anti-VEGF 2'-OMe RNA aptamer (C) were clearly separated on the RP-HPLC chromatogram. The PEGylated anti-VEGF RNA aptamer was fractionated by collecting the samples with elution times between *ca.* 100 min and *ca.* 110 min, and purified again by RP-HPLC. The PEGylated anti-

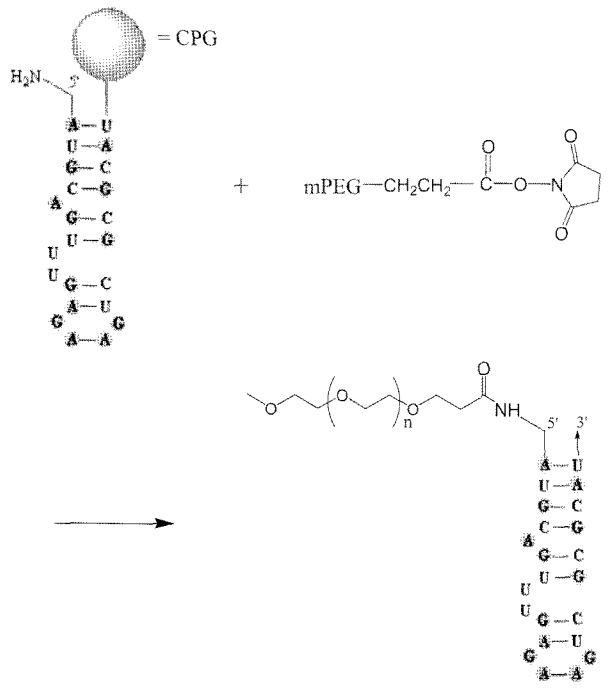

Figure 1. Schematic representation of the synthesis of PEGylated anti-VEGF 2'-OMe RNA aptamer (CPG=Controlled Pore Glass).

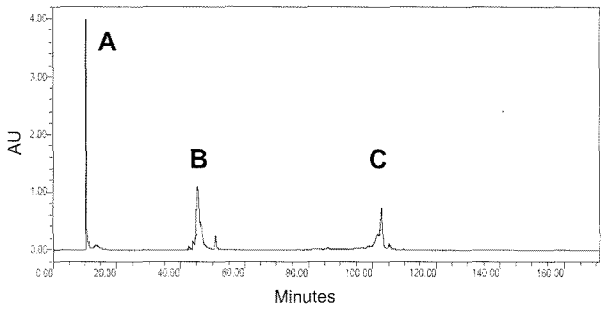

Figure 2. RP-HPLC of the PEGylation products after the conjugation reaction for an hour: (A) un-reacted PEG, (B) un-reacted anti-VEGF 2'-OMe RNA aptamer, and (C) PEGylated anti-VEGF 2'-OMe RNA aptamer.

VEGF RNA aptamer after fractionation appeared as a clear single peak on RP-HPLC.

The binding affinity of PEGylated anti-VEGF 2'-OMe RNA aptamer was assessed by SPR analysis, which reflects its biological activity indirectly. SPR has been widely used for the detection of bio-affinity adsorption between the biomolecules such as DNA, RNA, and protein.[8] As shown in Figure 3, the sensorgrams at five different concentrations represent for the binding affinity of anti-VEGF aptamer samples to VEGF coated on the BIAcore chip. The concentration of each VEGF aptamer sample varied from 30 to 500 nM. While the K_d value of non-modified anti-VEGF 2'-OMe RNA aptamer was 1.87×10^{-9} M, the K_d value of PEGylated anti-VEGF 2'-OMe RNA aptamer was 8.7×10^{-8} M. The sensorgram of PEGylated anti-VEGF RNA aptamer at a concentration of 500 nM appeared to be similar with that of anti-VEGF RNA aptamer without PEGylation at a concentration of 62.5 nM (Figures 3(A) and 3(B)). The results indicate that the binding affinity of anti-VEGF aptamer to VEGF became reduced drastically after PEGylation. Arithmetically, the binding affinity of anti-VEGF RNA aptamer was reduced to *ca.* 1/8 after PEGylation. One more thing to be mentioned is the binding characteristics of anti-VEGF RNA aptamer before and after PEGylation. Whereas the anti-VEGF RNA aptamer showed a rapid adsorption and a slow desorption, the PEGylated anti-VEGF RNA aptamer did a slow adsorption and a rapid desorption. Interestingly, there was no binding affinity at all for anti-VEGF DNA aptamer with the same sequence. The results imply that the anti-VEGF DNA aptamer has no therapeutic effect despite of having the same sequence with the anti-VEGF 2'-OMe RNA aptamer (Figure 3(C)). The SPR method was thought to be very useful for the analysis of binding characteristics of aptamers before and after PEGylation. Currently, we are developing a new branch type-PEGylation protocol for anti-VEGF aptamer therapeutics using symmetric doubler phosphoramidites.[8] *In vivo* tests of PEGylated VEGF aptamer therapeutics will be carried out and the biological activities

