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Enantioselective Sensors Based on Antibody-Mediated Nanomechanics

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The use of microfabricated cantilevers as bioaffinity sensors was investigated. Since many bioaffinity interactions involve proteins as receptors, we conducted studies of the magnitude, kinetics, and reversibility of surface stresses caused when common proteins interact with microcantilevers (MCs) with nanostructured (roughened) gold surfaces on one side. Exposure of nanostructured, unfunctionalized MCs to the proteins immunoglobulin G and bovine serum albumin (BSA) resulted in reversible large tensile stresses, whereas MCs with smooth gold surfaces on one side produced reversible responses that were considerably smaller and compressive. The response magnitude for nanostructured MCs exposed to BSA is shown to be concentration dependent, and linear calibration over the range of 1−200 mg/L is demonstrated. Stable, reusable protein bioaffinity phases based on unique enantioselective antibodies are created by covalently linking monoclonal antibodies to nanostructured MC surfaces. The direct (label-free) stereoselective detection of trace amounts of an important class of chiral analytes, the α-amino acids, was achieved based on immunomechanical responses involving nanoscale bending of the cantilever. The temporal response of the cantilever (Δ deflection/Δ time) is linearly proportional to the analyte concentration and allows the quantitative determination of enantiomeric purity up to an enantiomeric excess of 99.8%. To our knowledge, this is the first demonstration of chiral discrimination using highly scalable microelectromechanical systems.

Interactions of biomolecules with surfaces to which biomolecules are bound has significant biomedical and industrial applications. Many of the techniques that are currently used for investigation of biomolecular interactions in model systems, such as antigen−antibody complexation and cell receptor recognition of polypeptides and proteins, are based on protein adsorption and surface properties. Protein interactions on solid surfaces is a complex phenomenon and involves many dynamic steps, such as covalent and noncovalent bond formation between surfaces and proteins, lateral diffusion, and conformational rearrangements of adsorbed proteins.

The term biosensor generally is used to describe analytical devices that are used for measuring biological compounds, employ biological phases as receptors, or both. Detection is often accomplished following selective binding of a ligand, the analyte, to a bioaffinity receptor. During the past decade, microcantilevers (MCs) have been increasingly used as transducers in chemical[1−9] and biological sensing[10−16] including, as selective examples, antibody-based detection of prostate-specific antigen[14] and studies of DNA hybridization.[11,15] Intermolecular forces arising from analyte molecules adsorbing on surfaces or absorbing into surface-immobilized thin films are known to induce surface stresses. When these processes occur on the surfaces of microfabricated cantilevers, static mechanical bending of the cantilever can occur. The micrometer size of the transducer brings a few advantages relative to more traditional transducers, such as surface acoustic wave or

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quartz crystal microbalance devices, which explains the increasing use of microcantilever-based sensors.[3] The high surface stress-based sensitivity of MCs to ultratrace quantities of adsorbates affords much better mass sensitivity when compared to the aforementioned gravimetric resonating devices. A MC transducer converts the Gibbs surface free energy change created by analyte–surface interactions directly into measurable mechanical responses. Asymmetry is central to the functioning of MC sensors, wherein one side of the cantilever is made active while the opposing side is largely passive to analytes. The effect of surface stress change on cantilever bending is quantified by Stoney's equation,[17]

$$z_{\max} = \frac{3l^2(1-\nu)}{Et^2}\Delta\sigma \qquad (1)$$

where ν and E are, respectively, the Poisson ratio and Young's modulus for the cantilever, t is the thickness of the microcantilever, l is the cantilever effective length, and Δs is a analyte-induced differential surface stress ($\Delta\sigma_{\text{active side}} - \Delta\sigma_{\text{passive side}}$).

