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高感度化学発光イメージングのための酵素的デキストランプローブ

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Chemiluminescence (CL) detection of biological samples is an essential tool in many areas of scientific field because of its high sensitivity, high selectivity, simple instrumentation and economical alternative to colorimetric methodologies, such as enzyme-linked immunosorbent assays (ELISA). Therefore, there is increasing demand for a rapid and ultrasensitive detection of minimal amount of biological samples. In the conventional CL detection techniques, enzymatic CL reaction was combined with target-specific interaction, and biotinylated enzymes, such as alkaline phosphatase (ALP) and horseradish peroxidase (HRP), or avidin-enzyme fusion proteins have been utilized to obtain the target-specific signals. However, these probes normally contain one molecule of enzymes and the resulting CL signal was limited.

In this work, we have developed the dextran-based CL probes in which a number of biotin and ALP or HRP is grafted into the dextran molecule (average MW=2 x 10^6). The probes can form a macromolecular assembly through specific biotin-avidin interaction in the presence of avidin, and the biotinylated target molecule recognized by avidin protein should be captured by the assembly, resulting in the great enhancement of CL signal. We have synthesized a series of dextran-based probes containing various amount of ALP or HRP. The dextran-biotin-ALP probe (Dextran:ALP=1:20) exhibited about 2 folds higher CL intensity in 2 minutes in comparison with the dextran-biotin-HRP probe (Dextran:HRP=1:70), indicating the high efficiency of the dextran-biotin-APL as a signal probe. Furthermore, we have found that the incorporation of more ALP will enable the probe to show higher CL intensity. The synthesized dextran-based ALP probe will hopefully provide one of the most rapid and sensitive detection method for CL imaging of biological samples on a solid support.