Quantitative Affinity of Genetically Engineered Repeating Polypeptides to Inorganic Surfaces

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Binding kinetics of platinum-, silica-, and gold-binding peptides were investigated using a modified surface plasmon resonance spectroscopy (SPR). Platinum binding septa-peptides, quartz-binding dodecapeptides, and gold-binding 14-aa peptides were originally selected using phage or cell surface display libraries using the mineral or pure forms of these materials. All of the peptides were synthesized singly to investigate their binding kinetics and to assess quantitatively the specific affinity of each to its material of selection. The peptides were also postselection engineered to contain multiple copies of the same original sequences to quantify the effects of repeating units. SPR spectroscopy, normally using gold surfaces, was modified to contain a thin film (a few nm thick) of the material of interest (silica or platinum) on gold to allow the quantitative study of the adsorption kinetics of specific solid-binding peptides. The SPR experiments, carried out at different concentrations, on all three materials substrates, resulted in Langmuir behavior that allowed the determination of the kinetic parameters, including adsorption, desorption, and equilibrium binding constants for each of the solids as well as free energy of adsorption. Furthermore, we also tested multiple repeats of the peptide sequences, specifically three repeats, to see if there is a general trend of increased binding with increased number of binding domains. There was no general trend in the binding strength of the peptides with the increase of the repeat units from one to three, possibly because of the conformational changes between the single and multiple repeat polypeptides. In all cases, however, the binding was strong enough to suggest that these inorganic binding peptides could potentially be used as specific molecular linkers to bind molecular entities to specific solid substrates due to their surface recognition characteristics.

Introduction

Peptides with inorganic surface binding ability have been selected by using combinatorial biology techniques, namely phage display[1] and cell surface display[2] methods that have been adapted for materials science.[3−5] Inorganic binding peptides with affinity toward Au,[3] Ag,[5] Pt,[6] ZnO,[7] GaAs,[4] TiO_2,[8] and others were successfully selected by a number of research groups and us. Since their discovery, many of these inorganic binding peptides have been used in practical applications such as nanoparticle synthesis,[3,9,10] molecular linkers,[11] immobilization platforms,[12−14] and assembly to create nanostructures.[6] Despite the ever increasing utility in both materials and medicine, an in-depth understanding of the quantitative affinity and binding kinetics of many of these peptides has not been fully realized. Characterization of these peptides has been performed primarily using qualitative methods such as fluorescence microscopy or direct colony/plaque counting, while the peptide was displayed on the host phages or cells. Only a limited investigation has been accomplished to determine the solid binding of peptides using quartz crystal microbalance (QCM),[14−16] atomic force microscopy (AFM),[6,17] surface plasmon resonance spectroscopy (SPR),[18] and fluorometric peptide assays.[19] In a few cases, we and others have probed possible molecular mechanism of the binding especially due to secondary structure changes, obtained experimentally, for example, by NMR or by mutational analysis.[19−21] Computational analysis of the peptide−surface interactions was also carried out utilizing molecular dynamics and molecular mechanics in simplified environments.[22,23] Despite these preliminary studies, there has not been any global understanding of the engineered peptide recognition of inorganic solids, and the question of how biocombinatorially selected peptides bind to solids has so far been elusive. This investigation has been undertaken to create a quantitative body of knowledge that would provide a better insight in the mechanism for a more robust design of these molecular building blocks as a utility to a wide range of practical technological molecular and nanoscale systems.[6]

There are numerous methods to determine protein−protein, protein−small molecule, and protein−carbohydrate interactions; however, most of these methods are difficult to adapt for monitoring affinity of peptides to solid surfaces. For example, there has been considerable work carried out in the literature on protein adsorption on solid particles; however, these are usually "bulk" experiments and mostly limited to high protein concentrations and, therefore, do not offer the capability for any real time monitoring and quantification.[20,24,25] As an alternative method, SPR spectroscopy has become a major technique to monitor adsorption kinetics, thermodynamics, and real-time monitoring of molecular interactions at a gold surface.[26,27] The SPR signal is based on the effect of the collective excitation of the electrons at the metal-dielectric interface on the wavelength shift of the reflected light from a glass prism.[28] Because of the changing surface resonance, the shift in the wavelength increases as the molecular binding on the metal surface of the SPR chip progresses, thereby allowing the monitoring of the molecular adsorption and coverage on

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the surface. SPR spectroscopy is highly sensitive to the adsorbed (and desorbed) amount of molecules on the surface, making the method a useful tool to monitor adsorption of bio- and synthetic molecular systems on the chip surface.[29,30] The technique is frequently utilized to investigate molecular interactions where one biomolecule is immobilized to the surface using a metal-binding synthetic linker, for example, a thiol molecule, and the other end is free to interact with molecular entities within the SPR chamber. We have recently successfully employed a SPR approach using genetically engineered metal binding peptides, such as gold-binding and platinum binding, and obtained their Langmuir adsorption behavior.[16,18] Quartz crystal microbalance is also another frequently used technique to monitor the adsorption and desorption processes.[31] This technique relies on the change in the resonance frequency of quartz crystal coated with a inorganic surface of interest (which is generally gold). The change in the frequency of the quartz crystal represents the amount of mass deposited on the quartz crystal. Besides gold coating on quartz crystal, platinum, silica, hydroxyapatite, and different polymer coatings are also commercially available surfaces. The real time monitoring of the adsorption and desorption using QCM was carried out in many different studies toward understanding the nature of molecular interaction both qualitatively and quantitatively.[31,32] Because both techniques can be quantitative, SPR and QCM have also been used together to monitor the validity of the molecular adsorption on solids in the same system.[33] More qualitative other techniques, such as fluorescence, have also been used in conjunction with SPR or QCM to monitor molecular adsorption.[34,35] In fact, in our research, as a trend, we first qualitatively categorize the originally selected peptides rapidly using the FM technique in terms of their binding characteristics, and then apply either SPR or QCM, or both,[16] to obtain more quantitative data, such as the adsorption parameters of kinetics or thermodynamics.

