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Biochemically Responsive Smart Surface

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Abstract

A design of smart surfaces responsive to biochemical analytes is demonstrated in the example of mixed monolayers of biotin/fluorocarbon. The contact angle of aqueous solutions on such surfaces decreases upon streptavidin binding and can be used in detecting this protein. The specificity of the effect is confirmed by lack of the contact angle change by streptavidin blocked with biotin and by BSA.

Keywords

hydrophobicity switching; biotin; streptavidin; biochemically responsive surface

INTRODUCTION

Materials that can respond selectively to their environment or to a certain stimuli by switching one or more of their critical properties are called “smart”[1]. One of the properties that have received special attention is wettability. A number of groups have demonstrated fabrication of smart surfaces that display wettability changes induced by temperature [2], light [3–6], electrical potential [7], fumes of solvents [8] and pH [9]. A contact angle of a liquid drop on such surfaces can be altered by the above mentioned stimuli and, for example, switch it from hydrophobic to hydrophilic.

The contact angle (CA) is the most common measure of wettability which describes the angle, θ , at the three phase contact line formed by a drop of liquid resting on a surface. The Young’s equation:

$$\cos\theta = \frac{\gamma_{sv} - \gamma_{sl}}{\gamma_{lv}} \quad (1)$$

relates it to the surface energies at the solid/vapor, γ_{sv} , the solid/liquid, γ_{sl} , and the liquid/vapor, γ_{lv} , interfaces.

The contact angle can vary between the two values: the maximum, called advancing CA, and the lowest, called receding CA. The former can be measured using the Sessile drop method either by increasing the size of the drop until no variations on the CA are observed or as a maximum CA in front of a droplet on a tilted surface. The receding CA is measured as a minimum angle while gradually removing liquid from the drop until the contact line begins to move backwards. It also corresponds to the minimum CA for a droplet on the tilted surface. If

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Supporting Information contains the effect of exposure time to SA solution on variation of the contact angles for Sessile drops on the B0 (F100). It is available free of charge via the Internet at <http://pubs.acs.org>.

manipulation with the droplet is problematic, the receding angle can be measured by observing its evaporation. When the volume of a droplet shrinks, its shape changes while the contact line stays initially the same but eventually, upon reaching the minimum possible receding angle, the contact line detaches and the droplet continues shrinking without further change in the shape.

The hysteresis between advancing and receding CA can vary significantly and is due to metastable states at the solid/liquid/vapor interface [10]. Surface roughness, chemical heterogeneity, molecular reorientation and penetration of the small sized liquid molecules into the voids of the solid surface have been identified among the numerous causes for metastable states on surfaces modified with organic molecules [11]. Notably, even small amounts of impurities on the surface (chemical heterogeneities) can lead to a large hysteresis [10].

Amino acid residues in the polypeptide chain of a protein vary in their hydrophobicity and the hydrophobic interactions between them as well as with the surrounding water are the driving force for proteins folding into their native state.[1] Except for the membrane proteins, the outer surface of a typical protein is usually enriched with hydrophilic residues, which make the protein water soluble. Hydrophobic surfaces can be rendered hydrophilic by covering them with proteins. Such coverage can be achieved by relying on the amphipathic properties of proteins – after a prolonged contact with a hydrophobic surface, they can change conformation to ‘bind’ in a nonnative form via exposing their hydrophobic residues to the surface. Such a binding is weak and is efficient at a very high protein concentration; it is nondiscriminative and forms a protein film that switches a hydrophobic surface into hydrophilic [12]. Different proteins have varying tendency of binding to hydrophobic surfaces, bovine serum albumin (BSA) been one example with a strong conformationally induced adsorption [13] that is often used for hydrophobic surface modification.[14]

To the best of our knowledge, there are no reports of surfaces that could be switched from hydrophobic to hydrophilic by specific interaction between the analyte proteins and their ligands on the surface. In the present work, we illustrate a design of such smart surfaces using the well-known couple biotin-streptavidin (SA), which is commonly used for protein micropatterning. We show here that the mixed hydrophobic-biotin surfaces respond specifically to the presence of the streptavidin analyte by lowering the contact angle at the surface.

