Biосpecific Adsorption of Carbonic Anhydrase to Self-Assembled Monolayers of Alkanethiolates That Present Benzzenesulfonamide Groups on Gold

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The biосpecific adsorption of proteins at interfaces that present appropriate ligands is important for anchorage-dependent cell culture,1 screening of combinatorial libraries,2 biosensors,3 and biocompatible surfaces.4 Fundamental studies of molecular recognition at surfaces by proteins have been limited by a lack of an experimental system having the requisite characteristics:5 (i) a method to prepare surfaces with excellent control—at the molecular scale—over the density and environment of ligands; (ii) an “inert surface” that resists nonspecific adsorption; (iii) a convenient and readily available analytical technique that can measure the adsorption of protein at sub-monolayer coverage in situ and in real time. Here we demonstrate that the combination of (a) mixed self-assembled monolayers (SAMs) of alkanethiolates on gold presenting oligo(ethylene glycol) moieties and ligands and (b) surface plasmon resonance (SPR) spectroscopy meets these requirements. We demonstrate the characteristics of this system using a model system comprising immobilized benzene sulfonamide groups interacting with bovine carbonic anhydrase (EC 4.2.1.1) (Figure 1).

We used bovine carbonic anhydrase (CA) in this work because it is a well-characterized monomeric protein (MW = 30,000) that binds para-substituted benzene sulfonamide ligands with equilibrium dissociation constants (Kd) of approximately 10^-4-10^-6 M. We prepared mixed SAMs comprising different mole fractions (y) of an alkanethiolate terminated in a benzene sulfonamide ligand (2):4 the corresponding ligand 3 binds CA with a value of Kd of 5 x 10^-3 M in solution. The tri-(ethylene glycol)-terminated alkanethiol 1 was used as the major component of the SAMs because it resists the nonspecific adsorption of protein (Figure 1).10 We measured binding of carbonic anhydrase to these SAMs using surface plasmon resonance (using a Pharmacia Biacore instrument) as described previously.11 In the SPR experiment, p-polarized light is incident on the back side of a gold-coated glass slide supporting a SAM, and the angle (θ) at which the reflected light shows a minimum intensity is measured.12 The value of this resonance angle depends linearly on the amount of protein adsorbed to the surface and is recorded as a function of time. For all experiments described here, a solution of buffer (10 mM phosphate, 150 mM sodium chloride, pH = 7.2, T = 25°C) was allowed to flow through the cell for 4 min, replaced with a solution of CA in the same buffer for 6 min, and then returned to the original buffer for 20 min.

Figure 2 shows SPR response curves for adsorption of CA (0.5 mg/mL, 10 μM) to mixed SAMs having values of θd ranging from 0 to 0.08: the amount of CA that adsorbed to the SAMs increased with the mole fraction of 2 in the mixed monolayer (θ2) (Figure 2). Assuming values for the cross-sectional areas of an alkanethiolate and CA of 21 and 1800 Å², respectively, a mixed SAM having a value of θ2 ≈ 0.01 would support adsorption of a complete monolayer of CA.13 The approximate value of θ2 = 0.08 that we observed to be limiting is not inconsistent with this model, since mixed SAMs comprising two alkanethiolates are not randomly mixed and may even contain phase-separated domains of each SAM.14 For all SAMs

(7) Alkanethiol 1 was synthesized as described: Pale-Grosdemange, C.; Simon, E. S.; Prime, R. L.; Whitesides, G. M. J. Am. Chem. Soc. 1991, 113, 12–20. Alkanethiol 2 was synthesized in eight steps; details will be described in a subsequent report.
(8) Substrates were prepared by evaporating an adhesion layer of titanium (1 nm) and gold (40 nm) onto glass cover slips (0.20-mm, No. 2, Corning). The metalized substrates were immersed in solutions containing mixtures of 1 and 2 for 12 h to give mixed SAMs. The values of θ2 were determined by X-ray photoelectron spectroscopy as described previously;10 we report estimates of θ2 for SAMs having values of θ2 < 0.05, due to the limited sensitivity of the XPS measurement.
(9) Bird, R. E.; Mrksich, M.; Gao, J.; Whitesides, G. M. Unpublished.

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Figure 1. Cartoon showing reversible adsorption of CA to mixed SAMs presenting ligands.

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(13) The value for the alkanethiolate was calculated assuming a well-ordered SAM where the alkanethiolates coordinate to the hollow 5-fold sites of a gold (111) surface. The thiols are arranged in a cubic symmetry with a S–S spacing of 4.97 Å; Strong, L.; Whitesides, G. M. Langmuir 1988, 4, 546–550. The value for CA was calculated from the dimensions of CA obtained from the crystal structure: Eriksen, A. E.; Jones, T. A.; Liljas, A. Proteins: Struct., Funct., Genet. 1988, 4, 274–282.
having values of $\chi_2 < 0.1$, the adsorption of CA was more than 90% reversible; the SPR response curves indicated, however, that there was a small amount of protein (0%) that remained irreversibly adsorbed to the mixed SAMs. The amount of CA that adsorbed irreversibly increased with $\chi_2$; CA did not adsorb to a SAM containing only I; CA adsorbed irreversibly to a SAM containing only 2. This set of observations suggests that the irreversible adsorption was due to phase-separated domains of 2 present in the mixed SAMs.

We measured the binding of CA—at concentrations ranging from 60 nM to 18 mM—to a mixed SAM having a value of $\chi_2 \approx 0.03$. Analysis of the rates of binding gave a value of $k_{eq} = 1.9 \times 10^4$ M$^{-1}$ s$^{-1}$. The region of the response curve representing dissociation of protein was fitted to an exponential decay function to give a value of $k_{off}$/k$_{on}$ of $5.4 \times 10^{-3}$ s$^{-1}$. The ratio of these two rate constants (k$_{off}$/k$_{on}$) provides a value for the equilibrium dissociation constant, K$_d$, of $2.8 \times 10^{-7}$ M for binding of CA to the SAM. This value indicates that binding of CA to analogous ligands on the surface and in solution (3) occurs with tighter binding in solution (K$_d$ = $5 \times 10^{-8}$ M). This difference may reflect unfavorable steric interactions between the adsorbed protein and the surface, or an entropic repulsion between the bound protein and the tri(ethylene glycol) terminated SAM.

The binding of CA (5 µM) to a mixed SAM having a value of $\chi_2 \approx 0.03$ was inhibited by the addition of the inhibitor 4-carboxybenzenesulfonamide (4) to the CA-containing solution; the level of inhibition increased with the concentration of soluble ligand (Figure 2A). The SAMs also resisted the nonspecific adsorption of protein from a solution containing a mixture of nine proteins (Figure 3B). When CA (5 µM) was added to this sample, however, SPR measured adsorption of protein to the SAM. This high level of specificity is due to the effectiveness of the tri(ethylene glycol) group at preventing nonspecific adsorption of protein.

This work provides a structurally well defined, synthetically flexible model system with which to investigate biomolecular recognition at surfaces. SAMs offer several advantages over traditional materials (for example, gel layers) as an inert


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