Available online at www.sciencedirect.com
ScienceDirect
Analytical Biochemistry 374 (2008) 263–271
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Force differentiation in recognition of cross-reactive antigens by magnetic beads

Tamara Y. Morozova [a], Victor N. Morozov [a,b,*]

[a] *Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region 142290, Russia*
[b] *National Center for Biodefense and Infectious Diseases, George Mason University, Manassas, VA 20110, USA*

Received 15 August 2007
Available online 8 December 2007

Abstract

Functionalized magnetic beads have been suggested recently as active labels for extremely rapid and highly sensitive immunoassay. Here we addressed the problem of specificity and cross-reactivity in such detection, which (unlike conventional immunoassay methods) cannot rely on a difference in the equilibrium binding constants to distinguish between closely related antigens. Microarrays containing spots of nine albumins from sera of different mammals (human, bovine, sheep, goat, pig, dog, rabbit, rat, and mouse) were tested for their interaction with magnetic beads functionalized with monoclonal antibodies against bovine or human serum albumin. It was demonstrated that the magnetic beads bound only those albumin spots to which antibody was reactive or cross-reactive in enzyme-linked immunosorbent assay (ELISA). The effect of cross-reactivity in the assay with magnetic beads detection could be decreased substantially by placing the array into a flow cell and subjecting the tethered beads to increasing shear flow, which removed beads first from the weakest cross-reactive antigens and then from more strong ones. Partial blocking of the antibody molecules on the bead surface was shown to reduce critical shear stress necessary to remove beads from the specific antigens, indicating that multiple antigen–antibody bonds held the beads on the array surface.

Keywords: Magnetic beads; Immunoassay; Antibody; Cross-reactivity; Microarray; Shear flow

Recognition of cross-reactive antigens in conventional immunoassay techniques is based on the difference in their binding equilibrium constants. The latter is determined by measuring the concentration dependence of the equilibrium binding for each antigen in a range of concentrations close to their dissociation constant, K_d. Reaching equilibrium at low concentrations may take hours for a good strongly binding antibody. The problem of reaching equilibrium is further aggravated in the heterogeneous assay where the reaction of immobilized antibody molecules with dissolved antigens is limited by the diffusion-controlled transport [1–3]. A relatively high limit of detection (LOD)[1] is another diffusion-related drawback of the conventional heterogeneous assays. Even the most sensitive sensor capable of detecting single molecules cannot have an LOD lower than the femtomolar level so as to avoid the extremely long time needed for the analyte and sensor to come into contact [4–6].

A principally new approach has been developed recently, resulting in a decreased LOD and a substantially

* Corresponding author. Fax: +1 703 993 4288.
E-mail address: vmorozov@gmu.edu (V.N. Morozov).

[1] *Abbreviations used:* LOD, limit of detection; ds-DNA, double-stranded DNA; BSA, bovine serum albumin; HSA, human serum albumin; GSA, goat serum albumin; SSA, sheep serum albumin; DSA, dog serum albumin; RbSA, rabbit serum albumin; MSA, mouse serum albumin; RtSA, rat serum albumin; PSA, pig serum albumin; IgG, immunoglobulin G; anti-mouse–IgG–AP, alkaline phosphatase-labeled goat anti-mouse–IgG; Ova, ovalbumin; StA–AP, alkaline phosphatase-labeled streptavidin; biotin–BSA, biotin-labeled BSA; DMP, dimethyl pimelidate dihydrochloride; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide ethanolamine; MES, 4-morpholineethanesulfonic acid; NHS, *N*-hydroxysuccinimide; PVA, poly(vinyl alcohol); pNPP, *p*-nitrophenyl phosphate; RF, radio frequency; ELISA, enzyme-linked immunosorbent assay; HF, hydrofluoric acid; CCD, charge-coupled device.

0003-2697/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2007.12.007

accelerated immunoassay by replacing diffusion with electrophoretically assisted delivery of analytes to antibodies or antigens immobilized on a surface [7–9]. The active immunoassay causes antigen–antibody reactions to occur within 5 to 10 min, resulting in 70 to 80% of all analytes in the sample bound to the surface [8]. Equilibrium in the latter case is established in a micron-thick layer of protein solution concentrated over a layer of immobilized macromolecules. A high effective concentration of antibodies in such a layer (C_a ~7 μM estimated from a typical surface density of immobilized antibody molecules in a spot ~100 ng/cm^2 according to Ref. [10]) results in binding of every single antigen molecule brought to the layer provided that $K_d << C_a$. Thus, in the active assay, any antigen with a K_d satisfying the latter condition will bind to the array independent of its concentration. How might closely related analytes be recognized under such nonequilibrium conditions?

