Available online at www.sciencedirect.com
ScienceDirect
Sensors and Actuators B 128 (2008) 560–565
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Electrical control over antibody–antigen binding

Elad Brod *, Shai Nimri, Boaz Turner, Uri Sivan

The Russell Berrie Nanotechnology Institute, Department of Physics, Technion-Israel Institute of Technology, Haifa 32000, Israel

Received 14 March 2007; received in revised form 4 July 2007; accepted 5 July 2007
Available online 19 July 2007

Abstract

We show that the binding of an antibody to its antigen can be controlled electrically in a reversible manner. The antibody–antigen interaction is monitored by an electrochemical surface plasmon resonance (SPR) instrument. The antigen is immobilized on the working electrode while the antibody is injected in solution. After binding, application of a bias more negative than −0.5 V versus Ag/AgCl reference electrode causes rapid detachment of the antibody molecules from the antigens. Removal of the applied voltage restores the antigen ability to bind antibody molecules. The mechanism underlying the reported phenomenon is traced to deprotonation of positively charged amino acids, particularly lysine, by hydroxyl ions generated at the electrode/solution interface. Our finding facilitates exquisite control over one of the main interactions responsible for biomolecular recognition, namely, the attraction between positively and negatively charged residues. Potential applications to diagnostics and sensing are briefly discussed.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Antibody–antigen interaction; Non-covalent interactions; Surface plasmon resonance; Hydrogen evolution reaction

1. Introduction

Gaining an electrical control over the non-covalent interactions responsible for molecular recognition is an important milestone on the way to realize a functional interface between biology and electronics. Such control will facilitate triggering and suppression of biological pathways by electronic signals presented to the system and hence supplementing biology with the remarkable computing power and programmability of electronics.

In the following we show that an antibody–antigen interaction, a paradigm for molecular recognition, can effectively be modulated through electrical control over proton concentration in the vicinity of the interacting molecules. Peptide antigens are immobilized on a gold electrode immersed in solution. Antibodies are injected into solution and bind the peptides. Application of a bias more negative than −0.5 V (relative to Ag/AgCl electrode), causes rapid detachment of the antibodies from the peptides. When the bias is removed the antibodies rebind the peptides.

Specific binding of an antibody to its antigen depends on several interactions, including the electrostatic attraction between oppositely charged amino acids on the two molecules. In biologically relevant buffers, the short screening length requires an exquisite spatial matching of the charged residues on the two molecules, but when such matching occurs, the resulting attraction free energy is large. The electrostatic attraction between amino acids is sensitive to local proton concentration since the latter determines their side chain protonation state and thus, their net charge. Amino acids with acidic side chains turn negative above pH ≈ 4 and neutralize at pH ≤ 3. Amino acids with basic side chains are positively charged at natural pH and neutralize under alkaline conditions.

Two antibody–antigen pairs were examined in this work. Studies of their binding (with no applied bias) show that the pairs dissociate monotonically with pH in the range 7.2–10. Above pH 10 no binding is observed. This range of pH values hints to deprotonation of lysine as the mechanism underlying dissociation. Indeed, both peptides carry this amino acid.

Local pH next to a gold cathode can effectively be modulated by the hydrogen evolution reaction (HER) [1]. HER takes place

* Corresponding author.
E-mail address: elad.brod@gmail.com (E. Brod).

0925-4005/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2007.07.061

on an electrode/solution junction when a sufficiently reductive potential is applied to the electrode [2]. In alkaline solutions, hydrogen evolves from water [3]:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (1)$$

leaving behind hydroxyl ions that recombine with protons to produce water and thus increase the local pH [4,5]. The distance over which the pH deviates considerably from its native value can be approximated by the recombination length of hydroxyl ions, typically, between a few to hundreds nanometers depending on the pH and buffering capacity of the solution. This out-of-equilibrium effect is thus confined to the vicinity of the cathode. As soon as the bias is removed, the native pH profile is restored by proton and hydroxyl diffusion.

Given the observed antibody–antigen dissociation at basic pH values and the coincidence between an electrical triggering of that dissociation and HER, as observed in electrochemical measurements, we attribute the reported phenomenon to electrically induced enhancement of the local pH next to the cathode. The increased pH leads to deprotonation of lysines on the peptide antigens and consequently to a loss of the peptides electrostatic binding to their corresponding antibodies. When the bias is removed the native pH profile is restored, the lysines regain their charge, and the peptides resume their ability to bind their respective antibodies. Our conjecture is supported by corroboration of electrochemical and dissociation data, studies of the effect as a function of pH and bias, and mapping the effect of buffering capacity on the observed phenomenon. Since the effect is confined to the immediate vicinity of the electrode, small electrodes should facilitate spatial control over molecular recognition and protein function, which is yet another new dimension enabled by the reported phenomenon. Several potential applications reflecting these advantages are listed towards the end of the manuscript.

