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Research Article

Development and optimization of a new aptamer-based method for P-selectin (CD62p) measurement

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Abstract

Objectives: Disease-specific biomarkers are an essential tool for the efficient management of pathological conditions, including susceptibility determination, diagnosis, and preventive monitoring. P-selectin (CD62p) is selectively expressed after platelet activation, and is involved in thrombus formation and immune response. The serum and plasma level of CD62p increases in conditions such as heart attack, stroke, some immune diseases, and cancer. The aim of this study was to develop a new, aptamer-based method for CD62p measurement.

Methods: Aptamers can be used to target specific biomarkers based on their molecular shape. The systematic evolution of ligands by exponential enrichment (SELEX) process is a method to identify aptamers with a high affinity for a specific macromolecular target. This study explored using aptamers to measure CD62p. First, aptamers that specifically bind to CD62p were isolated using the SELEX method. The aptamers that demonstrated the highest binding affinity to the CD62p protein were used to coat 96-well plates. Next, the level of CD62p in human serum was measured using this aptamer and the test performance parameters of sensitivity, specificity, and precision were evaluated.

Results: Among the aptamers used, Apt-1, Apt-2, and Apt-3, bound to CD62p protein with high affinity. Apt-2 had the greatest binding affinity to CD62, demonstrating a binding constant of -9.6 kcal/mol, and a dissociation constant (Kd) of 18.15±2.36 nM. Bovine serum albumin was used in the specificity test as a negative control. No binding between the selected aptamers and this protein was observed. The performance showed that of intra-assay coefficient of variation (CV) was <6.52% and inter-assay CV was <3.96%. The recovery values were between 95.06% and 107.95%, and the linearity values were between 99.49% and 113.17%. Sensitivity was calculated at 0.30 ng/mL of CD62p.

Conclusion: The aptamer method to measure CD62p proved to be a sensitive, specific, time-saving, and low-cost option.

Keywords: Aptamer, CD62p protein, platelet, p-selectin, SELEX

Selectins are cell membrane glycoproteins that demonstrate calcium-dependent carbohydrate-binding activity. They are named according to the type of cell from which they were first isolated: platelet selectin (P-selectin, CD62p), endothelial selectin (E-selectin, CD62E), and leukocyte selectin (L-selectin, CD62L) [1-3]. CD62p is continuously synthesized in endothelial cells of the lung and the choroid plexus, megakaryocytes, and thrombocytes. It is stored within Weibel-Palade bodies of endothelial cells or alpha-granules of platelets. CD62p is expressed on the surface of activated endothelial cells or platelets through exocytosis of storage granules and their fusion with the cell membrane [4-6].

A soluble form of CD62p, which is regarded as a proteolytic fragment or a soluble splice variant absent the transmembrane domain, is found in serum and plasma. Overexpression of CD62p can occur as a result of several inflammatory disorders, including adult respiratory distress syndrome, acute lung injury, ischemia-reperfusion injury, Gram-negative sep-

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tic shock, thrombotic diseases, malaria, systemic sclerosis, connective tissue disease, or rheumatoid arthritis. In addition to these inflammatory diseases, increased plasma levels of CD62p have been detected in cardiovascular diseases, stroke, diabetes, and cancer. The measurement of serum and plasma CD62p levels is crucial for the diagnosis, treatment, and follow-up of these diseases [7-9].

Aptamers are chains of nucleic acid oligonucleotides comprising 25 to 50 nucleic acid bases. They can be produced using the systematic evolution of ligands by exponential enrichment (SELEX) technique. Oligonucleotide aptamers generated using random libraries have the potential to provide therapeutic effects for a large number of targets. The establishment of an oligonucleotide library would contribute greatly to research and a variety of applications [10]. Aptamers, also known as chemical antibodies, bind to their targets with high specificity and affinity in a 3-dimensional shape. Aptamer technologies have been used for several medical and biological purposes, including in vitro diagnosis, imaging, and targeting therapies [11].