EXPERIMENTAL

Materials

(3-Aminopropyl)trimethoxysilane (APTS) was obtained from Aldrich. 2H,2H,3H,3H-Perfluoro undecanoic acid was obtained from Fluorous Technologies. Biotin-LC-LC-COOH and N - (3 - dimethylaminopropyl) – N' – ethylcarbodiimide (EDC) were obtained from Anaspec. Streptavidin (SA), a 53 kDa protein, was obtained from Invitrogen. Bovine serum albumin (BSA), a 69 kDa protein, was received from Sigma. They have similar pI = 5 and 4.7, respectively. Methanol and ethanol, both of absolute grade from Aldrich, were used as received. Glass slides were cleaned with Piranha solution (30% H₂O₂, 70% H₂SO₄) during 20 minutes at 70°C (Piranha solution is explosive, use with caution), washed with copious amounts of distilled water and dried in an oven for 30 min at 115°C.

Preparation of aminated surfaces

The first step for surface modification is the silanization of cleaned glass slides with ethanol solution of aminopropyl trimethoxysilane (APTS) [15,16] for 12h at room temperature and with constant shaking. This reaction produces amino groups for further steps and is prone to multilayer growth in low polarity solvents, but if a proper solvent is used, this problem can be

minimized and practically avoided. In our experience, silanization using a 2% solution of APTS in ethanol results in a monolayer coverage, as judged by the surface density of amines. Evaluation of the amino group surface density was performed using the method of Moon et al [16] and it was established that at least 6 h were necessary to attain monolayer coverage, *ca.* $3 \times 10^{14}/\text{cm}^2$. [15,17] No further increase beyond monolayer coverage was detected for up to 24 h treatment (in contrast with nonpolar solvents). Silanization for all surfaces reported in this paper was performed using 12h of treatment in ethanol ensuring a close to monolayer coverage. Afterwards, slides were washed with ethanol and methanol and finally cured for over 1h at 115°C.

Mixed biotinylated and fluorinated surfaces

To prepare mixed monolayers, mixtures of two carboxylic acids in different proportions were reacted with the amino groups of the aminosilane layer using EDC coupling reagent. Perfluoric acid was chosen to minimize the passive adsorption of proteins.[7,18] The biotinylated acid consisted of D-biotin attached to a long linker (LC-LC-COOH). The purpose of the long linker is to extend the biotin moiety above the fluorinated monolayer to ensure its interaction with streptavidin (SA). Solutions of biotinylated and fluorinated carboxylic acids, both of 50 mM concentration, were mixed in a desired proportion to make 100 μL and diluted by ethanol to the final volume of 2 mL. The cleaned glass slides were deposited inside and, after adding 30 mg of EDC, the entire solution was agitated at room temperature for at least 6h. The slides were finally washed with ethanol and methanol and dried by purging with N_2 . We will refer to these mixed monolayers in accordance with the mole fraction of the carboxylated biotin in the carboxylic acid mixture used during the preparation, *i.e.*, B20 was prepared using a mixture of 0.2 mole fraction of biotin and 0.8 of the fluorinated acid. Note that the actual fraction of the biotin moiety in the surface monolayer can differ from that because of the possible reactivity difference for different carboxylic acids. The surface F100 (B0) corresponds to a solely fluorinated monolayer. Figure 1 shows a schematic of the expected monolayer configuration.

Contact angle measurements

The CA measurements were carried out using a home made apparatus consisting of a microscope connected to a digital camera, a horizontal beam holding the microscope parallel to the surface, a light dispersive plate, and a three-axis moving platform. Most experiments were conducted without control of humidity or temperature; the latter was typically within the range between 20 and 25°C. Each series of experiments representing by a graph was conducted on the same day to ensure the same humidity. Water vapor saturated environment used in some experiments, was achieved by placing a modified glass substrate on a support inside of a glass rectangular cuvette, the bottom of which was filled with DI water. When used, this condition almost eliminated the droplets' evaporation (a very slow Kelvin evaporation due to a small droplet size still took place).