Normally carrying out the SPR experiments on a given material surface is not possible because of the intrinsic limitations of the surface plasmon phenomena. Because of this, silver and, most frequently, gold surfaces are used to generate the surface plasmon effect. In these studies, generally, SPR analysis is performed using a glass slide coated with a thin film (45−50 nms) of gold.[28−30] Other materials, even Pt or Pd, have poor plasmonic properties.[36] Therefore, this problem limits the applicability of the SPR technique to monitor molecular adsorption on other practical solid surfaces such as Ti, Pt, Cu_2O, ZnO, Al_2O_3, silica, and others, all interesting for materials and medical applications. To overcome this difficulty, we developed a protocol that provides the design principles for the coating (usually via vacuum deposition) of an additional ultrathin layer of target material, of any type, that is, metal or oxide, on to the Au chip still providing the SPR signal from the underlying gold layer. The chip allows monitoring of molecular adsorption on this new ultrathin-film material at the very top surface facing the analyte solution. Protocols similar to ours have been developed using a sol gel technique for silica.[37] In a very recent study, using this approach, we monitored adsorption of Pt-binding peptides on to a 2 nm thick Pt-coated surface.[18]

Using the surface modified and properly prepared gold, platinum, and silica substrates, herein we carried out SPR to quantitatively characterize the binding kinetics of genetically engineered peptides for inorganics (GEPI),[6] synthesized to have the original molecular conformations displayed in the library coat protein either in the cell (for Au) or phage (for silica and Pt). Two quartz-binding peptides (QBP1 and QBP2) and two Pt-binding peptides (PtBP1 and PtBP2) were used and binding activities to their respective solids were compared to binding of a gold-binding peptide, GBP1[3], that we have extensively studied in our previous work.[6,16,18,20] The five peptides are known (qualitatively) to bind to their respective materials strongly as have been already determined by fluorescence microscopy. In this work, we have also tested the hypothesis that multiple repeats of the peptide sequences, which we call "repeating polypeptides"[3], known to bind to a specific material may have improved binding. The resulting data, therefore, were analyzed from two different perspectives, namely, to assess the effects of multiple repeats compared to the single-repeat peptides and evaluating the specific affinities quantitatively on each of the corresponding materials.

Materials and Methods

Selection of the Binding Peptides. *Phage Display.* Target materials (quartz, platinum) were cleaned using ethanol (95%), and equilibrated using phosphate/carbonate (PC) buffer overnight. After this procedure, the phage library housing 2×10^9 different randomized peptide sequences were brought into contact with the target materials. The library was incubated with the target Pt or quartz powder (obtained from pure quartz mineral) in potassium−carbonate (PC) buffer containing 0.1% detergent. Unbound phage were removed by washing with phosphate carbonate buffer containing 0.1% detergent. Specifically, bound phage were eluted from the surface using elution buffers; the eluted phage pool was amplified with *Eschericia coli* ER2738. The amplified phage were then purified. The amplified and purified phage were used for additional panning rounds; after each round, the phage were grown on solid media and single clones were selected by picking single phage plaques. DNA of single phage clones were isolated and sequenced. Because the insert position of the random sequences are known, the peptide sequenced displayed on each clone can be obtained from the DNA sequences (see Supporting Information for details).

Cell Surface Display. Gold binding protein was selected using the cell surface display. In this approach,[3] the *Eschericia coli* was used as the host cell for carrying the peptide exposed to the environment of the cell. The randomized peptides were displayed on the membrane protein of *E. coli*, multiporin, a fusion protein, which does not have any affinity for gold in its native form. Multiporin was then cloned to make a large number of copies (millions). The library has a diversity of 5×10^6 peptides. Each copy carries a segment, which encodes the random peptide sequence. For biopanning experiments, the cells displaying the random peptide sequences were brought in contact with gold powder and, after selection of the weak and non specific binders, strongly bound cells were harvested and the gene expressing multiporin protein was sequenced to obtain the information of DNA sequence encoding the putative gold binding peptide (see Supporting Information for details).

Peptide, Buffer, and Solutions. The highest affinity peptides obtained from the selection were synthesized using standard *Fmoc* solid phase peptide synthesis techniques and purified using C-18 reverse phase liquid chromatography (RPLC) to a level >95% (United Biochemical Research, U.S.A.). The peptides were synthesized without a blocking group either at the -N or -C termini. Peptide solutions, containing the five different GEPIs, PtBP1, PtBP2, QBP1, and QBP2, for SPR measurements were prepared in PC buffer (55 mM KH_2PO_4, 45 mM Na_2CO_3 and 200 mM NaCl). The fifth peptide, GBP1, was prepared in phosphate buffer (1:3 mixture of 10 mM KH_2PO_4, 10 mM K_2HPO_4, and 100 mM KCl). The pH of both buffers were adjusted to pH 7.5 using 0.1 M HCl and 0.1 M NaOH. None of the peptides were studied in their zwitterions form, and the pH of the buffer solution used was not at the isoelectric points of the peptides. Because histidine has a -NH_2 group on the side chain, it may contribute to the protonation of the GBP1. In fact, GBP1 can be protonated at pH 6.5 and the total charge becomes +1, while at pH 7.5, it is lower than +1.

Table 1. Amino Acid Sequences of Inorganic-Binding Peptides and their Physicochemical Properties

peptide	sequence	MW (g/mol)	charge (*e*)[a]	pI (pH unit)	GRAVY[b]
l-GBP1	MHGKQATSGTIQS	1446.6	+1	8.52	−0.743
l-QBP1	RLNPPSQMDPPF	1398.6	0	5.84	−1.142
l-QBP2	QTWPPPLWFSTS	1446.6	0	5.52	−0.542
c-PtBP1	CPTSTGQAC	886.9	0	5.51	−0.100
c-PtBP2	CQSVTSTKC	956.1	+1	8.06	−0.133

[a] *e*: elementary charge. [b] GRAVY: grand average hydropathicity.