Increasing understanding of the stereoselectivity of drug actions is causing regulatory agencies to establish guidelines for the development of stereoisomeric drugs and to demand that manufacturers specify their stereochemical purity. Since 1988, the U.S. Food and Drug Administration has requested not only the quantitative determination of the stereoisomeric composition of chiral drugs but also stereochemically specific identity tests and the pharmacologic and pharmacokinetic characterization of individual enantiomers.[18] Although the majority of drugs presently in development are chiral, analytical methods for the precise and sensitive determination of stereoisomers lag behind. One reason is that commonly used methods for enantiomer discrimination, such as chromatography and NMR, are relatively slow and generally not suitable for high-throughput analysis. Furthermore, chiral selectors for the direct differentiation of enantiomers are not tailor-made for a specific analyte, and the identification of a suitable selector is still a tedious trial-and-error exercise.[19] Moreover, such selectors generally exhibit only moderate stereoselectivity and show considerable cross-reactivity, which limits their applicability for sensitive sensing.[20–23]

We have recently demonstrated that the excellent stereoselectivity of suitably raised antibodies enables the detection of enantiomeric impurities with high sensitivity.[24,25] As early as 1928, Landsteiner and van der Scheer had shown that antibodies can differentiate between the enantiomers of chiral compounds.[26] However, this stereoselective potential was largely ignored by stereochemists, and only a few applications for the detection and separation of enantiomers have appeared.[27–29] Recently, we have demonstrated that nanostructuring of the active sides of MCs can magnify analyte-induced responses by 2 orders of magnitude or more in both gaseous and liquid environments.[4,5,13] MCs nanostructured on the active side were functionalized with self-assembled monolayers or thin films of cavitand receptors to provide chemical selectivity. In addition to the analytical significance of increased responses with functionalized nanostructured cantilevers, complications arising from analyte interactions at nontreated cantilever surfaces are rendered negligible using this methodology. Herein, the adsorption of two common proteins on nanostructured as well as on smooth gold MC surfaces was initially studied. Subsequently, a novel sensor is described for chiral discrimination that combines the exquisite stereoselectivity of the antibody–antigen interaction with the sensitivity of nanomechanical surface stress detection using functionalized MCs.

To produce a stereoselective nanomechanical immunosensor, we covalently immobilized monoclonal antibodies as a receptor layer on the nanostructured sides of microcantilevers. The antibodies used were raised in such a way that they selectively bind to either D- or L-α-amino acids; they are furthermore "group-specific"; i.e., they recognize a wide variety of amino acids with different side chains.[30,31] The α-amino acids represent one of the most important classes of substances in nature that incorporate a stereogenic center and, therefore, exemplify an excellent model system to demonstrate chiral discrimination. A very large number of α-amino acids have been isolated from biological and nonbiological sources. Furthermore, the α-amino acids are of utmost importance in the food, chemical, and pharmaceutical industries, e.g., as chiral building blocks. In this work, the stereoselective interaction of the anti-amino acid antibodies with amino acid analytes in an aqueous environment induced nanoscale mechanical bending of the cantilever, which was detected (Figure 1) by measuring the deflection of a laser beam reflected from the cantilever tip onto a position-sensitive detector.[4,13] Although an array of cantilevers is shown in this figure, we chemically treated all the cantilevers the same and simply recorded the response of a single randomly chosen MC within an array.

EXPERIMENTAL SECTION

Reagents. Experiments were performed using commercially available silicon array MCs (seen in Figure 1) coated with aluminum and having dimensions 400-μm length, 100-μm width, and ~1-μm thickness (Mikro Masch Co., Sunnyvale, CA). Chromium, gold, and silver metals deposited on the cantilevers were obtained from Kurt J. Lesker, Gatewest, and Alfa Aesar Co., respectively, at a purity of 99.9%. 2-Aminoethanethiol hydrochloride (AET), glutaraldehyde (GA), the salts employed for the preparation of buffer solutions, the amino acids, and all other reagents were purchased from Sigma or Fisher at highest available purity