Various methods have been developed for the measurement of P-selectin. The first was flow cytometry, first created in the mid-1980s, but use is limited due to the need for expensive equipment and specialized personnel [12]. Researchers subsequently developed an enzyme-linked immunosorbent assay (ELISA)-based method for P-selectin measurement [13]. Modern analytical methods, such as matrix-assisted laser desorption ionization/time-of-flight mass spectrometry, high-performance liquid chromatography, and surface plasmon resonance, are also used for P-selectin measurement. However, these methods also involve some parameters that can be prohibitive, such as expensive equipment, pre-treatment process, significant time requirements, and low precision [14, 15]. In recent years, a new measurement method for P-selectin using the aptamer-protein interaction has been developed. Compared with protein-protein interaction-based methods, aptamer-protein interaction-based methods have demonstrated many superior features, such as low molecular weight, high specificity, selectivity, and excellent thermal properties [16].

This study was designed to explore the use of a new aptamer-based method to measure P-selectin. First, aptamers that demonstrated binding to P-selectin were selected using the SELEX method. Three aptamers with the greatest affinity were then plated into 96-well plates for P-selectin measurement. Performance parameters such as sensitivity, specificity, and reproducibility were calculated. The results suggest that this may be a sensitive, specific, time-saving, and low-cost means to measure CD62p.

Materials and Methods

Building aptamer for P-selectin using SELEX

The oligonucleotides and primers were purchased from Integrated DNA Technologies (Coralville, IA, USA). Recombinant P-selectin protein (rCD62p) was obtained using a commercial product (228-11332-2; RayBiotech Life, Inc., Peachtree Corners, GA, UA). All of the procedures were performed at room temperature. The single-strand DNA (ssDNA) aptamer library selected, 5′-GATAGAATTCGAGCTCGGGC-N25- GCGGGTCGACAAGCTTTAAT-3′, was heated to 95°C for 5 minutes and then cooled at room temperature for 30 minutes to enable refolding in 200 µL of SELEX buffer (20 mM Tris at pH 7.5 with 150 mM NaCl, 1 mM MgCl2, 2.7 mM KCl, and 0.005% NP-40). The aptamer was incubated with 1 µg of 6X histidine-tagged rCD62p and nickel-nitrilotriacetic acid (Ni-NTA) beads for 30 minutes and shaken. Next, a centrifuge was used to remove the unbound ssDNA bound to the beads and 6X histidine-tagged rCD62p. The pellet was incubated for 30 minutes with appropriate concentrations (500 nM for cycle 1, 100 nM for cycles 2–5, 10 nM of cycle 6 and 1 nM for cycle 7) of 6X histidine-tagged rCD62p protein. After washing them 3 times with SELEX buffer (1 mL), the beads containing the rCD62-bound sequences were incubated with a solution of 1 M NaCl and 10 mM NaOH for 5 minutes and shaken before phenol extraction. The eluted samples were amplified using a polymerase chain reaction (PCR) method:Taq polymerase in 2X PCR master mix using forward (5′-GGAGCGTTAGCCAAGGC-3′) and reverse (5′-AACCGCGGACAAAGTGCTCC-3′) primers (95°C for 5 min, 20 cycles (95°C for 30 s, 58°C for 30 s and 72°C for 30 s) and 72°C for 3 min). After ethanol precipitation and centrifugation to recover the PCR product, the ssDNA aptamers were prepared. The ssDNA were purified using ethanol precipitation and phenol extraction. Aptamer quantitation was performed using repeated nanodrop and propagation procedures. The quantification result was used for the next SELEX selection cycle. The purity of the products obtained after SELEX was checked in 1% agarose gel using ethidium bromide in tris-acetate-EDTA buffer.

Determination of binding affinity

The selection buffer (SB) used was 100 mM NaCl, 20 mM Tris-HCl (pH 7.6), 2 mM MgCl2, 5 mM KCl, and 1 mM CaCl2. Each aptamer candidate was heated. To examine the aptamer binding ability, a premix containing 1 nM of the aptamer and 250 nM of extra MgCl2 in SB was prepared for each oligonucleotide. After that, 85 µL of the premix was added to the microplate wells containing 10 µL of SB or 1 nM rCD62p solution in SB, each in triplicate, and this mix was incubated at room temperature for 30 minutes, followed by the addition of 5 µL of SYBR Green I solution to the SB in each well. Incubation for 2 hours followed. After that, the fluorescence of the SYBR Green I (excitation at 480 nm, emission at 520 nm) was recorded using the microplate reader. Measurements were repeated twice. The DNA concentration of 5 nM was fixed. The bank library values were used as a non-binding control. Measurements were performed in triplicate. The Kd measurement of the concentration of rCD62p varied from 0.5 to 300 nM for Apt-1, Apt-2, Apt-3, and the fluorescent signal was plotted against the rCD62p concentration. The Kd was calculated by

