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Peptide-Surface Adsorption Free Energy Comparing Solution Conditions Ranging from Low-to-Medium Salt Concentrations

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Abstract

Peptide-adsorption studies in the biomaterials field are typically performed in phosphate-buffered saline (PBS) to represent the physiological environment. However, the study of peptide adsorption in pure water or low-salt media (e.g., 10 mM potassium phosphate buffered water; PPB) is of interest for many other applications in the broader field of biotechnology. In previous studies we used surface plasmon resonance spectroscopy (SPR) and atomic force microscopy (AFM) to determine the standard state adsorption free energy of peptide adsorption ($\Delta G^{\circ}_{\text{ads}}$) and the force required for peptide desorption (F_{des}), respectively, for a wide range of surface chemistries in PBS and showed that these two parameters were strongly correlated. The objective of this present research was to repeat these studies in PPB rather than in PBS in order to determine the influence of the differences in salt concentration between these two environments and also to determine if the same correlation between $\Delta G^{\circ}_{\text{ads}}$ and F_{des} held for peptide adsorption in PPB. The results from these studies show that $\Delta G^{\circ}_{\text{ads}}$ and the correlation between $\Delta G^{\circ}_{\text{ads}}$ and F_{des} in PPB are not significantly different than in PBS for the evaluated set of peptide-surface systems.

Keywords

adsorption; peptides; atomic force microscopy; surface plasmon resonance; thermodynamics

On a fundamental level, protein adsorption behavior can be considered to be represented by the combination of the individual interactions among the amino acid residues making up a protein (of which there are 20 different types coded for by DNA), the solvent environment (i.e., water molecules, salt ions, pH, and temperature), and the functional groups presented by a surface. These types of interactions can be characterized by the change in the standard-state adsorption free energy ($\Delta G^{\circ}_{\text{ads}}$) because it provides a direct assessment of the primary thermodynamic driving force that characterizes the overall tendency of a peptide to adsorb to a surface.^[1] This parameter could then be used to provide a level of understanding of the sub-molecular events that govern protein adsorption behavior.^[2]

Wei and Latour previously developed an experimental method for the characterization of peptide adsorption behavior using surface plasmon resonance spectroscopy (SPR) in a manner that minimizes the effects of peptide-peptide interactions at the adsorbent surface and provides a direct method of determining bulk-shift effects, thus providing accurate values of $\Delta G^{\circ}_{\text{ads}}$.^[3] SPR was selected for these studies because it is one of the most sensitive and directly applicable methods to determine adsorption free energy,^[4] it is inherently suitable for use with gold-alkanethiol self-assembled monolayer (SAM) surfaces,

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and it has been widely applied in recent years to study both peptide and protein adsorption behavior.^[5] After initial development, Wei and Latour applied their method to characterize the adsorption behavior of a large series of different peptide-SAM systems including 8 different zwitterionic host-guest peptides (TGTG-X-GTGT, where G and T are glycine and threonine, with X = V, G, F, W, K, D, T, and N using the standard single-letter amino acid code); with charged NH_3^+ and COO^- groups at the beginning and end of the peptide chain, respectively (i.e., charged N- and C-termini at pH 7.4) on eight different SAM surfaces (Y-SAM with Y = CH_3OH , NH_2 , COOH , OC_6H_5 , $(\text{OCH}_2\text{CH}_2)_3\text{OH}$ (PEG), NHCOCH_3 and COOCH_3) with static water contact angle values ranging from 16° to 110° (see the Supporting Information (S.I.), Table S.1) to represent common functional groups contained in organic polymers. These studies were conducted at 25°C in phosphate-buffered physiological saline (PBS; 140 mM NaCl, 10 mM Na_2HPO_4 , 2.7 mM KCl and 1.8 mM KH_2PO_4 , pH 7.4).^[6] Values of $\Delta G^\circ_{\text{ads}}$ determined from this previous study are presented in the Supporting Information, (S.I.), Table S.4.

