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Advanced Chemiluminescent Immunoassay for the Early Diagnosis of Pancreatic Cancer

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Abstract. A biosensor capable of rapidly quantifying trace levels of CA 19-9 using an enzyme-free immunoassay with 1,1'-oxalayldiimidazole chemiluminescent (ODI-CL) detection was developed. The highly sensitive enzyme-free immunoassay with ODI-CL detection can rapidly quantify CA 19-9 in human serum. This research demonstrates how the biosensor can be applied as an advanced bioanalytical method for the early diagnosis and accurate prognosis of pancreatic cancer. We anticipate that the biosensor can also be used to rapidly quantify various different tumor markers for the early diagnoses of human cancers

Keywords; Carbohydrate antigen 19-9 (CA 19-9), 1,1-Oxalyldiimidazole chemiluminescence (ODI-CL) ; Enzyme free immunoassay ; Tumor marker ; Pancreatic cancer

1. Introduction

Pancreatic cancer is among the ten most common cancers in humans. Pancreatic cancer spreads quickly throughout the body. This is because it is difficult to early diagnose pancreatic cancer, and it is very hard to treat when found in its later stages [1,2].

Currently, the concentration of carbohydrate antigen 19-9 (CA 19-9) existing in blood is quantified for the early diagnosis of pancreatic cancer [3]. Various analytical

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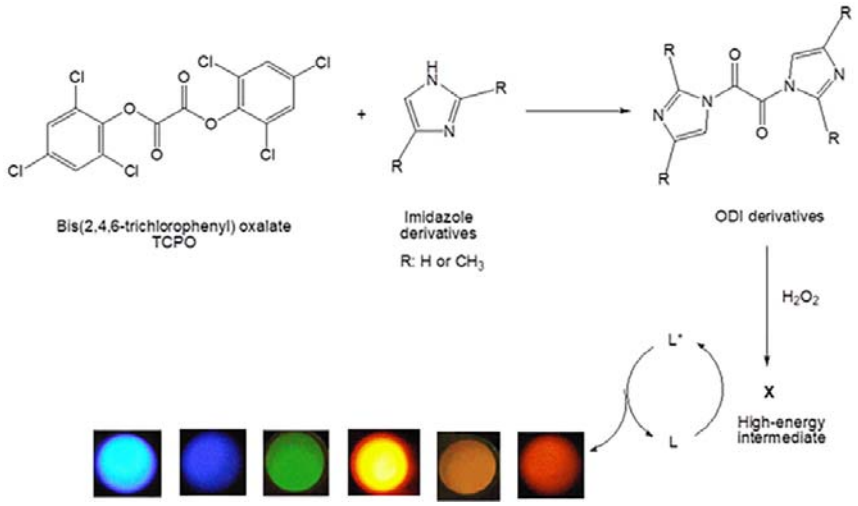
methods with a specific detection have been reported. An optical sensor such as chemiluminescence, colorimetric, and fluorescence are widely applied as a detection



Scheme 1. Reaction mechanism of ODI-CL. X is high-energy intermediate. L is lumnophore in the ground state. L* is luminophore in the excited state. R is H or CH3.

method for the quantification of CA 19-9 in human serum. It is well-known that chemiluminescence is more sensitive than other optical sensors because the background of the former is much lower than those of the latter. In particular, 1,1'-oxalyldimidazole chemiluminescence (ODI-CL) has been widely used for analyses of biomarkers in a sample because it is applied as a detection method of enzyme immunoassays [4] using horseradish peroxidase (HRP) and alkaline phosphatase (AP) as well as enzyme free assay [5] using a DNA aptamer conjugated with fluorescence dye. Scheme 1 shows that the color of light emitted from ODI-CL reaction is dependent on the chemical and physical properties of luminophore (L, fluorescence dye) used as a receiver in ODI-CL reaction. This is because luminophore (L*), formed after receiving energy from high-energy intermediate (X) formed from the reaction of ODI and H2O2, emits light.