Table 1. The range of Kd and ΔG values for the 3 aptamers

Aptamer number	Sequence (5′-3′)	ΔG value (kcal/mol)	Kd value (nM)
Apt-1	5′-GGAGCGTTAGCCAAGGCCGCTTGATTAACCGCGGACAAAGTGCTCC-3′	-13.8	41.99±6.96
Apt-2	5′-GGAGCGTTAGCCAAGGCCGCGGCGTAAACCGCGGACAAAGTGCTCC-3′	-9.6	18.15±2.36
Apt-3	5′-GGAGCGTTAGCCAAGGCTACGGGATTAACCGCGGACAAAGTGCTCC-3′	-15.0	64.18±8.33

entering the binding data to a one-site saturation equation using Graphpad software 7.04 (GraphPad Software Inc., San Diego, CA, USA).

Direct ELONA test protocol

Enzyme-linked oligonucleotide assay (ELONA) bioassay testing was performed to examine target detection. rCD62p and commercial Human sodium citrate plasma (Sigma P9523, USA) were diluted to a final concentration of 20 µg/mL in phosphate-buffered saline (PBS; 1.16 g Na2HPO4, 0.1 g KCl, 0.1 g K3PO4, 4.0 g NaCl in 500 mL distilled water pH 7.4) or another carbonate buffer (3.03 g Na2CO3, 6.0 g NaHCO3 in 1000 mL distilled water pH 9.6) and 50 µL of the CD62 dilution was added to the wells of the plate via pipetting. Albumin is a protein found in high levels in serum or plasma, and therefore bovine serum albumin (BSA) was used for matrix modeling in this study. BSA-Fraction V, (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) was prepared at a concentration of 50 µg/mL in PBS, dilutions were made, and the wells were loaded. The plate was covered with adhesive plastic film and incubated at room temperature for 2 hours. After discharge into the wells, it was washed with 200 µL PBS. The remaining protein-binding sites in the coated wells were blocked by adding 200 µL of blocking buffer (serum, non-fat dry milk, casein, gelatin in 1% PBS). The plate was covered with adhesive plastic film and incubated for 2 hours at room temperature for incubation. After discharge into the wells, it was washed with 200 µL PBS. The detection process began with adding 100 µL of the aptamer. The plate was covered with plastic film and incubated for 2 hours at room temperature. After discharge into the wells, it was washed 4 times with 200 µL PBS. Once 100 µL of HRP solution was added per well, it was allowed to incubate for 15 minutes. Next, 100 µL of stop solution was added to all of the wells. The absorbance was measured at 450 nm wavelength.

ELONA kit validation and quality testing

Validation tests generally found in kit studies and in the International Conference on Harmonization (ICH) Q2 Validation of Analytical Procedures were applied to the aptamer-based CD62 test. ELONA validation tests are based on the sensitivity, specificity, intra and inter-assay coefficient, recovery, and linearity of variation. Validation tests were performed using recombinant rCD62 and the efficacy measured was using a commercially purchased human sample (human sodium citrate plasma, P9523; Merck KGaA, Darmstadt, Germany). CD62 measurement was calculated using the limit of detection (LOD) equation: LOD=3.3 σ/Slope (σ=the SD of the response at low concentrations, slope=the slope of the calibration curve) to examine sensitivity.

Results

SELEX studies for P-selectin

After 15 cycles of positive-negative selection, 3 molecular probes were identified that were highly specific for P-selectin. The elutions used in making the selection were controlled using agarose gel. After sequencing, 3 aptamers for CD62p were identified using bioinformatic analysis. The sequences of these aptamers are provided in Table 1. Since aptamers can be thought of as "shape" libraries, potential secondary structures for each of the selected aptamers were generated and drawn for each of the predicted structures (www.aptamy.com; Anhui Huaheng Biological Engineering Co., Ltd., Anhui, China) (Fig. 1).

Aptamer binding affinity

To determine the aptamers' binding affinity to rCD62p, rCD62p was incubated with total concentrations of the aptamers and the fluorescence of individual well was measured using SYBR Green I (Fig. 2). All of the aptamers evaluated showed high-affinity binding to the epitopes: Apt-1 (Kd=41.99±6.96 nM), Apt-2 (Kd=18.15±2.36 nM), and Apt-3 (Kd=64.18±8.33 nM) (Table 1). The Gibbs energy measurement of the energy required to separate the bonded structure was -13.8 kcal/mol, -9.6 kcal/mol, -15.0 kcal/mol for Apt-1, Apt-2, Apt-3, respectively.