While SPR proved to be very useful for measuring $\Delta G^\circ_{\text{ads}}$ to characterize peptide-surface interactions, this method is largely restricted for use with materials that can readily form nanoscale-thick films over the respective sensor surfaces.^[7] On the contrary, AFM has been widely applied to characterize single biological molecular recognition processes because of its higher force sensitivity and the capability to operate under different solvent conditions on any macroscopically flat material surface such as polymers, ceramics or inorganic substrates.^[8–10] We have therefore developed a standardized AFM method^[11] (detailed methods for this standardized AFM method are provided in S.I., section S.V) to measure the desorption forces (F_{des}) between peptides and SAM surfaces under PBS solution conditions, and investigated how well values of F_{des} measured by AFM correlated with $\Delta G^\circ_{\text{ads}}$ values obtained by SPR^[11] to overcome the limitation that many material surfaces are not readily suitable for use with SPR.^[12] In order to use our host-guest model peptide for our AFM studies, we modified the peptide sequence by substituting cysteine (C) for the fourth glycine (G) amino acid of the peptide sequence (i.e., G8C substitution) in order to tether the host-guest peptide to the AFM tip via 3.4 kDa (ortho-pyridyl)-disulfidepoly (ethylene-glycol)-succinimidyl-ester (OPSS-PEG-NHS) tethers (Creative-PEGWorks, Winston Salem, NC) by an exchange reaction through the free thiol groups of the tether and the cysteine residue. In preparation for these experiments, SPR adsorption studies were first conducted with each of these model host-guest peptides for a wide range of peptide-SAM systems. No significant difference was found in the resulting $\Delta G^\circ_{\text{ads}}$ values, thus supporting that the TGTG-X-GTCT peptide could be used as an equivalent model system as TGTG-X-GTGT for the AFM studies^[11] (see S.I., section S.IV.c and Figure S.4). AFM studies were then conducted using the modified host-guest peptide for the same set of eight 'X' mid-chain amino acids and eight SAM surfaces that were used with our SPR studies in PBS solution. F_{des} values measured by AFM were then plotted against the $\Delta G^\circ_{\text{ads}}$ values determined by SPR to investigate the possible correlation between these two indicators of peptide adsorption affinity. The results of this comparison revealed that a strong, linear correlation does exist between these two parameters^[11]. The importance of this linear correlation between F_{des} and $\Delta G^\circ_{\text{ads}}$ is that it then can be used to estimate the $\Delta G^\circ_{\text{ads}}$ from F_{des} values measured by AFM for peptide interactions with material surface systems that are not readily amenable for use by SPR.^[7]

While PBS is an appropriate solution to simply simulate physiological conditions, which has been used for biomaterial application for decades,^[13] it contains a moderately high concentration of various salt ions, which may influence peptidesurface interactions. However, peptide-surface interactions are also important for a broad range of other areas of biotechnology^[14–19] that may involve solutions conditions more representative of pure water or aqueous solutions with much lower salt concentrations. This situation thus raises

the important experimental question: How does the presence of salt ions influence peptide-surface interactions for these model systems? While published reports in the literature suggest that salt concentrations should generally have minimal influence on peptide adsorption behavior for salt concentrations below 0.5 M,^[20] it is not clear that this general statement would hold for our peptide-SAM systems and SPR and AFM methods, especially for the negatively and positively charged COOH-SAM and NH₂-SAM surfaces, respectively, given the zwitterionic nature of our hostguest peptides. In order to investigate this question, we repeated the same set of SPR and AFM adsorption studies at 25 °C using our host-guest peptides on the same set of SAM surfaces, but with PBS replaced by nanopure water with only 10 mM potassium phosphate buffer (PPB) added for pH control (2 mM KH₂PO₄ 8 mM K₂HPO₄; pH 7.4). Potassium phosphate salt was chosen because of its high buffering capacity and it is one of the essential constituent in a wide variety of cell-culture media.^[21–25] Given that we previously found no significant difference with the G8C substitution, we used the TGTG-X-GTCT host-guest peptide for this new set of studies, thus enabling the same batch of synthesized peptide to be used for both of our SPR and AFM methods. Accordingly, SPR experiments were conducted with the same eight different ‘X’ mid-chain guest amino acid residues on the same set of eight different SAM surfaces in PPB at 25 °C, from which $\Delta G^{\circ}_{\text{ads}}$ was determined, and AFM experiments were then also performed for these same peptide-surface systems to measure F_{des} in PPB at 25 °C as well. We compared $\Delta G^{\circ}_{\text{ads}}$ values for peptide adsorption in PPB with our prior results obtained in PBS to evaluate the influence of salt concentration on adsorption free energy. We then also evaluated the relationship between $\Delta G^{\circ}_{\text{ads}}$ versus F_{des} to determine if the same correlation found under PBS conditions between these two parameters also holds for peptide adsorption/desorption behavior under PPB solution conditions.