Thus, we developed a biosensor capable of rapidly quantifying trace levels of CA 19-9 using an enzyme-free immunoassay with 1,1'-oxalayldiimidazole chemiluminescent (ODI-CL) detection. Detailed research results are reported in this manuscript.

2. Experimental

2.1. Chemicals and Materials

Biomarker CA 19-9, three different monoclonal antibodies of CA 19-9, and streptavidin were purchased from Meridian Life Science, Inc. Two additional monoclonal antibodies were purchased from Antibodies. Three different latex beads, capable of emitting a specific color (e.g., yellow-green, orange, red) in ODI-CL reaction, were purchased from Sigma. Magnetic bead conjugated with streptavidin was purchased from Thermofisher. HRP and biotin conjugation kits were purchased from Dojindo Molecular Technology. Bovine serum albumin (BSA), N-hydroxysuccinimide (NHS), 2-(4morpholino) ethanesulfonic acid (MES, 0.5 M, pH 5.5) buffer solution, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich. Bis(2,4,6-trichlorophenyl)oxalate (TCPO) and 4-methylimidazole (4MImH) were purchased from TCI America. Deionized water (HPLC grade), ethyl acetate (HPLC grade), isopropyl alcohol (HPLC grade), and 10×PBS (pH 7.4) were purchased from VWR.

2.2. Analytical method

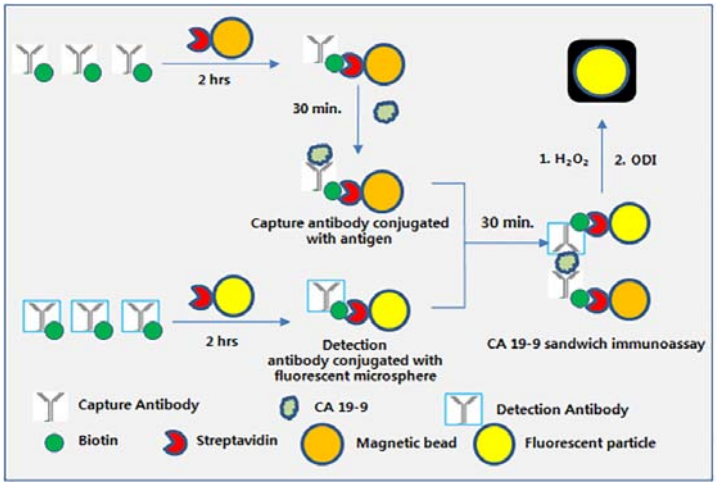


Fig. 1 Enzyme-free immunoassay with ODI-CL detection

As shown in Figure. 1, capture antibodies were immobilized on paramagnetic bead using the interaction between biotin and streptavidin. Also, detection antibodies were immobilized on luminescent latex bead using EDC and NHS. CA 19-9 in human serum was added into PBS (pH 7.4) solution containing the capture antibody and detection antibody. The final mixture was incubated for 30 min. at room temperature. After the incubation, the final products captured by a magnetic bar were washed 4 times using PBST. After that, the final products emitted light with the addition of ODI-CL reagents such as H2O2 and ODI. The brightness of light emitted in a sample was measured using a luminometer with two dispensers (Lumat 9508, Berthold, Inc).

3. Results and Discussion

3.1. Selection of latex bead

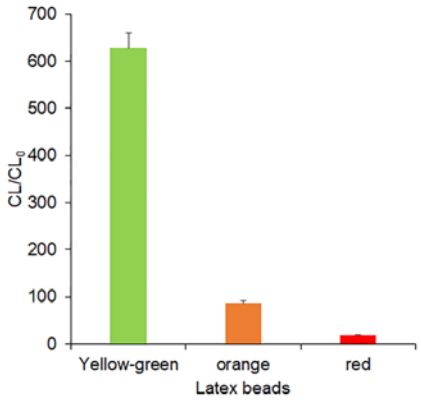


Fig 2. CL emissions of latex beads (0.025 %) in ODI-CL reaction

As shown in Figure 2, the brightness of light emitted in ODI-CL reaction was dependent on the property of the latex bead. Based on the preliminary research shown in Figure 2, we selected the yellow-green latex bead to develop the enzyme free immunoassay capable of rapidly sensing CA 19-9 in human serum. CL0 is the background measured in the absence of latex bead. CL is the relative CL intensity observed in the presence of latex beads (0.025 %) in PBS (pH 7.4).