Drops of approximately 1 μL were deposited on the surface using a Hamilton microsyringe. Movies and pictures of the drop's profiles were recorded every two minutes in most of the experiments. The images were analyzed using the contact angle plug-in (written by M. Brugnara [19]) in the ImageJ software. All measurements were done in triplicates. Cleaning of fouled surfaces by sonication (see text) was performed in Branson 1200 Ultrasonic Cleaner.

RESULTS and DISCUSSION

Our motivation was to investigate whether mixed hydrophobic surfaces can be triggered into hydrophilic by interaction with biochemical analytes. There are various possible applications for such a phenomenon, including electrical biosensors. We have demonstrated before [20–25] that nanoporous membranes, the surface of which is modified by organic monolayers, can

be made responsive to physical and (bio)chemical stimuli. When the surface is modified by a mixed layer of hydrophobic molecules and the hydrophobicity switching triggering elements, not only the ionic conductance [22,23] but the whole solution flow through the membrane can be switched by the stimulus[24–25]. Whether or not it is possible to realize such switching with biochemical analytes is the driving force behind this investigation, where streptavidin (SA) is used as a representative protein.

Streptavidin is a tetrameric protein that has four sites for binding biotin.[26] Binding of biotin to SA is among the strongest noncovalent interactions known. It has a very small dissociation constant, estimated between 10^{-15} M [27] and 4×10^{-14} M [28], and a long dissociation time, up to 3 days, making it an essentially irreversible reaction. The biotin binding site is buried quite deep into streptavidin. [29,30] Thus, an effective coupling between the two can be achieved only when the linker, by which biotin is attached to the surface, extends sufficiently enough, at least by 8 Å as measured from the carboxylate carbon of biotin.[29,30]. To ensure this and, at the same time provide enough hydrophobicity to the surface, the described above procedure for the mixed monolayer formation was chosen, where the surface was first aminated by APTS and then linked to carboxyl terminated molecules using the EDC coupling reagent. The LC-LC linker on biotin-LC-LC-COOH is sufficiently long to bind SA effectively [30].

To make the switching specific to the analyte (SA in our case), one needs to minimize the effect of a well known phenomenon of passive adsorption of proteins, which occurs even on hydrophobic surfaces [7,12,18]. Among the various options for handling this effect [31], fluorination, *i.e.*, surface modification using fluorinated molecules, was chosen for its relative simplicity. When compared to aliphatic surfaces, fluorinated surfaces show lower fouling by proteins, but even they eventually succumb to fouling at high concentrations of proteins, especially after prolonged exposures.

The effect of passive adsorption (physisorption).can be evaluated by measuring the contact angle (CA) of the droplets with different concentrations of a protein (e.g. SA) on the fully fluorinated surface B0 (F100). The simplest approach is to monitor the free standing sessile droplets' shapes in time upon their slow evaporation rather than to measure the advancing and receding angles. Besides providing more reproducible data for the receding angle, this approach also allows identification whether there is any delayed spreading of the droplets due to SA binding to biotin, similar to what happens with solutions of small amphiphile molecules [32].

Figure 2A demonstrates that for SA concentrations of 100 mg/L ($\sim 2 \mu\text{M}$) or higher there is significant nonspecific adsorption of SA to the fluorinated surface while the solution of lower concentrations, *e.g.*, 10 mg/L and lower, presents very minimal adsorption and almost matches the behavior of plain PBS buffer. At the same time, mixed monolayer modification, B30, has visibly changing receding contact angle down to SA concentrations of 100 $\mu\text{g/L}$, as shown in Figure 2B.

Figure 3 provides the time snapshots of PBS and SA containing droplets slowly evaporating on the surfaces modified with different solitary and mixed monolayers, B0 (F100), B25, B50, B75, and B100. The analysis of their contact angles, given in Figure 4, illustrates that all surface modifications (except for fully fluorinated F100) have dramatically different evolution of the PBS and SA containing droplets. The surfaces with higher content of biotin demonstrate lower initial contact angles as expected due to a more hydrophilic character of biotin. The CA values on each surface are almost identical for the two droplets at first but with time the PBS droplet starts shrinking upon reaching the corresponding receding CA. The streptavidin containing solutions, on the other hand, show continuous decrease of the contact angle within this time frame, which correlates well with the receding angles measured at different times after placing

the droplet on the surface. Obviously, this behavior is due to the specific interaction between SA in solution and the surface bound biotin.