Previous reports of electrical effects on biomolecular interactions included modulation of the activity of an enzyme immobilized on a membrane [6], redox reactions [7–9], dissociation of molecules by reducing covalent bonds [10], and electrophoretic and electrostatic control over DNA hybridization [11,12]. None of these reports demonstrated an electrical control over pH for the modulation of molecular recognition next to an electrode.

Non-electrical control over antibody–antigen interaction has been exercised through an anion induced conformational change in the antigen peptide [13] and the kinetics of the interaction has been controllably accelerated by application of high hydrostatic pressures [14].

We use a combination of electrochemical setup and an SPR instrument to simultaneously modify the pH in the vicinity of the antibody–antigen pair, and monitor the resulting change in binding. Electrochemical SPR experiments are widely used to investigate ion adsorption [15], Faradaic processes [16], double layer charging [17,18], and redox proteins [19]. Additionally, Heaton et al. [12] investigated effects of voltage on DNA hybridization kinetics and Hodneland and Mrksich [10] designed a self-assembled monolayer that selectively, though irreversibly, releases covalently bound biotin upon application of a reductive potential. In contrast to the latter reference, in our case the antibody is released in response to a change in the protonation states of the antigen rather than breaking of covalent bonds. The effect reported here is, hence, reversible.

2. Experimental

The experiment was carried out using a ProteOn XP (lab prototype) SPR instrument developed by Bio-Rad Haifa LTD. The measuring system (Fig. 1) included six 450 μm wide, 100 μm high channels carrying running solutions in contact with the chip top surface. The chip (Fig. 1b) comprised a glass prism whose top surface was coated with a thin adhesion layer of chromium followed by a 50 nm thick polycrystalline gold layer, patterned to yield working, reference, and counter electrodes. Silver was electroplated on the central part of the reference electrode from 0.5 M $AgNO_3$ solution. Electrochemical chlorination of the silver with 1 M HCl solution produced an Ag/AgCl layer, several microns thick, on top of the electrode. Unless specified other-

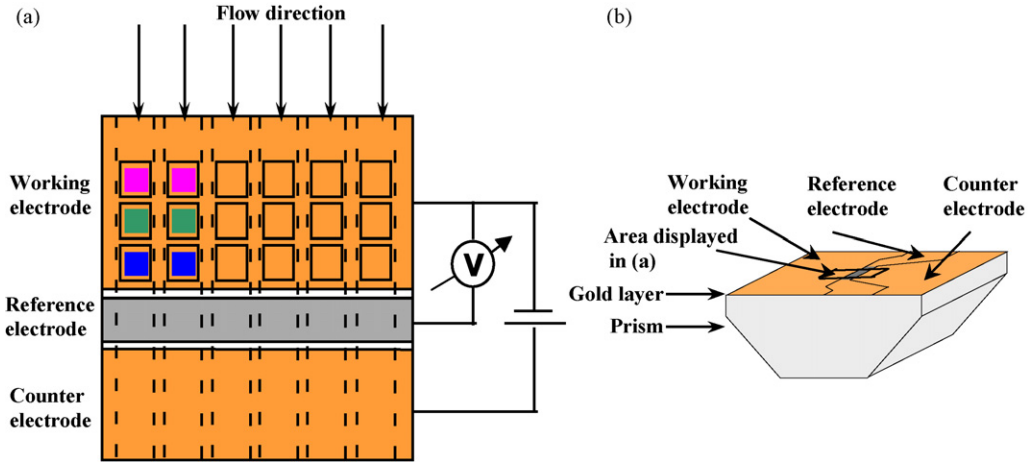

Fig. 1. (a) Monitored area of the SPR chip and electrical circuit. The areas in contact with the six fluid channels are marked by dashed lines. Areas of interest (AOI) are denoted by squares and colors correspond to different curves in Fig. 2. (b) An overall view of the patterned chip. The area covered by panel (a) is indicated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Download English Version:

https://daneshyari.com/en/article/741581

Download Persian Version:

https://daneshyari.com/article/741581

Daneshyari.com