As seen in Table 1, the aptamer with the lowest KD value was Apt-2. Apt-2 was selected as a candidate aptamer because of its high binding affinity to CD62p.

Sensitivity and specificity test results

The binding affinity of Apt-1, Apt-2, and Apt-3 was measured separately in equal concentrations using the SYBR green method with BSA as a negative control. The aptamers selected were determined to be specific for CD62 and not specific for BSA using a comparison of the fluorometer signals. A high degree of binding was demonstrated between CD62 protein and the aptamers, and the greatest affinity was seen in Apt-2, which was statistically significant (Fig. 3a).

Specificity and sensitivity tests were performed first with a constant concentration of Apt-2 and different concentrations

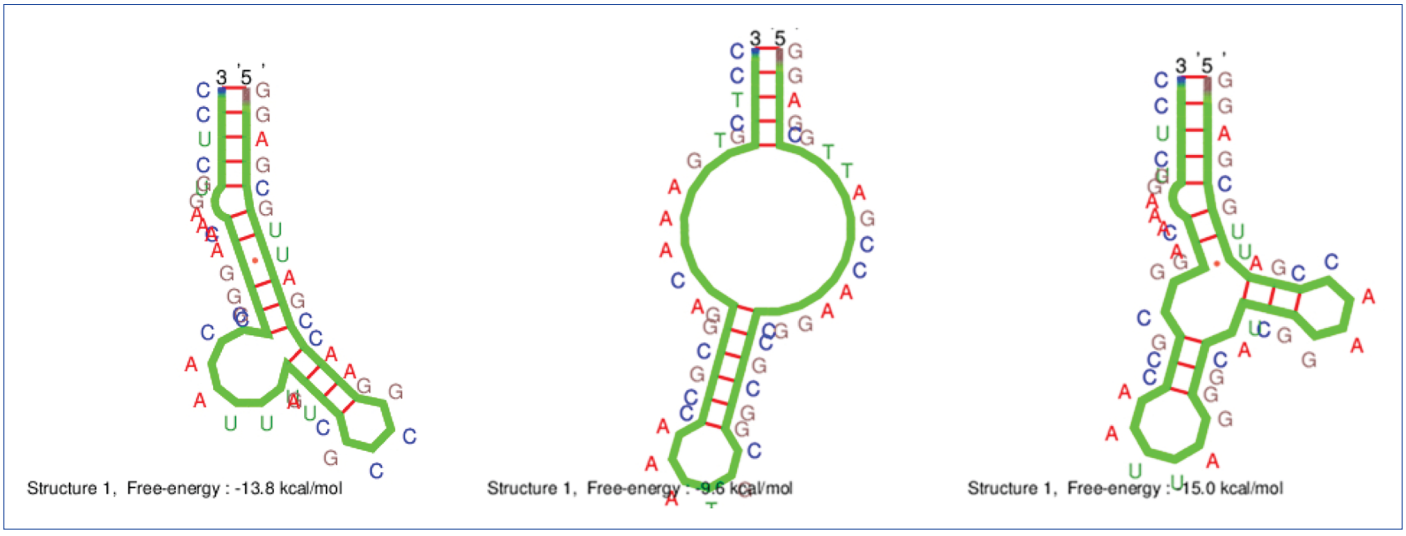

Figure 1. The genetic map of (a) Apt-1, (b) Apt-2, and (c) Apt-3.