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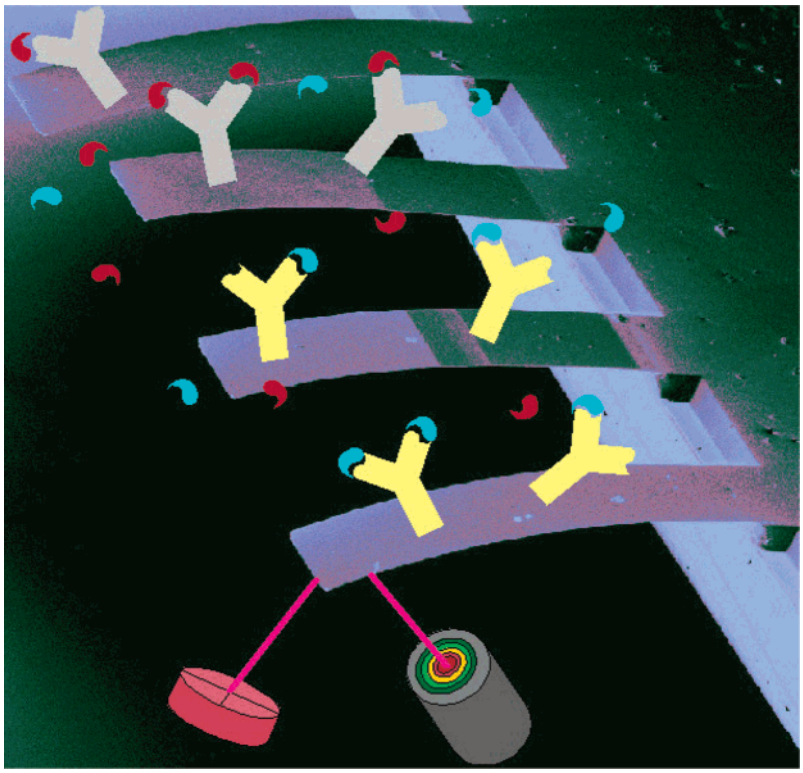

Figure 1. Micrograph of silicon cantilevers (100 μm × 400 μm × ~1 μm thick) in a one-dimensional array used in this work. Included in the figure is a schematic depiction of the optical detection system and surface-immobilized antibodies (gray and yellow symbols), which bind only enantiomers of the correct configuration (red and cyan symbols).

and used as received. The *p*-amino-D-phenylalanine was purchased in the hydrochloride form and all other amino acids were purchased in free acid form (quoted purities ranged from 98 to 99.5%). Water used to prepare solutions was obtained from a Barnstead E-pure water filtration system. The proteins, human immunoglobulin G (hIgG, reagent grade) and bovine serum albumin (BSA), were also from Sigma.

Monoclonal anti-D- and anti-L-α-amino acid antibodies and BSA conjugates of D- and L-phenylalanine were produced as previously described.[29,31] Briefly regarding the antibodies, eight-week-old BALB/c mice were immunized with *p*-azo-D- or L-phenylalanine–protein conjugates in complete Freund's adjuvant. Splenocytes from mice exhibiting strong immune responses were fused with NS0 myeloma cells using poly(ethylene glycol). Subsequently, hybridomas were selected in hypoxanthine/aminopterin/thymidine medium. The supernatants were screened by using noncompetitive enzyme-linked immunosorbent assays. Hybridomas that produced suitable antibodies were cloned by limiting dilution, and the antibodies were purified by ammonium sulfate precipitation and ion exchange chromatography. The antibodies were produced at the DeKalb laboratory location, shipped via next day air transport to the Knoxville laboratory location, and stored frozen until used.

Cantilever Surface Modification. The process of creating nanostructured surfaces on MCs is described in detail elsewhere.[4] Briefly, the original thin aluminum layer on the MCs was removed by dipping them into 1 M NaOH solution for 2 min. The cantilevers were then cleaned in a piranha bath (75% H_2SO_4, 25% H_2O_2) for 30 min, followed by thorough rinsing in deionized water (*Caution*: piranha solution reacts violently with organics). The MCs were then placed into a physical vapor deposition chamber (Cooke Vacuum Products, model CVE 301, South Norwalk, CT) to deposit appropriate metallic films on one side of the cantilevers using thermal evaporation. In this manner, a thin adhesion layer (~4 nm) of chromium was deposited and followed by evaporation of a thin (~15 nm) gold layer. Without stopping the evaporation of gold, coevaporation of gold and silver at approximately equal rates created a composite Au/Ag film of ~50-nm thickness. Subsequently, the silver was chemically removed via oxidation from the film ("dealloying") using an aqueous solution of 0.2% (w/v) $HAuCl_4$. The resulting nanostructured surface was colloidal in nature.[4] MCs with smooth gold surfaces were prepared by depositing 30-nm gold onto a 4-nm chromium adhesion layer.

In our studies, some nanostructured MCs were chemically modified by immersing them for 18 h in a 1 mM aqueous solution of AET producing a self-assembled monolayer of AET on the cantilever surface. Following thorough rinsing in deionized water, the amino groups were then derivatized with the cross-linker by immersing the cantilever in a 5% (w/v) solution of GA in water for 5 h.[32] The chemically treated cantilever was then allowed to soak in a large amount of water for 1 h to remove any nonspecifically bound GA on the nanostructured and silicon sides of the cantilevers.