SPR Experiments. The SPR measurements were made with a dual channel instrument (Kretschmann configuration) developed by the Radio Engineering Institute, Czech Republic.[28,30] The instrument can detect changes at a level of 0.0001 refractive index unit. Unlike Au and Ag, noble metals such Pt and Pd, or dielectrics such as SiO_x, do not have a measurable SPR signal. To overcome this difficulty, based on the result of a numerical method we developed, a very thin film (a few nms thick) of Pt or SiO_x was prepared on the Au substrate, allowing the sufficient generation of an SPR signal, where the thicknesses were predetermined using the numerical model.[18]

Results and Discussion

Following the biopanning experiments using platinum and silica powders two silica binding peptides (QBP1 and QBP2), and two platinum binding peptides (PtBP1 and PtBP2) were isolated. Gold binding peptide was isolated previously from a cell surface display library.[3] The amino acid (aa) sequences of the peptides are given in Table 1.

To mimic the peptides in their original molecular architecture as they are displayed on pIII coat protein of the phage library, the QBPs were synthesized as 12-amino acid sequences in a linear form while the PtBPs were synthesized in 7-aa long cyclic form. The GBP1 was displayed on the *Escherichia coli* cell surface as 14-aa linear form and, thus, synthesized as such. To examine whether tandem repeats of the peptide increases the binding affinity, the peptides were also synthesized in three-repeat multimers for further testing. As a special case for Pt binding peptides (that are originally displayed in constrained form via cysteines at N and C termini, linear one repeat forms) were prepared to probe the effect of conformational constraints on binding affinity.

Two strong platinum binders (PtBP1 and PtBP2), identified from a set of 37 original binders,[18] were tested for their binding affinity, both in the linear (l) and constrained (c) forms and in 1- and 3-repeat tandem sequences. Based on the observed binding behavior, the SPR data for platinum binding peptides were fitted to two different adsorption models. The adsorption data from c-PtBP1, 3l-PtBP1 and l-PtBP2, c-PtBP2 were fitted to a simple Langmuir adsorption model, and those from l-PtBP1 and 3l-PtBP2 were fitted to a biexponential Langmuir model. It is known from our earlier CD studies that these peptides have different secondary structures in solutions.[20] We attribute different binding behavior of constrained and linear forms of the same peptide sequences to possibly be a result of differences of their conformations leading to different molecular recognition of the solid and, hence, different adsorption behavior.

For the first Pt-binding peptide, that is, PtBP1, the equilibrium constants (K_{eq}) and, therefore, the free energy of adsorption of the constrained and singly linear forms of the PtBP1 are slightly different, the constrained one being a stronger binder (Table 2). According to our observations, this may be due to the fact that the constrained form of this peptide has a higher adsorption rate (k_a), 20 times, than that of the linear form, while both having the same desorption rate (Figure 1A,C). To test whether the 3-repeat form of the linear peptide has any effect on the binding kinetics, we also studied the adsorption behavior. Again, as shown in Figure 1E and in Table 2, 3l-PtBP1 has equilibrium constant and free energy of adsorption similar to those of the single repeat form of this Pt-binding peptide. It is interesting to note that the 3-repeat polypeptide has about twice the adsorption rate as well as twice the desorption rate of those of the single-repeat peptide, resulting in similar overall equilibrium behavior. These results suggest that the tandem repeat does not necessarily improve the adsorption behavior of a solid-binding peptide. No change in the adsorption behavior may be due to the conformational freedom (assuming various equilibrium molecular conformations) of the linear forms of these peptides as adsorbed on the solid surfaces. It should be noted also that, the constrained forms of these peptides have two cystein residues at either terminals. These amino acids, in the free forms, are known to influence binding to metal surfaces. In the present peptides, cysteins are used to form the loop and, therefore, they do not necessarily contribute to the adsorption behavior of the peptide (see below).

The SPR experiments that were carried out using the second Pt-binding peptide, that is, PtBP2, produce a new set of interesting results (Figure 1B,D,F and Table 2). First of all, the constrained and the linear forms of this peptide have similar equilibrium constants (0.09 and 0.16 M^{-1}, respectively) and, hence, similar free energy of adsorption (−6.7 and −7.1 kcal/mol, respectively). The adsorption and desorption kinetics of the linear peptide were several times faster than those of its constrained form (Figure 1B,D). This result is interesting and is just the opposite of what was found in the case of PtBP1, that is, solid-binding and -unbinding behaviors of this peptide (see above) are much faster than those of its linear counterpart. Interestingly, the 3-repeat linear tandem sequence of this peptide has considerably higher equilibrium adsorption behavior than the single repeat form (Figure 1F). Correspondingly, the 3l-PtBP2 binds to Pt substrate about 30% more than its single repeat peptide (Figure 1D,F, Table 2). Again, this result is opposite what was found for the PtBP1 case where these parameters corresponding to the single and 3-repeat forms hardly changed.

From the above observation, we see that the differences in the adsorption behavior of the platinum-binding peptides may be a result of their molecular structural differences. The effect resulting from the constrained forms on the binding affinity of the platinum binding peptides cannot be concluded as a universal behavior. On the one hand, we observed the binding affinity of PtBP1 was increased by creating a constrained structure; however, this was not true in the case of PtBP2, where there was no substantial change in the adsorption behavior. Similarly, the use of tandem linear repeats did not result in the same adsorption behavior of these concatemers; that is, while the 3l-PtBP1 had no change in its equilibrium adsorption behavior, PtBP2 had substantial increase. One reason for these observations is that the solid binding of a peptide is not only controlled by the amino acid sequences, but it may also depend on the secondary structure for, at least, the platinum-binding peptides. A case study was carried out in this laboratory to attempt to correlate binding and structure of PtBP1[18] in the linear and constrained forms. The CD experiments indicated the presence of extended helical polyproline type II (PPII) secondary structure, which has been noted in short peptides that contain Pro, Ala, and Gln. Thus, although both peptides feature the same integral sequence and possess some degree of random coil, the