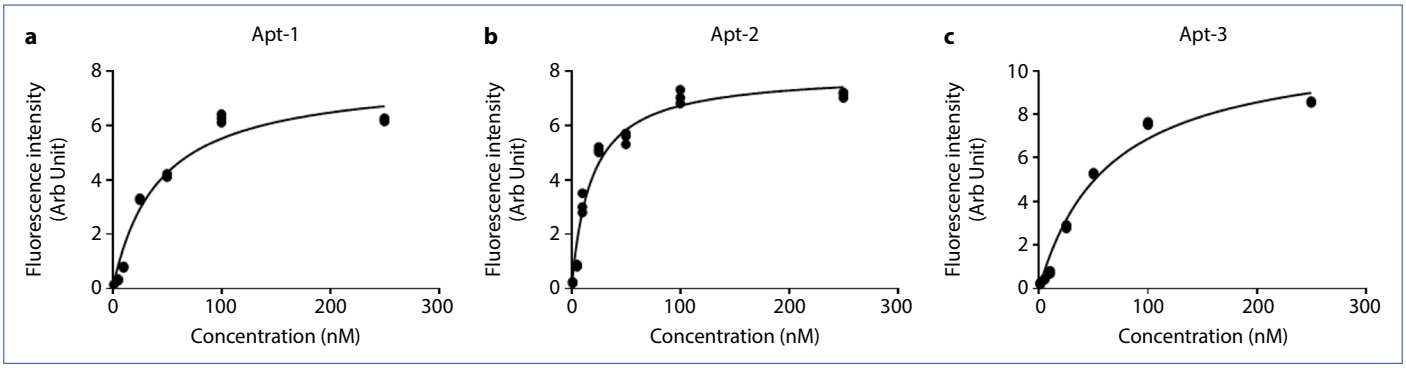

Figure 2. The binding equilibrium of (a) Apt-1, (b) Apt-2, and (c) Apt-3.

Table 2. Intra- and inter-assay value of enzyme-linked oligonucleotide assay test (accuracy and precision)

	Intra-assay			Inter-assay		
Sample	1	2	3	1	2	3
Measurements	6	6	6	6	6	6
Average±SD (ng/mL)	6.023±0.241	9.014±0.625	2.146±0.187	6.162±0.407	9.224±0.240	2.676±0.072
%CV	4.00	6.86	8.71	6.60	2.60	2.69

CV: Coefficient of variation

of recombinant CD62p and BSA. Next, the procedure was performed with different concentrations of Apt-2 and a constant concentration of CD62p and BSA and the color change was measured (Fig. 3b). Apt-2 binding to CD62p demonstrated a high level of specificity and sensitivity, in contrast to the BSA binding. The BSA and CD62p concentration used was 0.5-50 ng/mL. Sensitivity was calculated at 0.30 ng/mL.

Intra- and inter-assay test results

A CD62p ELONA test was evaluated using 6 replicates of 3 samples in the same assay. The intra and inter-assay performance showed that the average intra-assay coefficient of variation (CV) was 6.52%, and the average inter-assay CV was 3.96% (Table 2). The intra-assay results were quite low and effective when compared with the accepted rates of general ELISA tests (intra-assay: CV <10%, inter-assay: CV <12%). The results showed that the sample measurements were comparable.

Recovery tests results

The recovery test is used to determine whether the indicator to be measured is affected by different sample matrices. Protein-rich matrices, such as serum and plasma, can affect the ability to accurately measure a marker. In this study, BSA, which replicates the protein abundant in human serum and