Immobilization of the anti-amino acid antibodies was achieved by dipping the functionalized cantilevers into 50 mg/L solutions of antibody in phosphate-buffered saline (PBS) for 4 h. Antibodies to both D- and L-α-amino acids were separately immobilized on the functionalized surfaces of different cantilevers from different

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arrays. In one instance, the resonance frequency of the MC was measured before and after the immobilization process. The frequency component of the horizontal output of the detector was measured using a SR 770 FFT Network Analyzer (Stanford Research Systems, Sunnyvale, CA). The resonance frequency of the MC was then isolated and monitored for changes. The adsorbed mass was then estimated using eq 2,[3] where k is the

$$\Delta m = \frac{k}{4\pi^2}\left(\frac{1}{f_1^2} - \frac{1}{f_o^2}\right) \qquad (2)$$

MC spring constant [$k = (Ebt^3)/(4l^3)$], b is cantilever width, and f_1 and f_0 are resonant frequencies after and before treatment, respectively. After being washed with PBS, the functionalized microcantilevers could be stored in PBS at 4 °C for several days without loss of performance.

Instrumentation. The microcantilever deflection measurements were carried out using the optical beam deflection technique as previously described.[5] Our apparatus included a 5-mW diode laser (Coherent Laser Corp., Auburn, CA) operating at 632 nm, a spatial filtering and focusing system, and an in-house-built position-sensitive optical detector. The output of the detector was displayed and recorded using a multichannel digital recorder (Stanford Research Systems). Data were collected at 1 Hz and then running averaged over 32−180 data points to generate the figures appearing herein. This essentially eliminated noise in the raw data and also short-duration random fluctuations in the baseline. In all cases, the smoothing was determined not to alter the shapes of our slowly developing true response curves. The conversion factor for converting output voltage to MC tip deflection was determined by displacing the detector using a micrometer and measuring the resulting change in output voltage. With knowledge of the optical geometry, it is possible to report cantilever tip deflections in nm. With our arrangement, the effects of sample-related changes in refractive index on apparent deflections are eliminated.[5] The cantilever system was mounted inside a 150-μL-volume Teflon flow cell that was imaged using a Watec CCD camera. The camera facilitated aligning the focused laser beam to reflect off the cantilever tip (Edmund Industrial Optics, Barrington, NJ). Analyte solutions were delivered to the flow cell via a system of vessels connected to three-way valves allowing for switching between different solutions. The flow rate was generally adjusted to 0.1 mL/min. The entire apparatus was placed on a vibration isolation table (Newport Corp., RS2000) located in a thermally controlled environment. Measurement of pH was performed using an Orion SA 520 pH meter (Thermo Orion, Beverly, MA).

All analyte solutions were prepared in PBS solvent, which was also used as a background solution. MCs mounted in the flow cell were initially allowed to equilibrate in PBS until the signal was stable. For our purposes, tensile (compression of the nanostructured surface) and compressive (expansion of the nanostructured surface) responses involve bending away from and bending toward the silicon side of the MC, respectively.

RESULTS AND DISCUSSION

Stresses Induced by Protein−Surface Interactions on Different Surfaces. It is important to note that the static bending of MCs is not a direct consequence of mass loading, as is the case with traditional gravimetric resonating devices. Previous mechanisms describing the static bending of MCs include models based on true surface-confined phenomena and associated stress changes, as properly described by eq 1, and models based on the swelling of thin films on the cantilever surface caused by intermolecular interactions between analyte and the film.[3] In the case of protein receptors on MCs, elements of both models are probably operative. Subtle changes in the conformational stability of the proteins due to relatively small numbers of affinity interactions may give rise to relatively large effects.

