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Research paper

Two-color, 30 second microwave-accelerated Metal-Enhanced Fluorescence DNA assays: A new Rapid Catch and Signal (RCS) technology

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ABSTRACT

For analyses of DNA fragment sequences in solution we introduce a 2-color DNA assay, utilizing a combination of the Metal-Enhanced Fluorescence (MEF) effect and microwave-accelerated DNA hybridization. The assay is based on a new "Catch and Signal" technology, i.e. the simultaneous specific recognition of two target DNA sequences in one well by complementary anchor-ssDNAs, attached to silver island films (SiFs). It is shown that fluorescent labels (Alexa 488 and Alexa 594), covalently attached to ssDNA fragments, play the role of biosensor recognition probes, demonstrating strong response upon DNA hybridization, locating fluorophores in close proximity to silver NPs, which is ideal for MEF. Subsequently the emission dramatically increases, while the excited state lifetime decreases. It is also shown that 30 s microwave irradiation of wells, containing DNA molecules, considerably (~1000-fold) speeds up the highly selective hybridization of DNA fragments at ambient temperature. The 2-color "Catch and Signal" DNA assay platform can radically expedite quantitative analysis of genome DNA sequences, creating a simple and fast bio-medical platform for nucleic acid analysis.

1. Introduction

Identification of living organisms (eukaryotes, bacteria, viruses etc.) by means of quantitative analysis of their specific DNA sequences, which represent different genomes, is a challenging aim, which faces many scientists today. It also concerns the search and detection of different microorganism mutations and strains of pathogenic bacteria and causes of severe diseases in humans. Significant progress in the last few decades was achieved by the discovery and implementation of the PCR approach for genetic material analysis (Mullis, 2003; Saiki et al., 1985). This method is exceptionally sensitive to a small amount of DNA in solution, due to a huge amplification of the selected DNA sequence by numerous cycles of replication. In other words, the PCR method is based on an *artificial increase of the amount of DNA, containing the specific target sequence*, which then can be easily detected by common analytical methods. Despite the obvious advantage of PCR in DNA detection this approach has several disadvantages (Bae and Sohn, 2010; Chiminqgi et al., 2007; Tonooka and Fujishima, 2009), e.g. sensitivity to DNA material contaminants, misreading, quite high cost of analysis, reagents and time to fulfill experiments, and, most importantly, limits in its application as a general fast and easy Point-of-Care method of specific DNA sequence quantification.

Another approach for DNA quantitation is based on the *direct detection* of a small amount of DNA in solution, i.e. *without amplification of the DNA material*. It is based on detection of the

Abbreviations: MEF, Metal-Enhanced Fluorescence;NP, Nanoparticle;SiF, Silver island film;ssDNA, Single stranded DNA;PCR, Polymerase chain reaction;RCS, Rapid Catch and Signal technology.

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bright emission of dyes bound to nucleic acids (Dragan et al., 2010a,b; Lakowicz, 2006). Most popular chromophores for this approach are ethidium bromide, PicoGreen and Syber Green I, which bind DNA non-covalently and dramatically increase their fluorescence yield. For example, the last two chromophores increase their brightness almost 1000-fold upon binding to double-stranded DNA (Cosa et al., 2001; Dragan et al., 2010a,b; Singer et al., 1997; Zipper et al., 2004). It makes them extremely sensitive to a small (<ng/ml) amount of DNA in solution. Moreover, recently we have shown that in the presence of silver nanoparticles, due to the Metal-Enhanced Fluorescence (MEF) effect, the sensitivity of PicoGreen and Syber Green I to dsDNA can be significantly further increased and become comparable to the sensitivity of the PCR technique, i.e. to be in the range of ~pg/ml (Dragan et al., 2010). The great benefit of this approach is its fast, DNA-specific and inexpensive method of DNA quantitation. Disadvantages of this approach include a lack of DNA sequence specificity, which makes it unfeasible to employ directly in analysis of genome-specific DNA samples.