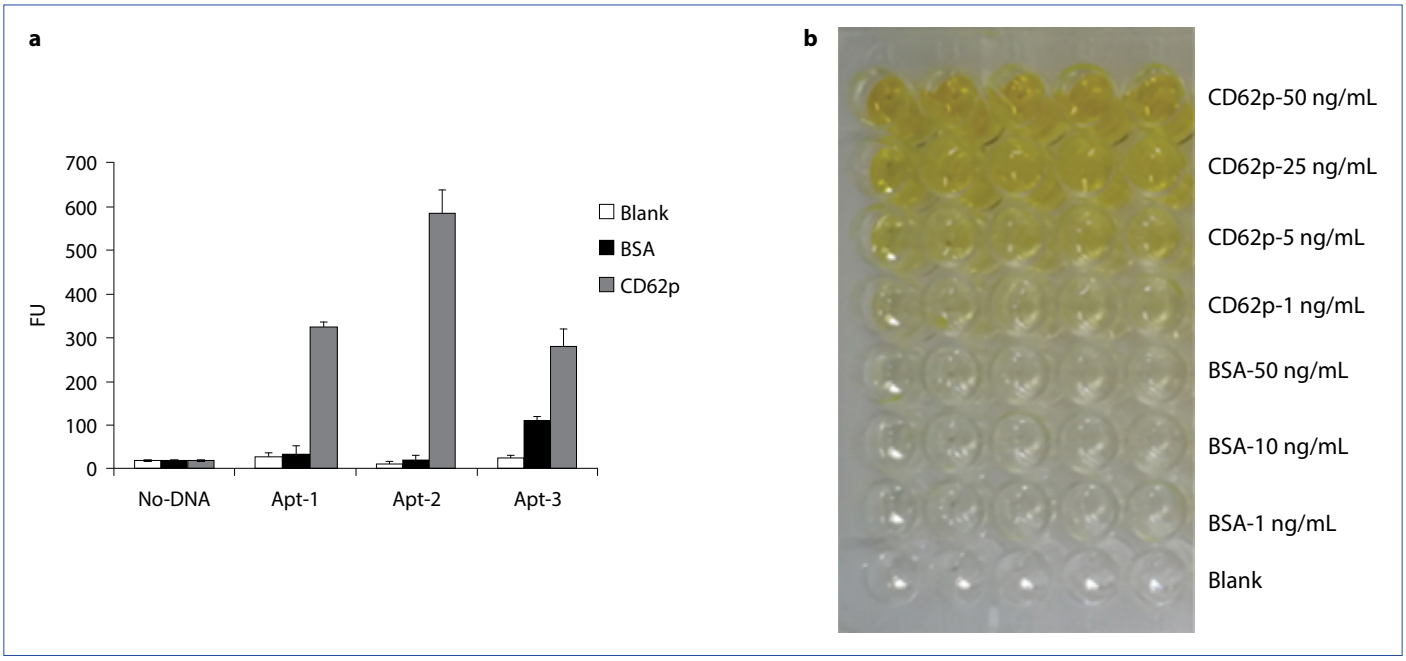

Figure 3. (a) Results of aptamer binding to bovine serum albumin (BSA) and CD62p using SYBR green method; (b) Well images of enzyme-linked oligonucleotide assay testing of specificity and sensitivity.

Table 3. Recovery results of enzyme-linked oligonucleotide assay test for CD62p measurement

Sample	1	2	3	4	5	Negative
Obtained value (ng/mL)	15.84±0.43	4.66±0.14	2.36±0.17	1.95±0.09	1.09±0.05	0.004
Expected value (ng/mL)	16	4.5	2.25	1.8	1.1	0
% Recovery	95.06±1.81	103.58±3.12	104.84±7.55	107.95±5.05	98.94±4.80	NA

NA: Not applicable.

plasma, was used as matrix. The results of the measurements of CD62p in BSA were calculated and presented in Table 3.

Linearity test results

Linear dilution experiments are used to demonstrate the operability of the test at different dilution rates. The analysis helps to measure the dynamic range for human samples. A well-developed diagnostic test should provide information about the precision of the results, even when samples are diluted. This study included the preparation of a sample dilution buffer (ratio 1, 1:2, 1:4,1:8). Linearity is important for the accurate measurement of the concentration across the dynamic range of the CD62p assay (Table 4). Acceptable linearity values in ELISA tests can vary between 70-130%.

CD62p detection results in human plasma

CD62p ELONA was performed using commercial healthy human plasma; therefore, the CD62p levels were low. In real-life human samples, CD62p levels increase with the activation of platelets. Modeling of a more clinically representative example was performed by adding rCD62p to the plasma test

Table 4. Linearity values of enzyme-linked oligonucleotide assay test for CD62p measurement

Dilution	Obtained value (ng/mL)	Expected value (ng/mL)	% Expected
1	24.87	25.0	99.49
1/2	12.39	12.5	99.10
1/4	7.18	6.25	114.88
1/8	3.54	3.12	113.17

samples. Plasma was used rather than serum, since CD62 is released from platelets. Commercial human plasma contains 4% trisodium citrate as an anticoagulant for platelet function. Color changes were observed by adding recombinant rCD62p to the commercial control plasma (Fig. 4).