The resulting $\Delta G^{\circ}_{\text{ads}}$ comparisons between peptide adsorption in PPB versus PBS from SPR are presented in Figure 1. The data for each individual peptide-SAM system are presented in the S.I. (Table S.4 and S.5), which reveal significant differences in peptide adsorption behavior as a function of both the peptide and surface types. Two lines are plotted in this figure: a solid line, which represents the linear regression of the experimental data points and a dotted line, which represents what the regression line would be if perfect agreement existed between the $\Delta G^{\circ}_{\text{ads}}$ values in PPB compared with PBS, with a slope of 1.0 and yintercept of zero. Statistical comparison between these two lines using a Student’s t-test at the 95% confidence level ($\alpha = 0.05$) shows no significant difference in either the slope ($p = 0.12$) or the y-intercept ($p = 0.33$), thus indicating that the differences in salt composition and concentration between PPB and PBS do not substantially influence peptide adsorption behavior for this set of 64 different peptide-surface systems. Of particular interest, this finding holds for both the charged SAM surfaces (i.e., negatively charged COOH-SAM, red-triangle data points; and the positively charged NH₂-SAM, blue-square data points) as well as for the non-charged SAM surfaces (green diamond data points). This observation primarily indicates that the presence of monovalent Na⁺ and Cl[−] salt ions in solution from 0 to 140 mM concentration in the presence of 10 mM phosphate buffer has negligible influence on peptide adsorption behavior.

We then investigated the relationship between $\Delta G^{\circ}_{\text{ads}}$ determined by SPR versus F_{des} measured using our standardized AFM method to determine whether the same correlation previously determined under PBS conditions also holds under PPB conditions. Data for each individual peptide-SAM system are presented in the S.I. (Table S.6 and S.7), which again reveal significant differences in peptide adsorption behavior as a function of both the peptide and surface types. Figure 2 presents this comparison. As shown in Figure 2a strong linear correlation (dashed trend line with $R^2 = 0.88$; blue-diamond data points) is observed for the 64 peptides-SAM systems in PPB in a manner that is essentially indistinguishable with the linear relationship found with the data set in PBS (solid trend line with $R^2 = 0.89$; red-

triangle data points). Comparison between regression lines for the data sets in PBS and PPB again shows no significant difference in either the slopes ($p = 0.68$) or intercepts ($p = 0.19$) at the 95% confidence level ($\alpha = 0.05$), thus indicating negligible influence of the differences in the salt compositions and concentrations between these two solution environments along with consistency in the correlation between F_{des} measured by AFM and $\Delta G^{\circ}_{\text{ads}}$ determined by SPR using the applied experimental methods. A combination of these two data sets provides an overall correlation equation of $\Delta G^{\circ}_{\text{ads}} = -0.067(\pm 0.002) \times F_{\text{des}} + 0.44(\pm 0.08)$, (mean of fitting coefficient $\pm$ 95% C.I.), $R^2 = 0.89$; (data plot with correlation line and equation shown in S.I., Figure S.7). This correlation equation thus provides the means to determine effective values of $\Delta G^{\circ}_{\text{ads}}$ for material surfaces that are not readily amenable for use with SPR by conducting measurements using our standardized AFM method to measure F_{des} and then applying this correlation equation to estimate $\Delta G^{\circ}_{\text{ads}}$ for these systems in either PBS or PPB solution. This capability is of interest to provide a common basis for comparing peptide interactions for a broad range of materials surfaces that can be tested by either SPR or AFM. It is also particularly important for providing the kinds of data that are needed for the evaluation, modification, and validation of empirical force field parameters that are required for the accurate simulation of peptide and protein adsorption behavior to material surfaces, which we are working on in a synergistically coordinated parallel set of complementary studies.^[26, 27]