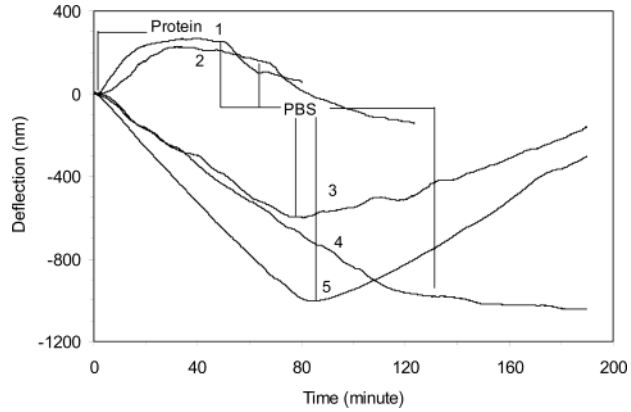

Figure 2. Time trace of cantilever deflections resulting from physical adsorption onto the gold surfaces as prepared (1−3, 5) and chemical attachment via GA linkage (4) of 50 mg/L BSA (1, 5) and human IgG (2−4) on smooth (1, 2) and nanostructured (3−5) MCs.

In an initial series of experiments, the behavior of nanostructured cantilevers when exposed to proteins was studied. When MCs with smooth gold surfaces were exposed to BSA and hIgG, adsorption of protein caused substantial cantilever deflection that corresponds to a compressive surface stress change, whereas nonspecific binding of BSA and hIgG onto a nanostructured surface induced a large tensile surface stress change (see Figure 2). The behavior of the asymmetrically nanostructured MCs is in stark contrast to that of the cantilevers with smooth surfaces. In comparison to smooth gold, 50-nm dealloyed gold coating increases MC deflections by roughly a factor of 3. In fact, we consistently observe enhancements in MC deflections upon nanostructuring of previously smooth MC surfaces that sometimes reach orders of magnitude and exceed the increase in surface area.[4,5,13] Microscopic investigations of the surfaces that are nanostructured by the dealloying process reveal its colloidal-like morphology.[4] Consistent with our MC observations, theoretical and experimental studies have shown that stresses caused by inter- and intramolecular interactions in surface-confined colloids may exceed that on smooth surfaces by several orders of magnitude.[4,33]

Depending on the nature of the protein and the protein−surface interaction, the observed bending can be compressive or tensile.[12,13] The kinetics of cantilever deflections was comparatively slow in the case of nanostructured gold coating, indicating that slow postbinding protein rearrangement contributes to MC deflections.[12] With the nanostructured surface, the direction of the MC deflections due to nonspecific binding of protein is just the opposite from the direction of MC deflection using a smooth

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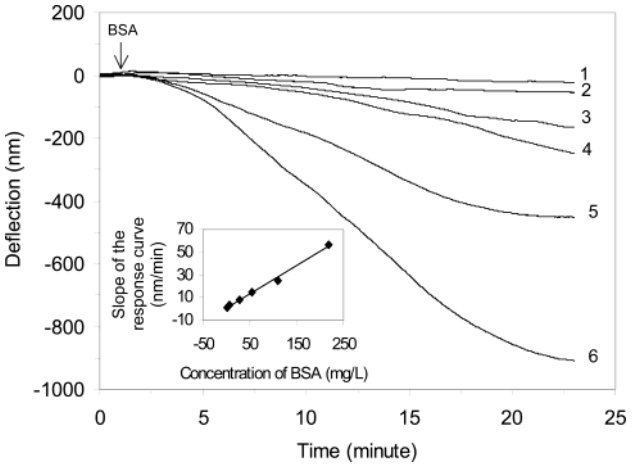

Figure 3. Concentration-based deflections of a nanostructured MC exposed to different concentration of BSA (1−208 mg/L) in PBS. (1) 1.1, (2) 5.4, (3) 27, (4) 54 , (5) 109, and (6) 208 mg/L. The inset shows the concentration-dependent slope of the response curve in a wide range of BSA concentration (1−208 mg/L).