Discussion

This study of CD62p was conducted due to the clinical importance of CD62p in common complications and disorders, including coronary artery disease, stroke, diabetes, and malignancies. Early detection of CD62p can lead to better treatment

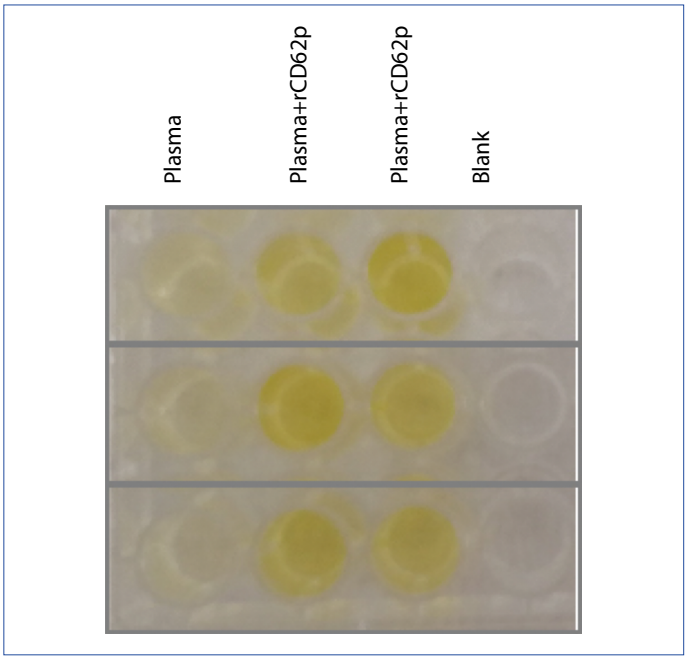

Figure 4. Well images of enzyme-linked oligonucleotide assay test to measure CD62p level in human plasma.

or longer survival for patients with chronic diseases, such as cancer, cardiovascular disease, and diabetes. CD62p is clinically detected using human P-selectin solid-phase sandwich ELISA testing that measures the presence of the target-specific antibody pair. The difficulties associated with antibody optimization are a disadvantage of sandwich ELISA testing. Only products specifically tested for sandwich ELISA must be used, since each antibody should react with a specific epitope on the target protein and not react with its partner. Also, antibodies are not thermally stable and activity loss can result with repeated denaturation. Another disadvantage is that antibodies can produce immunogenicity. In antibody-based systems, every production process is both expensive and does not have the same specificity. The development of tests that can be measured directly and provide consistent results is important. Aptamer-based ELONA has none of these defects and can detect protein with accuracy, specificity, and high sensitivity, and furthermore, is non-immunogenic and more temperature stable [17, 18].

Aptamer-based methods to detect biomarkers rely on the selection of aptamers that specifically bind to that biomarker. The most commonly used technique for this selection is the SELEX method. The process includes changing various physical parameters, such as pH, temperature, or salt concentration. Several studies in the literature have examined DNA and RNA-based aptamers that bind specifically to p-selectin. Researchers have isolated a DNA aptamer that selectively binds to P- and E-selectin using the SELEX method and used it to monitor the metastasis formation of cancer cells [19]. The sequence of this aptamer, which contains 89 bases, is 5′-GCCTGTTGTGAGCCTCCTAACGATTTGGATTT GGGGTGGAGGGTATGGTTTGTGCTGCGTTCATTTCCCATGCT-TATTCTTGTCTC-3′ and the binding constant for P-selectin is Kd=95±18 nM. In another study, an RNA-based aptamer (ARC 5690) was used to treat cell sickle disease via decreased adhesive blood cell interactions [20]. ARC 5690 contains 33 bases and the sequence is 5′-CUCGCAGACAACCGGAU GAAAUCGACCGGAG-3′. The binding constant of ARC5690 for P-selectin is approximately 15 pM. A thiosulphate-modified aptamer (ESTA-1) that specifically binds to E-Selectin was designed as a different approach to monitor tumor vascularity [21]. ESTA-1 contains 73 bases and the sequence is 5′-CGCTC-GGATCGATAAGCTTCGATCCCACTCTCCC GTTCACTTCTCCT-CACGTCACGGATCCTCTAGAGCACTG-3′. The binding constant of ESTA-1 for E-selectin is 47 nM. Studies of P-selectin-specific aptamers in medicine are increasing [22-24]. It has been reported that the ARC5692 aptamer was used as a P-selectin inhibitor to prevent venous thrombosis formation [25]. Our study indicated that Apt-2 had the greatest binding affinity with CD-62. It contains 48 bases and the sequence is 5′-GGAGCGTTAGCCAAGGCCGCGGCGTAAACCGCGGACAAAG TGCTCC-3′. The binding constant of Apt-2 for P-selectin was 18.15±2.36 nM. Apt-2 appears to have a lower equilibrium constant, especially compared with the aptamers reported in other studies [19]. The aptamer developed in this study demonstrated greater binding affinity to P-selectin.