Instead of measuring differences in the equilibrium binding, one can use the difference in kinetic constants to distinguish between closely related analytes. It is well known [11] that in a series of related antigens, K_d changes mostly due to changes in the dissociation constant, k_{diss}, and the difference in k_{diss} may be evaluated quickly by applying a force and measuring the bond lifetime under the load. The first practical application of such force differentiation was described by Blank and coworkers, who used a double-stranded DNA (ds-DNA) zipper as a molecular force sensor to discriminate between cross-reactive antigen–antibody bonds [12,13]. Atomic force spectroscopy demonstrated [11,14–16] that antigen–antibody bonds may be broken within milliseconds by applying a force of 200 to 500 pN, when the latter is applied rapidly (10^2–10^4 pN/s). Other techniques based on the use of magnetic beads [9,17,18], shear flow [19,20], and electric fields [21] have demonstrated that the force necessary to break a typical antigen–antibody bond drops to 2 to 10 pN when the bond is subjected to such a force for 1 to 2 min. Of all these methods, magnetic beads provide the most convenience because both magnetic and hydrodynamic forces may be employed and because the beads may also serve as highly sensitive labels capable of detecting single captured analyte molecules [9,22]. As demonstrated in our previous publication, 500 to 1000 protein molecules (or viruses) could be reliably detected within a few seconds by scanning microarray with functionalized magnetic beads [22].

It is important to understand whether such an active beads detection technique can recognize closely related antigens considering that not only the strength of the antigen–antibody bond mentioned above but also many other factors operate in a real bead-based assay. The roughness of the surfaces of both the array substrate and the beads, the length of the link that binds probe molecule and captured antigen to the surface, and the presence of multiple parallel bonds in the bead–array contact all are factors that may change the critical shear flow needed to break bead–array contact. To address the problem of specificity in a real active immunoassay experimentally, we studied a simple model system in which monoclonal antibodies specific for serum albumin were immobilized on magnetic beads and tested for their interaction with a multicomponent microarray composed of closely related antigens–albumins from different hosts. Monoclonal antibodies were chosen instead of polyclonals to avoid dealing with the great variety of dissociation constants that characterize binding of antigen to a polyclonal antibody. In this article, we show that even in the presence of multiple bonds in the bead–array contact, one can easily distinguish between closely related antigens by using force differentiation.

Materials and methods

Reagents

The following reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA): bovine serum albumin (BSA), human serum albumin (HSA), goat serum albumin (GSA), sheep serum albumin (SSA), dog serum albumin (DSA), rabbit serum albumin (RbSA), mouse serum albumin (MSA), rat serum albumin (RtSA) pig serum albumin (PSA), anti-mouse immunoglobulin G (IgG), monoclonal anti-BSA–IgG and anti-HSA–IgG, alkaline phosphatase-labeled goat anti-mouse IgG (anti-mouse–IgG–AP), ovalbumin (Ova), fish gelatin, alkaline phosphatase-labeled streptavidin (StA–AP), biotin-labeled BSA (biotin–BSA), dimethyl pimelidate dihydrochloride (DMP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide ethanolamine (EDC), *N*-hydroxysuccinimide (NHS), poly(vinyl alcohol) (PVA), sodium azide, sodium chloride, sodium cyanoborohydride, sodium phosphate, sodium sulfate, sodium carbonate, trehalose, triethanolamine, Tween 20, and *p*-nitrophenyl phosphate (pNPP, as Sigma FAST tablets), 4-morpholineethanesulfonic acid (MES).

Materials

Dialysis membrane from regenerated cellulose was obtained from Fisher Scientific (Pittsburgh, PA, USA). Nylon mesh was purchased from Small Parts (Miami Lakes, FL, USA). Dynal MyOne superparamagnetic beads functionalized with carboxyl groups (COOH beads) were purchased from Invitrogen (Carlsbad, CA, USA).

Manufacturing of multiantigen microarrays

All nine albumins were dialyzed against water, and protein concentrations were measured by UV absorption, using an extinction coefficient at 280 nm of $\varepsilon = 0.6$ for a 0.1% solution for all albumins except BSA ($\varepsilon = 0.66$), HSA ($\varepsilon = 0.55$), and MSA ($\varepsilon = 0.58$). Trehalose was added 10:1 (w/w) to each solution to protect proteins on electrospray deposition [23]. A nine-component albumin microarray was manufactured by electrospray deposition [24,25] of 3 μl of each 0.1% albumin solution through a polyester mesh (cat. no. CMY-0150D, Small Parts).