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Journal of Biotechnology 122 (2006) 16–27
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Effect of mixing on reaction–diffusion kinetics for protein hydrogel-based microchips

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Received 29 March 2005; received in revised form 11 July 2005; accepted 4 August 2005

Abstract

Protein hydrogel-based microchips are being developed for high-throughput evaluation of the concentrations and activities of various proteins. To shorten the time of analysis, the reaction–diffusion kinetics on gel microchips should be accelerated. Here we present the results of the experimental and theoretical analysis of the reaction–diffusion kinetics enforced by mixing with peristaltic pump. The experiments were carried out on gel-based protein microchips with immobilized antibodies under the conditions utilized for on-chip immunoassay. The dependence of fluorescence signals at saturation and corresponding saturation times on the concentrations of immobilized antibodies and antigen in solution proved to be in good agreement with theoretical predictions. It is shown that the enhancement of transport with peristaltic pump results in more than five-fold acceleration of binding kinetics. Our results suggest useful criteria for the optimal conditions for assays on gel microchips to balance high sensitivity and rapid fluorescence saturation kinetics.

Keywords: Protein hydrogel microchips; Reaction–diffusion kinetics; Peristaltic pump; Antibody–antigen interaction; Fluorescence measurement

1. Introduction

Biological microchips, or arrays of individual elements containing various probes (DNA, proteins, oligosaccharides, cells, etc.), serve as miniature tools for different research and practical purposes (for review see, e.g. Nature Genetics, 2002; Kolchinsky and Mirzabekov, 2002; Cutler, 2003; Seong and Choi, 2003; Stoll et al., 2004). Recently, three-dimensional hydrogel-based microchips with immobilized DNA and proteins were developed at the Moscow Institute of Molecular Biology (Rubina et al., 2003, 2004). Immobilization of probes within three-dimensional hydrogels offers many advantages over two-dimensional surface immobilization used by most microchip manufacturers. In particular, the increase in immobilization

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doi:10.1016/j.jbiotec.2005.08.010

Nomenclature

$[Ab_i]$	concentration of immobilized antibodies
$[Ag]_{sol}$	concentration of antigens in solution
D	diffusion coefficient
J_s	signal at saturation
K_{ass}	thermodynamic association constant
R	radius of semispherical gel pad
$\dot{V}$	volume flow rate generated by peristaltic pump
τ_B	saturation (or binding) time

capacity as compared with surface microchips, aqueous surrounding of immobilized compounds, absence of contacts with hydrophobic surface, and stabilizing effect of the gel appear to be especially important for proteins. Gel-based protein microchips were used for studies of protein–protein and protein–ligand interactions for enzymatic reactions and various immunoassays (Rubina et al., 2003; Dementieva et al., 2004; Rubina et al., 2005). The most important applications involve quantitative immunoassay of tumor-associated markers and biological toxins with the sensitivity comparable to or higher than that of standard immunological methods.

As a means of analysis, protein microchips should satisfy two conditions: (i) be sensitive enough to detect low concentrations of ligands or antigens and (ii) the time of analysis should be as short as possible. For gel protein microchips, the time needed for the saturation of observable fluorescence signals is mainly determined by hindered diffusion of antigens into the gel pads and their interaction with the immobilized antibodies. Although diffusion of proteins in different gels has been investigated rather intensively (Williams et al., 1998; Pluen et al., 1999; Ramanujan et al., 2002, and references therein), the study of protein kinetics combining both diffusion and molecular interactions within gel pads has been performed to a much lesser extent. However, for the latter problem we may relate to the previous results of the theoretical and experimental analysis of hybridization kinetics on the oligonucleotide microchips (Livshits and Mirzabekov, 1996; Sorokin et al., 2003; Bhanot et al., 2003; Dorris et al., 2003; Gadgil et al., 2004). The similarity should also be noted between this problem and the classical chemical engineering problem of transport to and into a catalyst particle. Among the extensive literature devoted to the reaction–diffusion kinetics, we may mention (Bird et al., 1960; Moelwyn-Hughes, 1971; Probstein, 1994; Schmidt, 1997; Froment, 2001).

The former investigations have shown that the characteristic hybridization time for the reaction–diffusion kinetics on the oligonucleotide microchips is about 10–20 h (and, as will be shown below, such assessment holds for the protein microchips as well). Therefore, the acceleration of saturation kinetics is highly desirable. Since we are interested primarily in mass application of protein microchip technology, we chose peristaltic pump as standard and inexpensive equipment for the acceleration of kinetics. Among other methods to accelerate the rate of kinetics it is worth mentioning the application of electric field (Heaton et al., 2001; Oddy et al., 2001), acoustic waves (Toegl et al., 2003), and microfluidic devices with different types of mixing (Adey et al., 2002; Yuen et al., 2003; McQuain et al., 2004).

The goal of this work was to compare the reaction–diffusion kinetics on protein hydrogel microchips and its acceleration by mixing buffer solution with peristaltic pump. The experiments were carried out on microchips with immobilized antibodies under the conditions utilized for on-chip immunoassay. To design and interpret the experiments, we present the relevant theory and demonstrate a good agreement between the theoretical predictions and experimental observations. It is shown that the same factors that enhance the final fluorescence intensities result in longer time needed to reach equilibrium and saturation of fluorescence signals. Thus, our results provide useful criteria for the choice of parameters of gel microchips and reaction conditions, which balance high sensitivity and rapid fluorescence saturation kinetics.

2. Materials and methods

Insulin and monoclonal antibodies to insulin were purchased from Sigma (St. Louis, MO, USA); Cy3 fluorescence dye, monosuccinimide ester, Sephadex G-25 coarse, from Amersham Pharmacia Biosciences (Piscataway, NJ, USA); Micro Bio-Spin chromatography columns from Bio-Rad Laboratories (Hercules, CA, USA); glass slides for the fabrication of microar-