Table 2. Adsorption and Desorption Rates, k_a and k_d, Respectively, Equilibrium Adsorption Coefficients, K_{eq}, and the Free Energies of Adsorption, ΔG_{ads}, of Peptides on a Given Surface[a]

peptides	k_a^1 (M^{-1} s^{-1})	k_a^2 (M^{-1} s^{-1})	$k_d^1 \times 10^{-5}$ (s^{-1})	$k_d^2 \times 10^{-4}$ (s^{-1})	$K_{eq}^1 \times 10^6$ (M^{-1})	$K_{eq}^2 \times 10^4$ (M^{-1})	ΔG_{ads}^1 (kcal/mol)	ΔG_{ads}^2 (kcal/mol)
c-PtBP1	170 ± 3.44		5.00 ± 0.88		3.40 ± 0.89		−8.9 ± 0.1	
l-PtBP1	8.27 ± 0.92	10.2 ± 1.3	5.00 ± 0.57	3.00 ± 0.33	0.16 ± 0.04	3.39 ± 1.24	−7.1 ± 0.1	−6.2 ± 0.2
3l-PtBP1	16.88 ± 4.07		9.00 ± 1.00		0.19 ± 0.07		−7.2 ± 0.2	
c-PtBP2	5.32 ± 1.15		6.28 ± 1.55		0.09 ± 0.04		−6.7 ± 0.3	
l-PtBP2	21.8 ± 2.2		15.22 ± 5.0		0.16 ± 0.07		−7.1 ± 0.3	
3l-PtBP2	1603 ± 52	15.5 ± 4.9	26.11 ± 12	3.6 ± 0.5	6.73 ± 2.49	3.87 ± 1.24	−9.3 ± 0.3	−6.3 ± 0.2
l-QBP1	238 ± 1.0		200 ± 4.0		0.122 ± 0.03		−6.9 ± 0.1	
3l-QBP1	1912 ± 88		130 ± 1.0		1.48 ± 0.23		−8.5 ± 0.1	
l-QBP2	37.1 ± 2.7	484 ± 94	3.0 ± 0.2	200 ± 2	1.24 ± 0.1	3.2 ± 0.7	−8.4 ± 0.04	−6.2 ± 0.12
3l-QBP2	112.9 ± 11.49		10 ± 0.1		1.13 ± 0.12		−8.3 ± 0.1	
l-GBP1	1.4 ± 0.1 × 10^4	112 ± 21	890 ± 150	2 ± 0.01	1.56 ± 0.28	56 ± 10	−8.2 ± 0.1	−7.8 ± 0.1
3l-GBP1	2.9 ± 0.2 × 10^4	1.6 ± 0.2 × 10^3	773 ± 183	6.5 ± 3.7	3.91 ± 1.22	330 ± 190	−9.0 ± 0.2	−8.9 ± 0.3

[a] The values calculated using the biexponential adsorption model are labeled with the superscripts "1" and "2", representing the first and second part of the exponential, respectively.

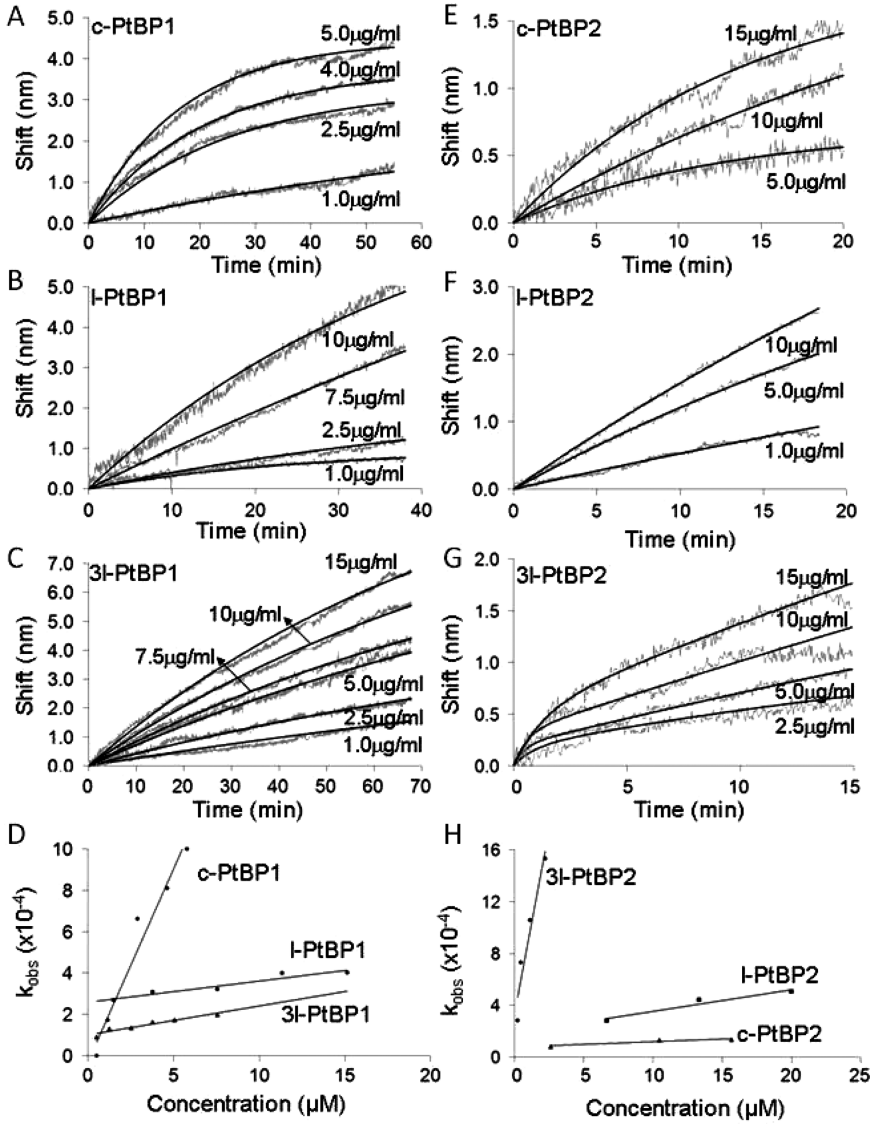

Figure 1. SPR spectral profiles of the concentration-dependent adsorption kinetics of the three different forms of the two platinum binding peptides; PtBP1 (a, b, and c) and PtBP2 (e, f, and g) for single constrained, single linear, and 3-repeat linear, respectively. The continuous lines represent the fit over the experimental data points. The concentration dependent behavior of apparent adsorption rates, k_{obs}, is shown in (d) and (h) for PtPB1 and PtBP2, respectively.

linear form of PtBP1 also adopts some degree of PPII structure in solution but the cyclic version does not. The PtBP2 lacks these amino acids, and therefore, the differences between the binding behavior to Pt of the l and c-forms do not follow the case for PtBP1.