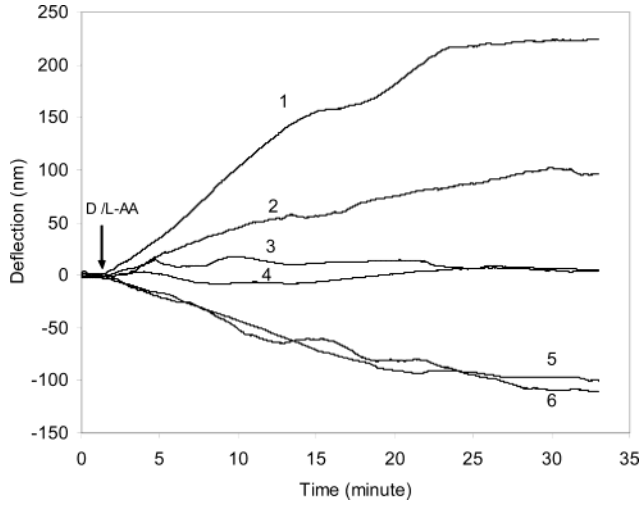

Figure 4. Time trace of cantilever deflections resulting from the binding of various free amino acids to MCs modified with covalently immobilized anti-L-amino acid antibody (1−4) or human immunoglobulin G (5, 6): (1) 50 mg/L L-tryptophan; (2, 5) 50 mg/L L-phenylalanine; (3) 50 mg/L D-tryptophan; (4, 6) 50 mg/L D-phenylalanine.

gold surface, which was also observed for pH responses,[5] biotin−albumin binding,[13] and thiolated DNA binding responses. As previously described,[5] this indicates a different chemical nature of the nanostructured surface resulting from the dealloying process. In contrast to smooth gold deposited by physical vapor deposition, the dealloying process imparts a positive charge to the surface. For example, Welland and co-workers reported slow compressive responses to IgG and slow tensile response to BSA on smooth gold AFM cantilevers.[12] Inconsistencies between the responses of MC prepared and stored differently can probably be attributed to the heterogeneity of protein samples, as well as differences in surface charge and adsorbed organics content that relate to the methods used to form the gold layers and its postformation history.

From Figure 2, it is clear that interactions of proteins with smooth and nanostructured gold surfaces produced cantilever bending that reversed when the protein solution was replaced by background buffer solution (PBS). To produce microcantilevers with stable and reusable protein coatings, the surfaces were chemically modified by AET and GA. As seen in Figure 2 (trace 4), a stable coating of IgG can be created by this procedure.

Figure 3 shows responses for silicon cantilevers with 50-nm nanostructured surface exposed to different concentrations of BSA for 20 min. The response of the cantilever induced by the binding of protein to the surface during the first 5 min following exposure increases directly with the concentration in the range of 1−208 mg/L BSA. The coefficient of variation (CV) tested via ($n = 4$) consecutive measurements of a solution of 54 mg/L BSA was 10%.

Chiral Discrimination Studies. Immobilization resulted in a cantilever resonance frequency decrease of ∼30 Hz (from $f_0 =$ 10 500 Hz; see eq 2), indicating less than 1 fmol of antibody was immobilized on the functionalized surface of a single MC. Since immobilization based on glutaraldehyde linkage occurs randomly via accessible amino groups, some of the α-amino acid binding sites of the antibody may be lost in the process. This potential loss in activity is expected to be far less than the gain in surface-bound antibody amount and response (see discussion above) resulting from surface nanostructuring.

The issue of response selectivity is central to the development of advanced sensors. High levels of specificity involving molecular recognition, e.g., antibody−antigen interactions, are generally considered desirable in analytical chemistry. However, inherent to this high-level specificity can be a lack of versatility. Consider as examples separation techniques that exhibit large numbers of theoretical plates. Differences in selectivity factors of less than 1% are more than adequate to baseline resolve injected analytes in capillary chromatography or capillary electrophoresis; greater levels of selectivity often waste analysis time. With chemical sensors, the effective plate count is one. Consequently, the analysis of mixtures or structurally similar compounds (e.g., enantiomers) becomes more challenging. Parallel or sequential exposure of sample components to the differently responding sensors in an array can be used with patterned recognition to glean analytical information, even when the range of selectivity factors for sensor−analyte combinations is not enormous. A unique characteristic of bioaffinity sensing using the anti-α-amino acid antibodies employed herein is that they provide high levels of enantioselectivity while being group-specific and, hence, represent very versatile reagents for sensing applications as well.

In the chiral discrimination experiments, the enantiomers of phenylalanine and tryptophan were injected over a microcantilever surface modified with anti-L-α-amino acid antibody (anti-L-AA) at a flow rate of 0.1 mL/min. The stereoselective interaction of the immobilized antibody with the L-enantiomers of these amino acids resulted in large positive responses, which corresponds to a compressive surface stress change (see Figure 4.). It is interesting to note the large magnitude of response to low molecular weight molecules that do not appreciably mass load the cantilever. In contrast to these large responses, no significant response was observed using the D-enantiomers as analytes. As seen in the figure, the injection of the enantiomers of phenylalanine over a nonspecific, control cantilever with immobilized hIgG caused a sizable tensile surface stress change but did not exhibit any stereoselectivity.

