Recent advances on an electric silicon based chip for analysis of nucleic acids

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Recently, several innovative designs for nucleic acid-based electrochemical sensing have appeared (1-4). These types of sensors combine nucleic acid layers with electrochemical transducers to produce a biochip. Electrochemistry-based chips offer sensitivity, selectivity and low cost for the detection of selected nucleic acid sequences (5). Nucleic acids are especially well suited for biosensing applications, because the base-pairing interactions between complementary sequences are both specific and robust (6). Thus, such electrochemical biochips can be used for detection of the presence of genes or mutant genes associated with diseases (7). They can be employed to early and precise diagnoses of infectious agents in various environments, or can be exploited for monitoring sequences for specific hybridization events (5).

This article reviews recent activity in our laboratories in developing a silicon chip-based electric detector coupled to bead-based sandwich hybridization as an approach to perform rapid analysis of specific nucleic acids. A microfluidic platform incorporating paramagnetic beads with immobilized capture probes is used for the biorecognition step. The protocol involves simultaneous sandwich hybridization of a single-stranded nucleic acid target with the capture probe on the beads and with a detection probe in the reaction solution. Such complexes are detected by their affinity-directed association to a reporter enzyme, alkaline phosphatase. The enzyme is used to catalyse the hydrolysis of p-aminophenyl phosphate to p-aminophenol. The product is an electrochemically reversible material that is utilized to generate amperometric signals in combination with the electric chip (Figure 1) (8).

The advantages of this method were demonstrated by its application in the detection of nucleic acid segments related to different genes. A detailed instrumental protocol for analysis of specific nucleic acids has been developed, as well as non-covalent and covalent immobilization procedures of the capture probe on paramagnetic beads (8). The protocol was previously characterized using an *in vitro* synthesized RNA oligonucleotide, and after that optimized and successfully employed for analysis of 16S rRNA in *E. coli* extract (Figure 2) (8), and for *B. cereus* identification (Figure 3) and phage DNA detection (Table I) (9). Because of secondary structure formation in the RNA target problem with low electric signal due to insufficient hybridization may appear. Then application of helper probes resulted in sensitivity improvement (Figure 2) (10). It has been shown that the capture and detection probe location at a site where secondary structure

Figure 1 – Outline of the bead-based sandwich hybridization on the electric biochip. The two alternative methods of immobilization (Model I and II) of the capture probe on paramagnetic beads are indicated

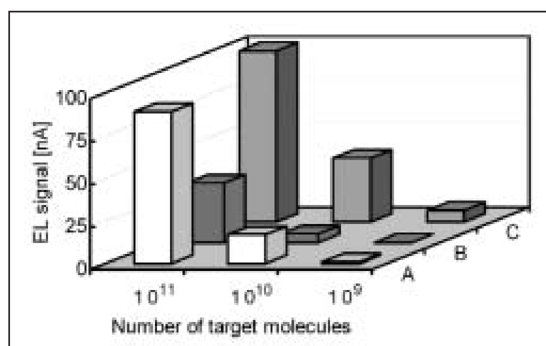

Figure 2 – Effect of capture probe to detection probe distance in the target analyte of 16S rRNA.
A – the 652 bases distance with helper probes (white bars);
B – as in A but without helper probes (gray bars);
C – the 50 bases distance without helper probes (striped bars)

formation is less likely, is an important factor (11). Several software tools are available for simulation of secondary structure configuration, but there is still a lack of confidence with respect to the applicability of these simulations to realistic sample conditions (12).

The biochip analysis of crude vegetative cell and spore lysates was of particular practical relevance. In this case, the analysis of DNA of enterotoxic *Bacillus* strains based on their toxin-encoding genes was done (our unpublished data).

The electric biochip assay was applied directly to ultra-sound or high-pressure disintegrated cells and spores after centrifugation. It has been demonstrated that the analytical protocol enables users to examine specific nucleic acid sequences in crude samples without any prior purification, thus a possible analyte loss during separation procedures is eliminated.

Usefulness of the procedure was also tested for specific detection of phage infections in bacterial cultures by determining the detection sensitivity for different phage DNAs in samples.

The standardized instrumental protocol was adapted for bead-based multiplex analysis of phage DNA (Table I). Particular phage DNA from an examinated sample was subjected to assays by using mixtures of capturing beads specific to different phages. No problems due to cross-reactivity of the probes were detected. Due to relatively low non-specific nucleic acid adsorption at the magnetic beads, high specificity of DNA hybridization was achieved. However, to reach good sensitivity also the background noise (which occurred due to unspecific binding of the enzyme-labeled conjugates to the bead surface) was eliminated. The use of a blocking reagent, like bovine serum albumin (BSA), to prevent unspecific binding in the system, was shown to be crucial and efficient.

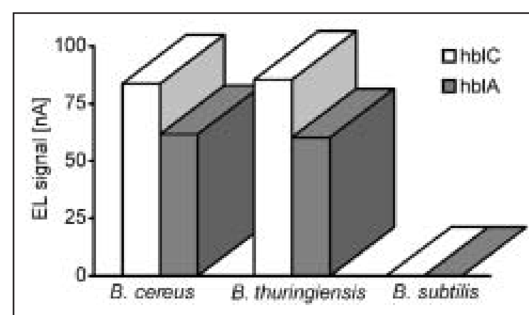

Figure 3 – Specificity of the electric biochip assay of two genes, hblC *(grey bars) and* hblA *(white bars), in* Bacillus *strains*

Table I – Bacteriophage DNA detection with single and multi-sensing magnetic beads by means of electric biochips

Sample	Average EL signal [nA]		
	Phage P1	Phage T4	Phage λ
Negative control with bacterial DNA	0.0	0.0	0.0
Single beads	19.9	22.1	45.5
Multiple beads	19.8	22.0	46.7

The potential of the new analytical platform for fast and sensitive analysis of nucleic acids has been demonstrated. The possibility to perform simple and rapid analyses of samples with respect to content of specific classes of species, for instance pathogenic organisms, or to monitor phage infections, proved the value of the new technique. A major breakthrough in this method was achieved by eliminating the PCR step from the assay. The present assay has met the requirements in terms of speed and sensitivity. Procedural time of 50 min and sensitivity of 10^7 target molecules per assay have been reached without the use of a nucleic acid amplification step. In addition, the biochip has been characterized by a very high specificity towards the target and no background noise.

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