Silica binding peptides were tested on a silica-coated SPR substrate for their binding affinity (see Supporting Information). The original sequences are linear 12 aa long peptide selected from phage display libraries. Also 36 aa long tandem repeats of these peptides were synthesized to determine the effect of

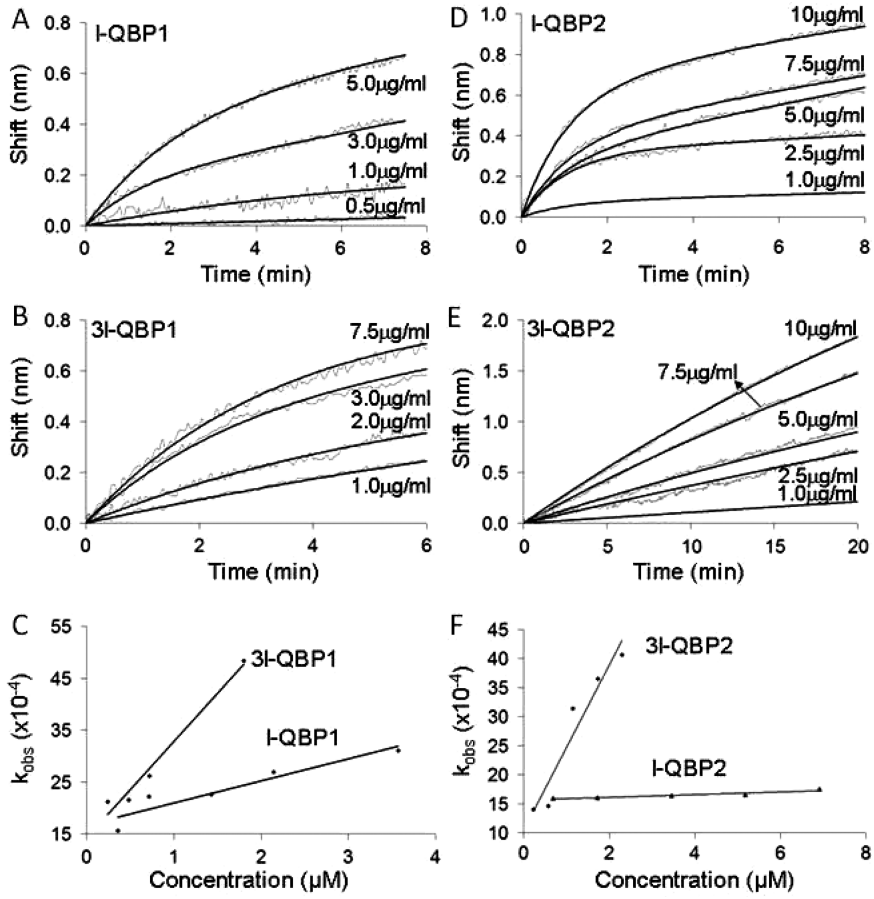

Figure 2. SPR spectral profiles of the concentration-dependent adsorption of three different forms of the two silica binding peptides; both linear, in single and three-repeat tandem forms, are shown in: (a) l-QBP1, (b) 3l-QBP1, (d) l-QBP2, and (e) 3l-QBP2. The continuous lines represent the fit over the experimental data points. The concentration dependent behavior of apparent adsorption rates, k_{obs}, is shown in (c) and (f) for QBP1 and QBP2, respectively.

multimerization on the binding affinity (Figure 2). Silica binders l-QBP1 and 3l-QBP1 have binding free energies of −6.9 and −8.5 kcal/mol, respectively (Table 2). The desorption rates for both peptides are closer although the adsorption rate of the tandem repeat is an order of magnitude faster leading to a 1.6 kcal/mol difference in their binding energies. The binding energy of QBP2 did not change as a function of increased number of repeats. Making a trimer of the QBP2 increased the adsorption rate of the original sequence almost the same as its desorption rate (Figure 2D,E), resulting in a virtually no change in the equilibrium adsorption constant (Table 2).

The exact mechanism(s) of the adsorption behavior of the peptide on to the solids investigated in this work are not yet clear. In general, adsorption of a peptide on a solid may be a result of chemisorptions or physisorption processes, involving hydrophobic or electrostatic interactions, hydrogen bonding or combinations of these forces. For example, observing the hydrophobicity[38] of the two Pt-binding peptides, we see that they are more or less the same (Table 1). Their equilibrium constants and the energy of adsorption are also similar in the linear form. However, between the c and l forms, peptides, having the same integral sequence, have significantly different adsorption behavior, suggesting an effect imposed by the molecular conformation in molecular recognition of the solid. This is also suggested from the multimerization, in which case while PtBP1 exhibits no change in free energy of adsorption PtBP2 shows an increase in this property, again possibly due to significant change in the molecular conformation of this concatamer. Between the two silica-binding peptides, the more hydrophobic QBP2 has a slower adsorption and desorption rates but higher binding constant and, hence, higher energy of binding than those of the QBP1 which is more hydrophilic dissolving in the buffer more easily. However, in the three repeat forms of these two peptides there is hardly any difference in the adsorption energies but much faster binding processes in the more hydrophilic peptide (i.e., 3l-QBP1). Finally, in the case of GBP1s, the 3-repeat form has an adsorption rate twice as fast and a slight increase (∼10%) in adsorption energy compared to those of the single repeat one, again, perhaps, a result of the conformational changes in the peptides when they are exposed to the solid surface (Figure 3). The conformational change and relaxation of the peptide on the solid surface may result in lattice matching with the underlying crystal surface of the solid (an important issue and a subject of another major work).[39]