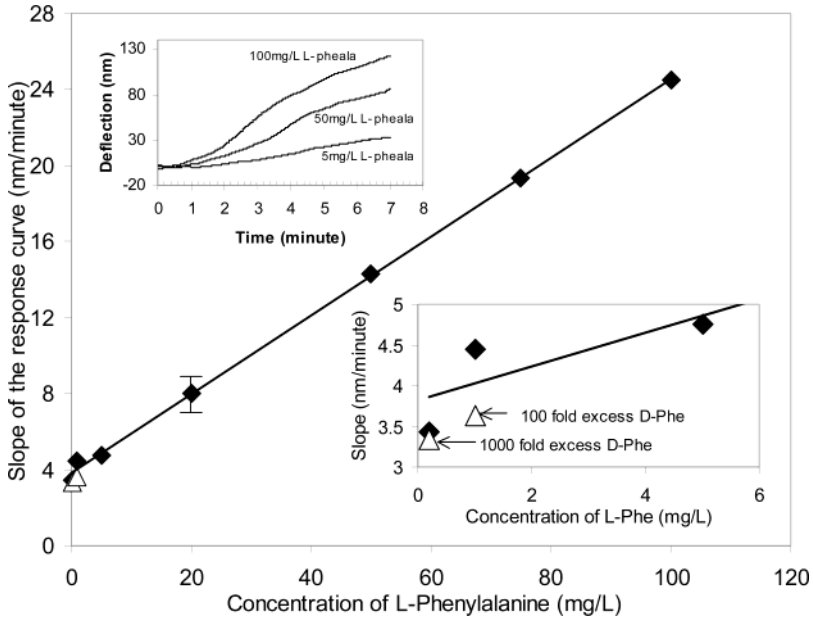

Figure 5. Analytical data that includes a calibration curve ($r^2 = 0.999$) based on the slope of the response curve obtained with an anti-L-AA functionalized MC. The cantilever was exposed to varying concentrations of L-phenylalanine as well as to mixtures containing a 100- and 1000-fold excess of D-phenylalanine. The lower inset shows the concentration-dependent slope of the curve in the lower range of concentration (≤5 mg/L L-phenylalanine) with the mixed-system data and the upper inset shows examples of concentration-based deflections of an anti-L-AA functionalized MC exposed to L-phenylalanine. The error bar indicates the standard deviation (with CV = 2%) for four replicate measurements using 20 mg/L L-phenylalanine.

Table 1. Maximum Deflections and Δ Deflection/Δ Time for Antibody-Functionalized Microcantilevers Exposed to Amino Acid Enantiomers

	anti-D-AA Ab on surface		anti-L-AA Ab on surface	
exposure to amino acid (concn, mg/L)	max deflection (nm)	slope of response curve (nm/min)	max deflection (nm)	slope of response curve (nm/min)
D-phenylalanine (50)	170	7.2	7	0.86
L-phenylalanine (50)	35	1.1	110	4.6
p-azo-D-Phe-BSA (20)	145	22	100	3.3
p-azo-L-Phe-BSA (20)	61	8	180	7
D-tryptophan (50)	56	5.1	5	0.64
L-tryptophan (50)	3	0.66	220	13
D-methionine (50)	160	7.5	6	−0.54
L-methionine (50)	10	0.2	195	7
D-valine (50)	130	5.2	10	−0.34
L-valine (50)	4	0.05	115	5.6
D-histidine (50)	114	6.5	13	1.1
L-histidine (50)	22	1.4	75	4.2
p-amino-D-phenylalanine-HCl (50)	385	32	−10	−1.44
p-amino-L-phenylalanine (50)	6	−0.86	117	9.9

