

DNA-BASED PROBE POTENTIALLY SUITABLE FOR THE DETECTION OF ACTIVE NF- κ B

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Abstract

The Nuclear Factor kappa B (NF- κ B), a transcription factor regulating a battery of inflammatory genes, has been indicated to play a fundamental role in the development of numerous pathological states^{1,2}. It has been demonstrated that anti-inflammatory agents, both steroid and non steroid, besides their principal mechanism of action, have a secondary mechanism with anti-NF- κ B effects^{3,4}. Thereafter, when the anti-inflammatory therapy is applied to an inflammatory disease, for which the detection of the efficacy of the therapeutic agent is particularly important, the monitoring of NF- κ B concentration (for example in cellular lysate) is an important target.

NF- κ B exists in two different forms, active and inactive form. The former is able to bind a specific sequence of DNA, the latter is complexed with the protein NF- κ B inhibitor (I κ B) and, therefore, is not able to bind the DNA. It is present in the active form in the nucleus of mature B cells and in some T cell lines. In most of the other cell types, it is present in the inactive form in the cytoplasm where it is activated in the presence of inflammatory events. Some consolidated techniques to quantify NF- κ B (e.g. ELISA) are not able to discriminate the active structure from the inactive one, and other methods (such as band shift assay) need laborious steps. Therefore, the detection of NF- κ B active form is extremely important in order to evaluate the therapeutic agent efficacy. The demand of a simple and direct method to evaluate the amount of active NF- κ B in a biological sample can be satisfied using a suitable and reusable biosensor.

In order to realize this device we designed and synthesized the sequence of DNA (the bioreceptor) able to specifically recognize NF- κ B (the analyte), and we immobilized this DNA sequence inside to a glass capillary tube. The evaluation of the correct DNA immobilization inside of the capillary was carried out. A compact semiconductor laser beam ($\lambda=635$ nm) was used as an excitation source and it was focused onto the capillary using a lens with 40 mm focal length. Fluorescence from the capillary was collected with a x5 microscope objective and detected with the CMOS microchip⁵ (Complementary Metal Oxide Semiconductor) that was perpendicular to the capillary. The determination of NF- κ B in the active form is based on the competition between a known solution of labeled protein with an appropriate dye (e.g. Cy5) and the biological sample under test.

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