The tandem 3-repeat form of the gold binding peptide, 3l-GBP1, was tested for its binding kinetics in a previous study.[16] We also studied the adsorption and supramolecular assembly characteristics of this GEPI on gold surfaces using atomic force microscopy.[39] Here we studied original sequence of the GBP1 in its singly repeating form to compare its binding characteristics of the multimerized form. The binding energies of the two polypeptides are −8.2 and −9.0 kcal/mol, respectively. The examination of the Figure 4 (and Table 2) reveals that the difference in the binding energy is caused by the faster adsorption of the 3-repeat peptide on the gold surface. Similar to the 3-repeat form, the adsorption data of the single repeat were fitted with a biexponential model that resulted in the two

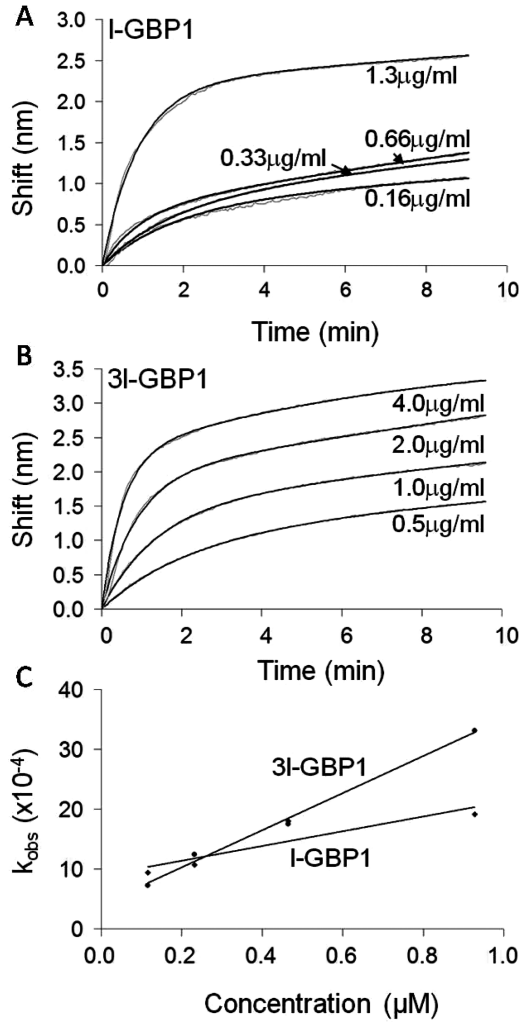

Figure 3. SPR spectral profiles of the concentration-dependent adsorption of the two different forms of the gold binding peptides, l-GBP1 and 3l-GBP1 (a) and (b), respectively. The continuous lines represent the fit over the experimental data points. The concentration dependent behavior of the apparent adsorption rates, k_{obs}, for the two peptides is shown in (c).

sets of adsorption, desorption and equilibrium constants, as calculated in Table 2.

Similar to these single and three-repeat forms of GBP1, the adsorption behavior of some of the peptides studied in this work, specifically, linear-PtBP1, 3-repeat linear PtBP2, and linear-QBP2, also exhibit biexponential behavior. This means that the adsorption of the peptide measured by SPR spectroscopy could be fitted by two parallel adsorption profiles, thereby producing two sets of binding and equilibrium constants (Table 2). The biexponential adsorption behavior may indicate two parallel adsorption processing taking place during the peptide binding, assembly, and coverage of the solid surface. Our original explanation[16] for the two-stage adsorption behavior of the 3l-GBP1 was that because the surface irregularities on the gold, such as grain boundaries versus grain interiors, have different surface characteristics, peptide binding and coverage could be different on these topologically (and energetically) different sites. There may be another explanation for this biexponential behavior. In a recent atomic force microscopy study,[39] performed again using 3-repeat GBP1, on an atomically flat Au(111) surface, we directly observed the adsorption behavior of the peptide and found a number of morphological changes taking place on the surface. During the ultrathin film formation (1 nm thick) that nearly covers the whole surface of the solid, peptides first form isolated islands that ripen with a branch morphology first forming a network structure which then widen to cover the surface. The quantitative analysis of this behavior leads to biexponential plots where the first exponential behavior corresponds to peptide island formation and the second corresponds to lateral growth of the molecular network, its coverage, and eventual formation of the molecule-thick film. This behavior may not necessarily correspond to the behavior of the peptides binding to their corresponding solids in this study, but could be a possible reasonable explanation which needs to be experimentally verified using detailed AFM investigation in each of these cases.

In our SPR studies, we examined desorption as well as adsorption profiles of the peptides binding to their complementary solids (Figure 4). In general, the peptides showed a low rate of desorption and very low desorption overall from the surface in all cases. In other words, peptide/solid interactions do not necessarily involve adsorption and desorption, and the high adsorption combined with low desorption may be general characteristics of the GEPIs with specific (as opposed to nonspecific) molecular recognition of solids. The adsorption behavior of GEPI on a solid substrate is different than the conventionally observed interactions between two biological molecules, in which case one of the proteins is bound to a solid surface, for example, through a thiol molecular linker, and the other stays in solution (an analyte), where the binding affinity is dictated by the differences in adsorption and desorption rate constants of similar magnitudes leading to an equilibrium. Here, GEPIs universally have low desorption profiles and, therefore, the adsorption process dominates the solid-binding event. Also, during the peptide adsorption onto a solid surface, there may be additional surface interactions involved compared to the well-known molecular interactions occurring in solution between molecules through recognition, resulting in a more complex peptide−solid binding process and causing variations in the Langmuir adsorption behavior, as described above. These processes may include peptide−peptide, peptide−solid interactions, surface diffusion, island formation, island diffusion, reorganization, and growth along with potential reconformation of the peptide on the surface leading to various supramolecular self-assembly on a given solid (metal, ceramic, semiconductor, etc.).[6,39] Furthermore, these ordered assemblies may not be too different than traditional self-assembled molecular conformations that form on metallic surfaces.[40]

A detailed understanding of the peptide assembly processes in the future, which may become possible through high resolution surface microscopy, spectroscopy and diffraction studies, may lead to better insights into the mechanisms of the quantitative adsorption studied here. Nevertheless, it is apparent from our comprehensive adsorption study that in vivo biocombinatorially selected short polypeptides have interesting adsorption behavior and the kinetics of binding can, to a certain degree, be controlled using tandem repeats, thereby providing means to manipulate binding behavior of the peptides. It should be pointed out that repetition of a given solid binding sequence may be introduced in several different ways. In this work, we introduced the peptide repeats in simple tandem form, where a given amino acid sequence is sequentially repeated without any interruption between repeats. One may also introduce the repeats in the form in which the sequences could have designed peptide linkers between them. The introduction of the linkers will inevitably affect the characteristics of the binding of the peptide to a given solid surface. Linker design by itself is a major task.

