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Simultaneous bioluminescence immunoassay of two targets in single well based on obelin color mutants

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Novel dual-analyte single-well bioluminescence immunoassay (BLIA) for two gonadotropic hormones and two forms of prolactin was developed on the base of Ca^{2+} -regulated photoprotein obelin mutants as the reporters. The mutants W92F-H22E and Y138F with altered colors and kinetics of bioluminescence signals were chemically conjugated with appropriate monoclonal immunoglobulins and thus displayed signals from targets. Bioluminescence of the reporters was simultaneously triggered by a single injection of Ca^{2+} solution and discriminated via bioluminescent signal spectral and time resolution. The developed microplate-based immunoassay allows detection of two targets in crude serum without additional manipulations (e.g., gel chromatography or PEG-precipitation for prolactin forms). Total prolactin bioluminescence immunoassay in standard, control, and clinical sera offers high sensitivity and reproducibility. The BLIA results show good correlation with those obtained by conventional separate RIA ($R^2=0.92$, $N=117$). For quantitative determination of macroprolactin in clinical samples, we offered a model analytical system, which reflects the processes taking place in serum at the formation of immunological complexes. As a result the dependence of green reporter signal on human immunoglobulin concentration was established, and further used as a calibration curve to detect macroprolactin. The results of the developed assay correlated well with traditional ones and were confirmed by gel-chromatography data. Similarly, follicle-stimulating (FSH) and luteinizing (LH) hormones were simultaneously quantified in clinical sera ($N=83$) with the help of calibration curves, obtained on the base of standard mixed sera. The results of bioluminescent analysis were in good correspondence with the RIA data ($R^2=0.94$ and $R^2=0.91$ respectively).

Biography

A N Kudryavtsev is a postgraduate student of the Institute of Biophysics of SB RAS (Russia). He graduated from the Siberian Federal University in 2011 and got the diploma in biology (MS Degree). His current research is mainly focused on the development of novel analytical approaches involving Ca^{2+} -regulated photoproteins as high-sensitivity reporters.

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