A remarkable improvement of this technique has been achieved by the combination of two approaches: microwave-accelerated sequence-specific hybridization of the target DNA with anchor DNA, immobilized on a metal surface, and the Metal-Enhanced Fluorescence (MEF) effect, responsible for the large enhancement of a DNA's fluorescent label. Whereas the interactions of fluorophores with metallic nanoparticles has been known for some years (Drexhage, 1970; Persson, 1978), the precise mechanism for enhanced fluorescence is still of some debate (Geddes, 2010). Some authors describe the interaction as due to a modification in the radiative decay rate of a fluorophore (Lakowicz et al., 2002; Lakowicz, 2006), while Metal-Enhanced Fluorescence (MEF), described by Geddes in 2002 (Geddes and Lakowicz, 2002), gave raison d'être to this effect as due to a near-field coupling between an excited state dipole and induced surface plasmons, the surface plasmons in turn radiating the coupled quanta (Geddes and Lakowicz, 2002). Fig. 1 shows a graphical representation of the general principles of MEF and our "Catch and Signal" technology. The MEF effect, i.e. enhancement of a fluorophore's brightness, exponentially depends on the distance between chromophore and metal nanoparticle, due to a short-range (0–30 nm) coupling of a chromophore's excited state electronic system with nanoparticle (NP) plasmons (Fig. 1a). As a result, only chromophores proximal to NPs increase their emission a hundreth thousand fold. Subsequently, hybridization is not only the event of a specific recognition of a target DNA but also the creation of the MEF pair (fluorophore–NP plasmons), which enhances the fluorescence signal. Duplex annealing puts a fluorescent label on a short (~7 nm) enough leash, relative to a NP, thereby in a perfect condition for intense MEF. A significant addition to this technology is microwave "heating" of the reacting system, which significantly speeds up the process of DNA hybridization (Aslan et al., 2006), which is an important attractive feature of any bio-assay. In our recent publications we have presented a platform 1-color DNA assay, based on the "Catch and Signal" technology, for the detection of genome-specific DNA and demonstrated the power of this technique by the detection of DNA from *Bacillus anthracis* spores and the vegetative cells (Aslan et al., 2008).

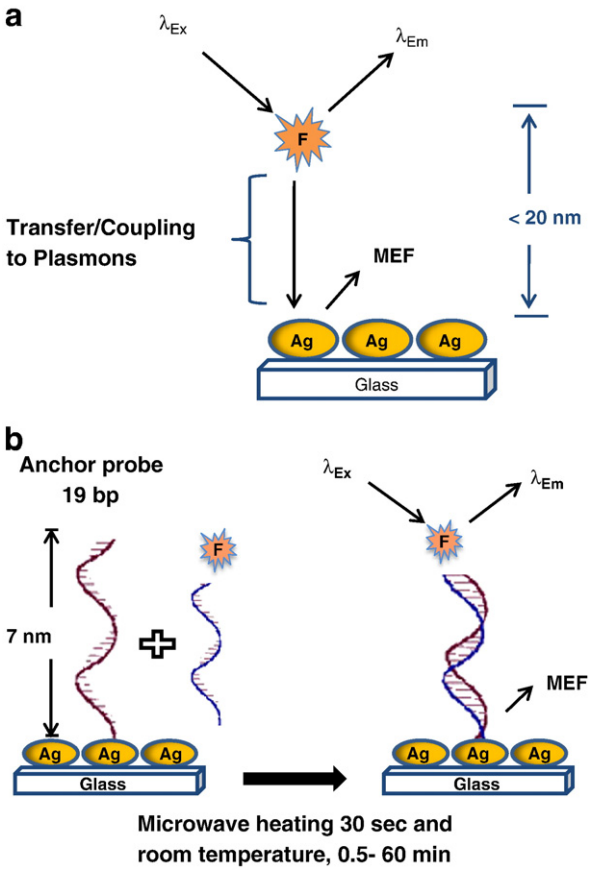

Fig. 1. (a) Graphical representation of the current interpretation of MEF. (b) MAMEF surface DNA capture assay, annealing in microwave cavity and the control assay run at room temperature, "Rapid Catch and Signal" technology.

In this short communication we present further development of the "Catch and Signal" technology—principles of a 2-color DNA assay for the simultaneous detection/quantification of two genome-specific DNAs in one well.

2. Materials and methods

2.1. Materials

Silver nitrate (99.9%), sodium hydroxide (99.996%), ammonium hydroxide (30%), *D*-glucose and premium quality APS-coated glass slides (75×25 mm) were obtained from Sigma-Aldrich.

2.2. The 19 base DNA sequences

The 19 base DNA sequences used in this study are fragments of Chinese hamster ovary (CHO) Alu sequence. Oligonucleotides were purchased from Integrated DNA Technologies, Inc.:

"Target" Green-DNA:

5′-/Alexa488N/TTC TTT TGC TCA TAT CTC T-3′