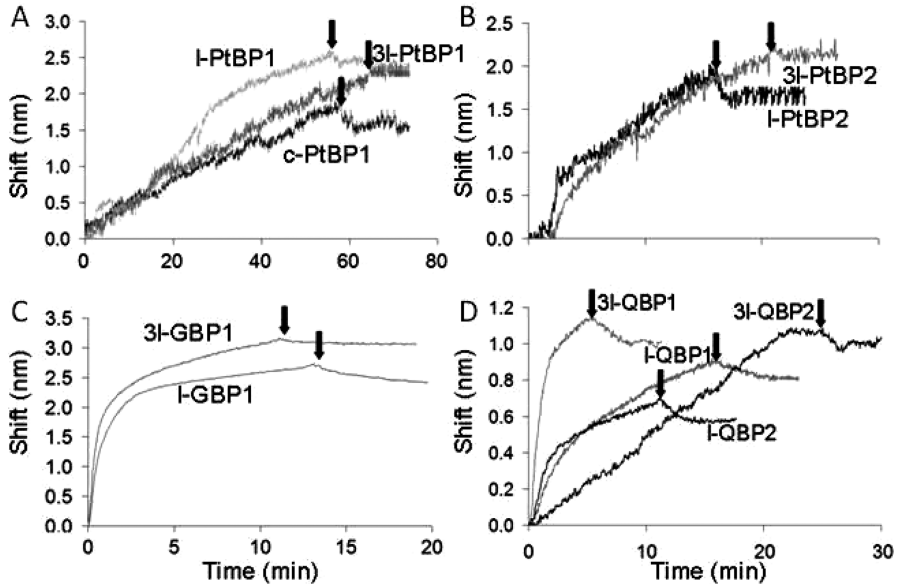

Figure 4. Experimental SPR profiles of the solid binding peptides that include the desorption behavior for (a and b) platinum-binding peptides on Pt substrate, (c) gold binding peptides on a gold substrate, and (d) quartz-binding peptides on a silica substrate. The arrows on the profiles indicate the times at which the flow of protein solution is ceased and the flow of buffer caused desorption of nonspecific binding of the peptides form the surface, resulting in a descending profile. For clarity, only single concentrations were used.

The number of amino acids used in the linker, its flexibility or rigidity, or intentional twists, turns, and bends in the linker are all considerations that our group is currently pursuing on.

Finally, the question whether and how a given genetically selected peptide sequence binds to a given material and not others is fundamentally significant for understanding molecular mechanisms of peptide recognition of solids. Also, from a more practical point of view, the knowledge material selectivity of a solid-binding peptide is necessary if the peptide is to be used as a utility in applications that would require targeted- or directed-immobilization or -assembly of nanoentities (nanoparticles, functional molecules, or probes) in multimaterial systems.

Conclusions

In this work, using a modified surface plasmon resonance spectroscopy, we studied the molecular adsorption behavior and binding kinetics of several genetically engineered peptides on corresponding solid surfaces and determined quantitative binding parameters. The Pt-, quartz-, and Au-binding peptides were tested in their original form, heptapeptides, dodecopeptides, 14-aa peptides, respectively, as they are displayed on the phage display or the cell surface display libraries. These original sequences were also altered to test the effect of multiple, tandem repeats on binding affinity. The use of multiple repeats of a given peptide sequence did not have a uniform effect in binding behavior of all peptides, as in some cases it increased the binding affinity and in other cases the affinity was reduced. The ability to form tandem repeats of the solid-binding peptides, nonetheless, provides a new means to tune binding behavior of short peptides possibly due to the molecular conformations that influences their solid-binding affinity. The low desorption of the peptides from the selected solids surfaces compared with the generally observed rapid adsorption with high coverage and long-term stability may be a unique set of characteristics of these new classes of peptides. The knowledge of quantitative adsorption of solid binding peptides with specific amino acid sequences identified here and the way to manipulate their binding behavior using multimerization, could, therefore, be useful for a wide range of applications requiring material-specific molecular-linkers, -erectors, and -assemblers for utility in bioassays, biosensors, and biomolecular probing.