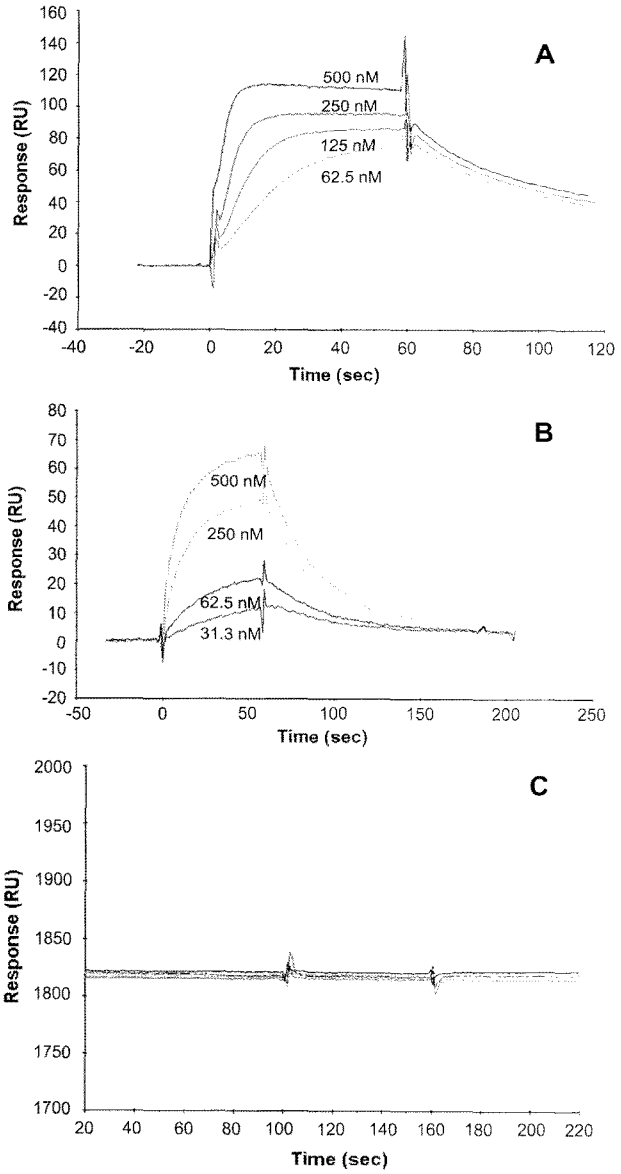

Figure 3. Surface plasmon resonance (SPR) of anti-VEGF aptamer samples to VEGF coated on the BIAcore chip: (A) anti-VEGF 2'-OMe RNA aptamer, (B) PEGylated anti-VEGF 2'-OMe RNA aptamer, and (C) anti-VEGF DNA aptamer.

will be compared with *in vitro* test results using the SPR method.

Conclusions

SPR analysis for the assessment of anti-VEGF RNA aptamer binding affinity was successfully carried out before and after PEGylation. Anti-VEGF 2'-OMe RNA aptamer was synthesized and PEGylated through the amide bond formation between the succinimidyl group of mPEG-SPA and the terminal amine group of anti-VEGF 2'-OMe RNA aptamer. According to the SPR analysis using a BIAcore 2000 instrument, the binding affinity of anti-VEGF 2'-OMe-RNA aptamer to the VEGF (K_d=1.87×10^{-9} M) decreased significantly after PEGylation (K_d=8.7×10^{-8} M). The PEGylated anti-VEGF RNA aptamer showed a slow adsorption and a rapid desorption. Interestingly, the anti-VEGF DNA aptamer having the same sequence with the anti-VEGF 2'-OMe RNA aptamer showed no binding affinity at all. The SPR method was thought to be usefully applied for the assessment of bio-conjugated aptamer therapeutics.

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References

(1) R. M. Boomer, S. D. Lewis, J. M. Healy, M. Kurz, C. Wilson, and T. G. McCauley, *Oligonucleotides*, **15**, 183 (2005).
(2) J. F. Lee, G. M. Stovall, and A. D. Ellington, *Curr. Opin. in Chem. Biol.*, **10**, 282 (2006).
(3) R. R. White, B. A. Sullenger, and C. P. Rusconi, *J. Clin. Invest.*, **106**, 929 (2000).
(4) G. R. Zimmermann, C. L. Wick, T. P. Shields, R. D. Jenison, and A. Pardi, *RNA*, **6**, 659 (2000).
(5) J. M. Healy, S. D. Lewis, M. Kurz, R. M. Boomer, K. M. Thompson, C. Wilson, and T. G. McCauley, *Pharm. Res.*, **21**, 2234 (2004).
(6) H. Dougan, D. M. Lyster, C. V. Vo, A. Stafford, J. I. Weitz, and J. B. Hobbs, *Nucl. Med. Biol.*, **27**, 289 (2000).
(7) T. G. McCauley, J. C. Kurz, P. G. Merlino, S. D. Lewis, M. Gilbert, D. M. Epstein, and H. N. Marsh, *Pharm. Res.*, **23**, 303 (2006).
(8) H. Kang, J. K. Park, Y. B. Seu, and S. K. Hahn, *Key Eng. Mater.*, **343**, 529 (2007).
(9) S. J. Hwang, M. S. Kim, J. K. Han, D. S. Lee, B. S. Kim, E. K. Choi, H. J. Park, and J. S. Kim, *Macromol. Res.*, **15**, 437 (2007).
(10) Y. K. Son, Y. P. Jung, J. H. Kim, and D. J. Chung, *Macromol. Res.*, **14**, 394 (2006).
(11) W. T. Chuang, U. S. Jeng, H. S. Sheu, and P. D. Hong, *Macromol. Res.*, **14**, 45 (2006).
(12) E. W. M. Ng, D. T. Shima, P. Calias, E. T. Jr. Cunningham, D. R. Guyer, and A. P. Adamis, *Nat. Rev. Drug. Discov.*, **5**, 123 (2006).
(13) F. Ducongé, C. D. Primo, and J. J. Toulmé, *J. Biol. Chem.* **275**, 21287 (2000).
(14) P. E. Burmeister, S. D. Lewis, R. F. Silva, J. R. Preiss, L. R. Horwirtz, P. S. Pendergrast, T. G. McCauley, J. C. Kurz, D. M. Epstein, C. Wilson, and A. D. Keefe, *Chem. Biol.*, **12**, 25 (2005).