As Figure 5 illustrates, upon evaporation of the droplet, biotin moieties act as “anchors” for SA binding and thus pin the contact line to its original position. When the volume of the droplet shrinks, the resulting contact angle decreases. With no biotin on the surface, the droplet decreases in size while maintaining the same shape as soon the CA reaches the receding angle value.

The specificity of biotin - SA interaction as responsible for the observed CA hysteresis can be confirmed by the lack of such CA changes with solutions of other proteins. To minimize the number of variables and to have a better comparison, we performed the experiments with streptavidin whose active sites were ‘capped’ with biotin. Capping was achieved by adding a slightly above the stoichiometric amount (5:1) of D-Biotin to the SA solution and thus rendering the protein inactive to binding with biotin on the surface. Figure 4B confirms that the variation of contact angle for the capped SA on a B30 is practically indistinguishable from that of PBS buffer in a dramatic distinction from the uncapped SA. Again, the distinction emerges as soon as the shrinking due to evaporation droplets reach their corresponding minimum receding contact angles. The solution with capped streptavidin does not demonstrate hydrophobicity switching and the droplet starts shrinking early with a large contact angle, while the uncapped SA binds specifically to the biotin on the surface, lowers its surface tension and pins the contact line until a much lower contact angle is realized.

Alternatively, the contact angle with dull PBS buffer (free of any protein) can be used to study binding of proteins to the same surfaces after their prolonged exposure to the protein solutions. This approach allows a convenient way of discriminating between specific binding and passive adsorption (physisorption). The fully fluorinated surface (B0) gets fouled after 30 min exposure to either SA or BSA solutions. Because of a longer exposure time, the contact angle drops from $108 \pm 2^\circ$ before to $78 \pm 6^\circ$ and $78 \pm 7^\circ$, respectively, for the two proteins (see Figure 6) due to their physisorption. The hydrophobic property fully recovers after 1 min sonication in 50% v/v of methanol. This treatment is mild enough not to denature the protein, at least, not streptavidin. As seen in Figure 6, partially biotinylated B30 surface experiences a significant drop of the contact angle after exposure to either SA and BSA solutions. The contact angle decreases from $100 \pm 9^\circ$ to $54 \pm 6^\circ$ and $71 \pm 10^\circ$ after SA and BSA, respectively. Remarkably, sonication of the BSA fouled B30 surface for 1 min in 50% methanol completely recovers while it has an insignificant change, to $56 \pm 7^\circ$, for SA treated B30 surface. More harsh conditions of 30 min sonication in pure methanol is likely to denature proteins more significantly, as is observed in recovery of the contact angle for SA treated B30 surface back to the original value of greater than 100° . Multiple uses of this procedure eventually deteriorate the surface properties, which is first revealed in lowering of the receding angle.

There are two questions worthy of further discussion. First, nonspecific binding of proteins (physisorption) occurs on all surfaces, with and without the biotin ligand, but it is a much slower process. When the kinetics of the contact angle variation with evaporation are measured after letting a streptavidin containing droplet to soak on that surface in saturated vapor, changes in the receding angle are observed with 10 mg/L of SA as well.[32] It requires additional at least 10 min to observe a significant effect for that concentration on F100 surface. During this time, biotinylated surfaces demonstrate a strong binding even with lower concentrations. In applying this method for sensing SA, one can eliminate the nonspecifically bound proteins by sonication, as explained above. Whether or not physisorption is a cooperative effect, would require additional studies and probably with a more appropriate technique.