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Supporting Information Available. Experimental procedures for phage display, cell surface display, substrate preparation for surface plasmon resonance studies, details of Langmuir adsorption isotherm calculations, curve fitting, and residual plots. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Smith, G. P. *Science* **1985**, *228*, 1315–1317.
(2) Wittrup, K. D. *Curr. Opin. Biotechnol.* **2001**, *12*, 395–399.
(3) Brown, S.; Sarikaya, M.; Johnson, E. *J. Mol. Biol.* **2000**, *299*, 725–732.
(4) Whaley, S. R.; English, D. S.; Hu, E. L.; Barbara, P. F.; Belcher, A. M. *Nature* **2000**, *405*, 665–668.
(5) Naik, R. R.; Stringer, S. J.; Agarwal, G.; Jones, S. E.; Stone, M. O. *Nat. Mater.* **2002**, *1*, 169–172.
(6) Sarikaya, M.; Tamerler, C.; Jen, A. K. Y.; Schulten, K.; Baneyx, F. *Nat. Mater.* **2003**, *2* (9), 577–585.
(7) Thai, C. K.; Dai, H. X.; Sastry, M. S. R.; Sarikaya, M.; Schwartz, D. T.; Baneyx, F. *Biotechnol. Bioeng.* **2004**, *87* (2), 129–137.
(8) Sano, K.; Sasaki, H.; Shiba, K. *J. Am. Chem. Soc.* **2006**, *128* (5), 1717–1722.
(9) Naik, R. R.; Jones, S. E.; Murray, C. J.; McAuliffe, J. C.; Vaia, R. A.; Stone, M. O. *Adv. Funct. Mater.* **2004**, *14* (1), 25–30.
(10) Mao, C. B.; Solis, D. J.; Reiss, B. D.; Kottmann, S. T.; Sweeney, R. Y.; Hayhurst, A.; Georgiou, G.; Iverson, B.; Belcher, A. M. *Science* **2004**, *303*, 213–217.
(11) Tamerler, C.; Duman, M.; Oren, E. E.; Gungormus, M.; Xiong, X. R.; Kacar, T.; Parviz, B. A.; Sarikaya, M. *Small* **2006**, *2*, 1372–1378.
(12) Naik, R. R.; Stringer, S. J.; Agarwal, G.; Jones, S. E.; Stone, M. O. *Nat. Mater.* **2002**, *1*, 169–172.
(13) Park, T. J.; Lee, S. Y.; Lee, S. J.; Park, J. P.; Yang, K. S.; Lee, K. B.; Ko, S.; Park, J. B.; Kim, T.; Kim, S. K.; Shin, Y. B.; Chung, B. H.; Ku, S. J.; Kim, D. H.; Choi, I. S. *Anal. Chem.* **2006**, *78*, 7197–7205.

(14) Sano, K.; Ajima, K.; Iwahori, K.; Yudasaka, M.; Iijima, S.; Yamashita, I.; Shiba, K. *Small* **2005**, *1*, 826–832.
(15) Chen, H. B.; Su, X. D.; Neoh, K. G.; Choe, W. S. *Anal. Chem.* **2006**, *78*, 4872–4879.
(16) Tamerler, C.; Oren, E. E.; Duman, M.; Venkatasubramanian, E.; Sarikaya, M. *Langmuir* **2006**, *22* (18), 7712–7718.
(17) Yamashita, K.; Kirimura, H.; Okuda, M.; Nishio, K.; Sano, K. I.; Shiba, K.; Hayashi, T.; Hara, M.; Mishima, Y. *Small* **2006**, *2* (10), 1148–1152.
(18) Seker, U. O. S.; Wilson, B.; Dincer, S.; Kim, I. W.; Oren, E. E.; Evans, J. S.; Tamerler, C.; Sarikaya, M. *Langmuir* **2007**, *23* (15), 7895–7900.
(19) Sano, K. I.; Sasaki, H.; Shiba, K. *Langmuir* **2005**, *21*, 3090–3095.
(20) Kulp, J. L.; Sarikaya, M.; Evans, J. S. *J. Mater. Chem.* **2004**, *14*, 2325–2332.
(21) Sano, K.; Shiba, K. *J. Am. Chem. Soc.* **2006**, *125* (47), 14234–14235.
(22) Oren, E. E.; Tamerler, C.; Sarikaya, M. *Nano Lett.* **2005**, *5*, 415–419.
(23) Tomasio, S. M.; Walsh, T. R. *Mol. Phys.* **2007**, *105*, 221–229.
(24) Rocha, S.; Thunemann, A. F.; Pereira, M. C.; Coelho, M. A. N.; Mohwald, H.; Brezesinski, G. *ChemBioChem* **2005**, *6* (2), 280–283.
(25) Joshi, H.; Shirude, P. S.; Bansal, V.; Ganesh, K. N.; Sastry, M. *J. Phys. Chem. B* **2004**, *108* (31), 11535–11540.
(26) Cocklin, S.; Jost, M.; Robertson, N. M.; D; Weeks, S.; Weber, H. W.; Young, E; Seal, S.; Zhang, C.; Mosser, E.; Loll, P. J.; Saunders, A. J.; Rest, R. F.; Chaiken, I. M.; *J. Mol. Recognit.* **2006**, *19* (4), 354–362.
(27) Day, Y. S. N; Baird, C. L.; Rich, R. L.; Myszka, D. G. *Protein Sci.* **2002**, *11*, 1017–1025.
(28) Homola, J.; Lu, H. B. B.; Nenninger, G. G.; Dostalek, J.; Yee, S. S. *Sens. Actuators, B* **2001**, *76*, 403–410.
(29) Rich, R. L.; Myszka, D. G. *J. Mol. Recognit.* **2005**, *18* (1), 1–39.
(30) Jung, L. S.; Campbell, C. T.; Chinowsky, T. M.; Mar, M. N.; Yee, S. S. *Langmuir* **1998**, *14* (19), 5636–5648.
(31) Bailey, L. E.; Kambhampat, D.; Kanazawa, K. K.; Knoll, W.; Frank, C. W. *Langmuir* **2002**, *18* (2), 479–489.
(32) Choe, W. S.; Sastry, M. S. R.; Thai, C. K.; Dai, H.; Schwartz, D. T.; Baneyx, F. *Langmuir* **2007**, *23*, 11347–11350.
(33) Hook, F.; Kasemo, B. *Anal. Chem.* **2001**, *73*, 5796–5804.
(34) Gestwicki, J. E.; Cairo, C. W.; Mann, D. A; Owen, R. M.; Kiessling, L. L. *Anal. Biochem.* **2002**, *305*, 149–155.
(35) Arima, Y.; Iwata, H. *J. Mater. Chem.* **2007**, *17*, 4079–4087.
(36) Kovalenko, S. A.; Lisitsa, M. P. *Semicond. Phys., Quantum Electron. Optoelectron.* **2001**, *4*, 352–357.
(37) Szunerits, S.; Coffinier, Y.; Janel, S.; Boukherroub, R. *Langmuir* **2006**, *22* (25), 10716–10722.
(38) Kyte, J.; Doolittle, R. F. *J. Mol. Biol.* **1982**, *157* (1), 105–132.
(39) So, C. 2008, unpublished.
(40) Brune, H. *Surf. Sci. Rep.* **1998**, *31*, 121–229.

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