



Methodology

Semi-quantitative detection of gene expression using bisbenzimidazole dye

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ABSTRACT. An electrochemical biosensor, using a disposable electrochemical printed chip aggregation by the bisbenzimidazole dye (Hoechst 33258), was used for detecting the expression of β -actin and RAGE genes. Using linear sweep voltammetry, the expression of these two genes in HeLa and HepG2 cell lines was determined based on anodic peak current, and the results were compared with conventional agarose gel electrophoresis. Total cellular RNA was reverse transcribed to complementary DNA, and amplification by PCR was carried out. Subsequently, the PCR products were subjected to detection by either electrophoresis or electrochemical biosensor. Precision of the electrochemical biosensor technique was acceptable (β -actin: CV = 1.875% for 10^4 copies and 4.684% for 10^9 copies; RAGE: CV = 2.253% for 10^9 copies, and 3.743% for 10 copies). In the electrochemical biosensor technique, the PCR products were measured in the same run with various concentrations of standards, and copy numbers of β -actin gene were interpolated from a standard curve. Copy numbers of the

β -actin gene were then compared between the two techniques. At the 95% confidence limit, the two methods had no significant differences and were significantly correlated ($y = -40383.0623 + 1.0233x$; $P > 0.10$). The electrochemical biosensor method was more sensitive than the conventional electrophoresis method because it could detect as low as 10 copies of the RAGE gene. The conventional electrophoresis method detected the RAGE gene at concentrations of at least 10^4 copies, and the linearity for semi-quantitative measurement was in the range of 10^6 - 10^9 copies. When the electrochemical biosensor was applied to detect the RAGE gene expression in both cell types, we found that HeLa cells expressed the RAGE gene about 2-fold higher than in HepG2 cells (relative value of 0.000905 vs 0.0004670). Therefore, this study suggests the potential modification of the electrochemical biosensor with the use of bisbenzimidazole dye (Hoechst 33258) for detecting gene expression.

Key words: Electrochemical biosensor; DEP chip; Hoechst 33258; Gene expression; RAGE; β -actin