Sensitivity and specificity are the principal parameters used to test the effectiveness of the measurement of a biomarker. If the biomarker to be measured is found in serum or plasma, a specificity test is usually performed using the most abundant protein in serum and plasma, typically serum albumin. Sensitivity can be expressed as the minimum amount of CD62 measured [26]. In this study, 3 aptamers were identified that specifically bound to the CD62 protein. Specificity studies indicated that these 3 aptamers did not bind to BSA, confirming that these were specific aptamers to measure CD62 levels in serum and plasma. Examination of the sensitivity values of ELISA-based commercial kits sold for P-selectin measurement (MyBioSource MBS722270, MyBioSource Inc., San Diego, CA, USA; Elabscience E-EL-H0917, Elabscience Inc., Houston, TX, USA; Thermo Fisher Scientific BMS-219-4, Thermo Fisher Scientific, Inc., Waltham, MA, USA) revealed a range of 0.1-0.2 ng/mL. The sensitivity value of the aptamer used in this study was 0.3 ng/mL, which is very close to the sensitivity values of the commercially available kits.

Precision is defined as the ability to provide the same result in replicates of the same sample. Acceptable precision values are an inter-assay CV result of <15% and an intra-assay result of <10%. The intra-assay average of the commercially available kits compared is a CV of <5%, 10%, and 7.8%, respectively. The intra-assay precision value of the CD62 measurement developed in this study was a CV of <6.52%. Similarly, the inter-assay averages of the commercially available kits is a CV of <7.25%, 10% and 5.4%, respectively. The inter-assay precision value of the CD62 measurement kit developed in this study was a CV of <3.96%. Our CD62 measurement had superior in-

ter-assay and intra-assay values in comparison with standard ELISA kits available on the market.

Linearity describes results that are directly proportional to the amount of analyte in the test sample within a specified range. Samples are generally diluted in proportions of 1:2, 1:4, 1:8, 1:16 for linearity calculations. The desired linearity values for an optimal kit are between 70% and 130% [27]. The linearity values of the commercially available kits are 96-108%, 93-109%, and 81.3-108.9%, respectively. The linearity values of the CD62 measurement kit developed in this study were 99.49-113.17%. These results are consistent with those considered appropriate in the literature.

Recovery testing helps to determine the analytical performance of a measurement. The assessment determines how analyte measurement will be affected in complex fluids, such as serum or plasma [28]. The recovery values of commercially available kits on the market are 94-103%, 93-109% and 69-81%, respectively. The recovery values of the CD62 measurement method developed in this study were 95.06-107.95%, which are very close to the published recovery values of commercially available kits.

Interference is defined as a substance or process that falsely alters an assay result. Examples include the presence of other antibodies or other substances. The hook effect is a change due to a very high amount of an analyte in the sample that causes a falsely low value. In the event of a suspected hook effect, serial measurements are made with dilution, and the change in the real value is measured. We determined that there was no hook effect in our aptamer and CD62p recombinant protein-binding experiments by examining the effect of a high protein content in the matrix using BSA. In addition, the measured values in the dilutions made in linearity studies were close to the standard values. We also explored whether the aptamer measurements were altered based on the matrix with the addition of recombinant CD62p to commercially available human plasma. Nonetheless, it may be beneficial to evaluate the measurements in clinical samples for potential interference or hook effect.

In addition to analytical performance effectiveness, low cost and rapid analysis are desirable features in a test. The average analysis time of the ELISA-based 96-well kits on the market is approximately 5 hours and the average price is approximately $600. Our CD62 testing method has the advantages of a shorter average measurement time and lower cost.

Conclusion

Innovative devices and new methods of analysis to be used in the diagnosis and treatment of various diseases continue to emerge. Many researchers have concentrated on options that offer high sensitivity and a short turnaround time. Aptamers have become a popular focus since they are specific and continuously produced oligonucleotides. The current methods used to measure thrombocyte function are expensive and it can take a significant amount of time to obtain the results. This study resulted in the development of an aptamer-based diagnostic method that can detect platelet activation in a short period of time and can be used in clinical studies as a potential means to provide accurate and early detection of P-selectin molecules involved in many chronic and acute diseases. This diagnostic ability could contribute to the development of additional innovative methods and diagnostic techniques with high accuracy, high specificity, and sensitivity.

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