In further experiments, chiral discrimination was also demonstrated for the enantiomers of *p*-aminophenylalanine, valine, methionine, and histidine using both anti-L-AA and anti-D-α-amino acid antibody (anti-D-AA) as surface receptors (Table 1). In all cases, the specific binding of the α-amino acids to the antibodies created compressive surface stresses, which may involve gradual conformational changes of the antibodies (note the time frame for the response in Figure 4); it is furthermore known that electrostatic, steric, and hydrophobic interactions can cause surface stress.[34] It is significant that only the free amino acid enantiomers with the "correct" configuration caused large responses with the corresponding antibody on the cantilever surface, while the results obtained with the opposite enantiomer generally were not discernible from short-term drifts in the baseline. Thus, only the L-enantiomers of the amino acids bound to anti-L-AA, while only the D-enantiomers bound to anti-D-AA. Large enantioselective responses were also observed when *p*-aminophenylalanine, covalently linked to the protein BSA was used as analyte; the response curves, however, indicate some nonspecific interactions between the sensor surface and the BSA conjugates. The selectivities demonstrated for the free amino acids (ratio of correct-to-incorrect enantiomer responses) presented in Table 1 may improve with dilution. Also, it is important to note that the incorrect enantiomer solutions may contain as much as 2% correct enantiomer impurity based on purities quoted by the supplier.

As regeneration is critical for the successful reuse of sensor surfaces, several solutions were tested for their ability to release bound amino acids without significantly affecting the binding capacity of the antibody surface. A unique property of the monoclonal antibodies used in these studies is their ability to provide a high level of enantioselectivity with relatively modest binding constants. This property permitted chiral discrimination by affinity liquid chromatography in an isocratic mode of operation in prior work[29] and efficient regeneration of the microcantilever surfaces, in this work, using a simple rinse with 10 mM, pH 9 phosphate buffer. Replicate regenerations of up to 10 were successfully tested; higher levels of regeneration may be possible.

The temporal response of the cantilever (Δ deflection/Δ time), induced by the binding of analyte to immobilized antibody during the first 5 min following exposure, was found to be directly proportional to the concentration of analyte. The linear relationship

(34) Safran, S. A. *Adv. Phys.* **1999**, *48*, 395−399.

between the slope of the response curve and the analyte concentration could therefore be used for quantification purposes. A working curve over the range of 0.2−100 mg/L of the correct phenylalanine enantiomer is shown in Figure 5. Since the antibodies exhibit no significant cross-reactivity to the "wrong" enantiomer, quantification was also possible in mixtures of enantiomers, thus allowing measurements of enantiomeric excess. For example, stereoselective interaction between the anti-L-AA antibody and L-phenylalanine at a concentration of 0.2 mg/L (∼1 μM) was not appreciably affected by the presence of D-phenylalanine at a concentration of 200 mg/L (see inset of Figure 5), which corresponds to an enantiomeric excess of 99.8%.

In prior work, we typically obtained measurement reproducibility with CV values of ∼10%.[5] Because of the limited availability of the immunological reagents used in these studies, large numbers of replicate analyses were not attempted. Nevertheless, using a single cantilever array and four replicate measurements on the same day, the reproducibility obtained for the immunomechnical MCs was very good (see Figure 5). Measurements of resonance frequencies of MCs from different array chips varied considerably for the MCs supplied for this work, indicating variations in thickness and hence responses (see eq 1). However, highly precise nanofabrication is possible, and when the nanostructuring and chemical treatment steps employed herein are performed simultaneously on large batches of MCs, good intraday reproducibility for large numbers of measurements should be feasible.

CONCLUSIONS

We have shown here that the combination of highly selective antibodies as chiral selectors with an efficient transducer system results in a potent sensor, which can be applied for the discrimination of a variety of α-amino acid enantiomers and for the detection of trace enantiomeric impurities. Since antibodies can be raised against virtually any molecule of interest, this approach can be readily extended to other chiral compounds. Moreover, the antibody-functionalized surface was found in these studies to regenerate with simple rinsing for at least 10 measurements. The utilization of nanomechanical surface stress detection as a direct transduction method allows extremely sensitive detection of biological interactions without the need of labeling, optical excitation, or external probes. In addition, microcantilevers are routinely microfabricated by standard low-cost silicon technology. The major advantages of using antibodies as chiral selectors with microcantilever sensors, over more traditional scale transducers such as surface acoustic wave and quartz crystal microbalance, resides in the superior sensitivity to minute quantities of analytes and an ability to microfabricate compact one- and two-dimensional arrays of cantilevers to facilitate simultaneous, hence high-throughput, measurements.

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