3.2. Selection of capture and detection antibodies

As shown in Figure 3(A), the efficiency of conventional ELISA for the quantification of CA 19-9 in human serum depends on the combination of capture and detection antibodies. We confirmed that the best combination of capture and detection antibodies was 2 and 3. The detection antibody was conjugated with HRP for the ELISA and ODI-CL enzyme immunoassay. Figure 3(B) shows that ODI-CL enzyme immunoassay, designed based on the previous reports, can quantify CA 19-9 in human serum using the capture (2) and detection (3) antibodies. Based on the results shown in Figure 3, we selected 2 and 3 as the capture and detection antibodies for the development of enzyme free immunoassay with ODI-CL detection for the early diagnosis of pancreatic cancer.

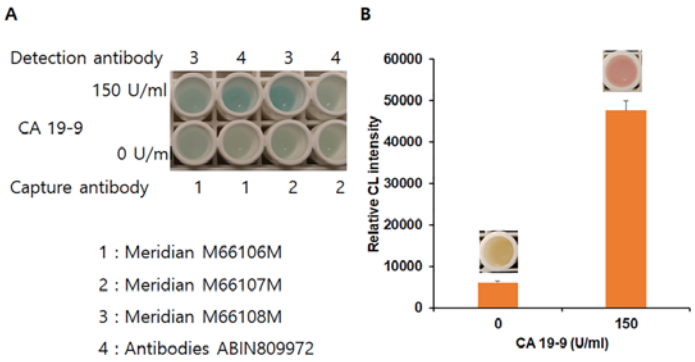


Fig 3. (A) Enzyme-linked immunosorbent assay (ELISA) used to select the best capture and detection antibodies, (B) ODI-CL enzyme immunoassay

3.3. Enzyme-free immunoassay for the quantification of CA 19-9 to early diagnose pancreatic cancer

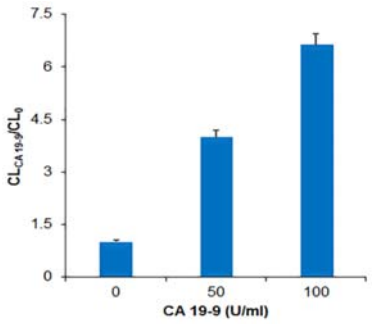


Fig 4. Ratio of CL measured in the absence and presence of CA 19-9.

As shown in Figure 4, the ratio of CL measured in the absence (CL0) and presence (CLCA 19-9) of CA 19-9 determined with enzyme-free immunoassay with ODI-CL detection is dependent on the concentration of CA 19-9 in a sample. Figures 4 shows that the relative CL intensity was proportionally enhanced with the increase of CA 19-9 activity. The biosensor operated with the enzyme-free immunoassay with ODI-CL detection was able to rapidly quantify CA 19-9 with a wide linear calibration curve capable of sensing from 0.0021 to 100 U/ml. The limit of detection (LOD = 3σ) of the biosensor was as low as 0.6 mU/ml. σ is the standard deviation of the average for backgrounds consecutively measured 20 times. The results indicate that enzyme-free immunoassay with ODI-CL detection can rapidly diagnose pancreatic cancer because the cut-off value (37 U/ml) is in the dynamic range of the new biosensor.

4. Conclusion

The highly sensitive enzyme-free immunoassay with ODI-CL detection can rapidly quantify CA 19-9 in human serum. The results indicate that the biosensor can be applied an advanced bioanalytical method for the early diagnosis and accurate prognosis of pancreatic cancer. Also, we expect that the biosensor can be applied to rapidly quantify various different tumor markers for the early diagnoses of human cancers.

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