Development of *in vitro* testing technology using biosensors based on chemiluminescent proteins

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Researchmap https://researchmap.jp/ng1

Abstract

We have been developing super bright chemiluminescent indicators for specific detection of biological substances in blood. "BABI" is a chemiluminescent indicator that binds to bilirubin, the causative molecule of jaundice in newborn babies, and changes its luminescence color from blue to green (Fig.1). Thrombastor" is an indicator of thrombin, which is important for the progression of blood coagulation. When digested by thrombin, Thrombastor changes its luminescence color from green to blue (Fig. 2). As a result of reacting each of them with mouse blood, we observed a change in luminescence color according to the concentration of the target. By capturing the emitted luminescence signal with a smartphone camera and analyzing the images, we demonstrated quantitative measurement of the concentration or activity of the target in the blood.

Background & Results

Blood tests are widely used in medical examinations as a common in vitro diagnostic method. However, there are various detection methods for different blood components, and the amount of blood required and the processing method vary depending on the detection sensitivity of the method used. In order to establish a rapid and simple method for blood testing, we attempted to detect bilirubin and thrombin in blood using chemiluminescent indicators.

Bilirubin is a metabolite of hemoglobin. Unconjugated bilirubin (UCBR), a component of bilirubin that is harmful to the human body, is easily elevated in the blood of newborns and causes jaundice. Because it causes serious symptoms, such as kernicterus, deafness, and cerebral palsy, UCBR is an important factor that need to be monitored. The chemiluminescent indicator BABI, which detects UCBR, changes its color from blue to green when it binds to UCBR (Figure 1). By capturing this color change with a smartphone camera and analyzing the image components, we succeeded in quantitatively measuring the amount of UCBR in mouse blood.

Thrombin is a serine protease that plays a central role in blood coagulation, and excessive activity increases the probability of blood coagulation and thrombus formation. Therefore, the measurement of thrombin activity is essential for the prevention of thrombosis such as cerebral infarction and myocardial infarction. Thrombastor, a chemiluminescent indicator that detects thrombin activity, changes its luminescence color from green to blue when cleaved by thrombin (Figure 2). By measuiring the color change by a smartphone camera, we also succeeded in quantitative measurement of thrombin activity in plasma.

Significance of the research and Future perspective

The measurement of blood components using chemiluminescent indicators can quickly provide results from a smaller sample than conventional detection methods. In addition, the cost of testing can be minimized because the measurement can be done with general-device such as a smartphone camera. Currently, there is a growing need for "point-of-care test" that does not limit the places of diagnosis. By taking advantage of the high penetration rate of smartphones and their high functionality, our chemiluminescent reagents are expected to be used as a point-of-care test method that anyone can easily implement.
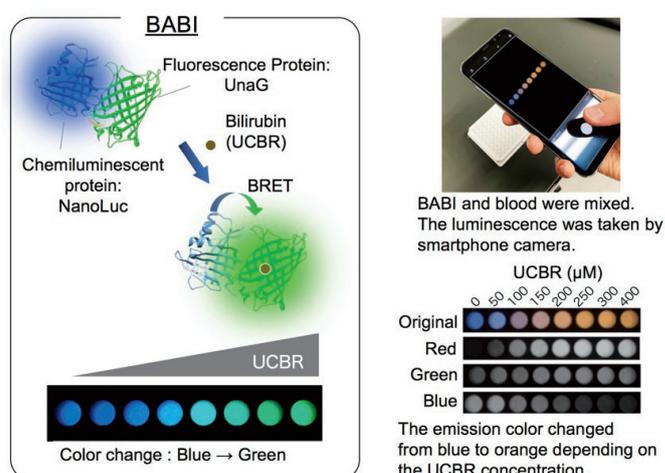
Figure 1. Measurement of blood bilirubin concentration with a smartphone camera
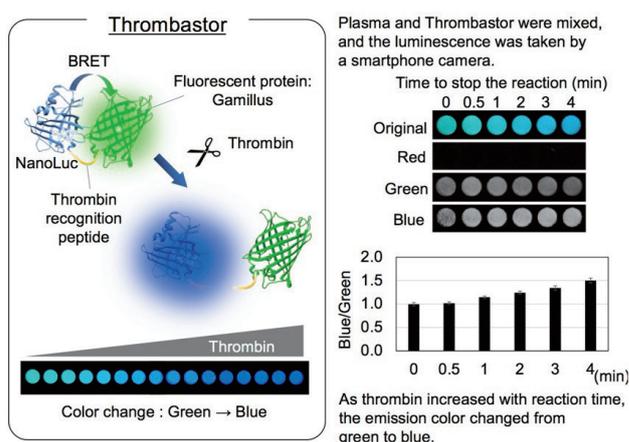
Figure 2. Measurement of plasma thrombin activity with a smartphone camera