The second question that motivated this work is about the contact line movement as a result of specific protein binding. We observe no such movement for either specific or nonspecific protein interaction with surface. Despite the large contact angle hysteresis upon SA binding, which exceeds 70° for B50, we do not observe the contact line movement outwards, *i.e.*, there is no delayed droplet spreading. Even on B100 surface, *i.e.*, when only biotin is present on the surface, no movement of the contact line is observed in 100% humid atmosphere over a 12 hours period with the SA concentrations of 10 mg/L or lower. This behavior is different from that of small amphiphile molecules. For the latter it has been established that the process, which primarily determines the spreading of surfactant solutions over hydrophobic substrates, is the transfer of surfactant molecules onto a bare hydrophobic substrate in front of the moving three-phase contact line [33]. This process results in a partial hydrophilization of the hydrophobic surface in front of the drop and determines the delayed spontaneous spreading. Indeed, it is easy to see from Eq.(1) that the decrease of only γ_{sl} and γ_{lv} resulting from the relatively fast adsorption of amphiphiles on solid/liquid and liquid/vapor interfaces cannot explain the switch from hydrophobic ($\theta > 90^\circ$) to hydrophilic ($\theta < 90^\circ$) behavior. Obviously, it can be realized only when γ_{sv} becomes greater than γ_{sl} , *i.e.*, when γ_{sv} increases in a vicinity of the three phase contact line as well.

Transfer of surfactants from the solution onto the solid–vapor interface just in front of the drop increases the local free energy but the total free energy of the system decreases. The process goes via a relatively high potential barrier and hence is considerably slower than the adsorption at liquid/solid and liquid/vapor interfaces, *i.e.*, the time scale for the droplet spreading is defined by the characteristic time of surfactant transfer from the drop onto the solid/vapor interface. If the latter is slow, the system can ‘get stuck’ in the metastable state for a very long time. Large proteins such as SA or BSA cannot follow this route directly because of the size; the only option left for them to affect the advancing contact angle is if the contact line can fluctuate itself thus exposing SA to the possibility of binding with biotin on the surface (see Figure 5). The alternative version would be with biotin ahead of the contact line fluctuating in and out of the droplet and occasionally ‘fishing-out’ SA from aqueous solution into the dry region. Both of these options are apparently too much of an uphill process and are not realized to a sufficient degree.

CONCLUSIONS

Mixed fluorinated surfaces with covalently bound biotin demonstrate smart active hydrophobicity switching, where specific binding of streptavidin from a low concentration solution can decrease the contact angle with water from being greater than 90° down to less than 60° . The effect is clearly visible in the receding contact angle for solutions of streptavidin while the advancing angle remains identical for the buffer and streptavidin solutions thus proving that advancement of large protein molecules onto hydrophobic surfaces ahead of the contact line even via help of specific interaction with ligands is a highly unfavorable process.

Streptavidin with blocked biotin binding sites and bovine serum albumin lack the active hydrophobicity switching but do show nonspecific binding by physisorption that can be eliminated by sonicating the exposed surface in 50% methanol solution for 1 min. Harsher treatment of 30 min sonication in pure methanol denatures these proteins and recovers hydrophobicity of these surface.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Representation of the surface modified with mixed monolayer of biotin-LC-LC and perfluoric acid. Biotin moiety must extend at least three bond lengths above the top of the fluorinated monolayer to ensure streptavidin binding.

Figure 2. Contact angle variations for droplets with streptavidin (SA) solutions of different concentrations on B0 (A) and B30 (B) surfaces.

Figure 3.

Time evolution of the slowly evaporating droplets without (left) and with 10 mg/L streptavidin solutions on the surfaces with different percentage of biotin (fluorocarbon is the remaining component of surface modification)

Figure 4.

Variation of the contact angle with time for Sessile drops with: PBS and 10 mg/L SA solutions on surfaces with different amounts of biotin moieties (A); solutions with 10 mg/L of free SA and 10 mg/L SA capped with biotin, both on B30 surface (B). The capped SA variation is practically indistinguishable from that of PBS buffer.

Figure 5. Illustration of the mechanism of the contact angle variation. The advancing contact angle is not affected by streptavidin binding onto biotin because neither biotin nor streptavidin can sway across the contact line and the thermal oscillations of the contact line are not sufficient either.

Figure 6.
Variation of the drop with **PBS** buffer on B0 (F100) and B30 surfaces after different treatments.