In summary, these multi-technique methods involving SPR and AFM provide experimental data for the characterization of peptide adsorption affinity to a wide range of surfaces. No significant difference in adsorption behavior was found between environmental conditions represented by either PBS or PPB solutions for either $\Delta G^{\circ}_{\text{ads}}$ values determined by SPR or the correlation between $\Delta G^{\circ}_{\text{ads}}$ values determined by SPR and F_{des} values measured by AFM. These combined results thus indicate that the presence of Na^+ and Cl^- ions over the range of about 0 to 140 mM and the slight differences in the phosphate buffer in the PBS and PPB solutions have negligible influence on peptide adsorption affinity for these host-guest peptide and functionalized SAM-surface systems, even for surfaces with charged functional groups. However, while these findings were consistent with some of the literature reports,^[20, 28] several other studies have suggested otherwise^[29, 30]. The differences in the adsorption response between these studies could stem from the differences in the electrostatic nature as well as the structure-forming tendency of the peptide used. For example, for the case of LK peptides (L: leucine & K: lysine)^[30], the propensity of forming helical structure is varied with the peptide length and the ionic concentration in solution, which in turn influenced the adsorption behavior of the peptides. In contrast, our peptides were specifically designed to exhibit random structure and to interact with the adsorbent surfaces through limited electrostatic interactions as the net charge in any of our peptides was at most ± 1 . The presence of weak electrostatic interactions for our systems are further supported by our previous results that our peptide-SAM interactions were mainly influenced by functional group hydrophobicity with specific interactions between the functional groups of the peptide and the SAM surfaces playing a secondary role.^[6, 11]

Based on these discussions, this combination of the presented SPR and AFM methods thus provides the ability to experimentally characterize and compare fundamental interactions that are important to understand protein adsorption behavior on material surfaces. In addition, results obtained from these multi-technique methods provide experimental data that are critically needed for the evaluation, modification, and validation of force field parameters to support the development of computational methods that are currently being designed to be able to be used to accurately predict protein adsorption behavior by molecular simulation.

Experimental Section

The unique custom-designed peptide model was used here. The host-guest model peptides, which were synthesized by Biomatik (Wilmington, DA) and characterized by analytical HPLC and mass spectral analysis with at least 98% purity, were designed with the amino acid sequence of TGTG-X-GTGT or TGTG-X-GTCT with zwitterionic end groups, where G, T, and C are glycine ($-H$ side-chain), threonine ($-\text{CH}(\text{CH}_3)\text{OH}$ side-chain), and cysteine ($-\text{CH}_2\text{SH}$ side chain), respectively. The X represents a “guest” amino acid residue, which was positioned in the middle of the peptide to best represent the characteristics of a mid-chain amino acid in a protein by positioning it relatively far from the zwitterionic end groups. The threonine residues and the zwitterionic end groups were selected to enhance aqueous solubility and provide additional molecular weight for SPR detection while the nonchiral glycine residues were selected to inhibit the formation of secondary structure, which, if present, would complicate the adsorption process.

The adsorption experiments from SPR in these studies were conducted using a Biacore-X SPR spectrometer (Biacore, Inc., Piscataway, NJ) in PBS or PPB. Briefly, the SPR sensorgrams were recorded in the form of resonance units^[3] as a function of time for six independent runs of peptide concentrations over each SAM surface at 25°C. The data obtained were then used to generate isotherm curves by plotting the raw SPR signal as a function of peptide solution concentration. The equations that were used for the determination of $\Delta G^\circ_{\text{ads}}$ from the adsorption isotherms were then derived based on the chemical potential of the peptide in its adsorbed and bulk solution state. Examples of adsorption isotherm and equations to calculate the free energy involved are shown in Fig. S. 2 & S.3 and Eq. (S.1) & (S.2)).

High-resolution desorption force measurements were done using AFM (MFP-3D instrument, Asylum Research, Santa Barbara, CA) with DNP-10 silicon nitride cantilever tips (Veeco Nanofabrication Center, Camarillo, CA). As indicated in Fig. S.1, the host-guest peptide sequences were tethered to AFM tips via a PEG cross-linker, a heterobifunctional polyethylene-glycol tether (3.4-kDA (orthopyridyl) disulfide-poly(ethylene-glycol)-succinimidyl ester (OPSS-PEGNHS), Creative PEGWorks, Winston Salem, NC). This molecule is composed of 77 ethylene glycol units with amine and thiol reactive end groups, thus enabling it to be used to covalently tether our peptide to silicon nitride tips. Force measurements were performed using our standardized AFM technique.^[7] Briefly, all force spectroscopy experiments were performed at room temperature in a fluid cell filled with droplets of PBS or PPB, pH 7.4. The functionalized tip with the peptide was then brought in contact with SAM surfaces for one second of surface delay and then retracted at a constant vertical scanning speed of 0.1 $\mu\text{m/s}$. Tips with PEG-OH (i.e., without peptide) were used as controls and a corresponding example of force-separation distance curve for our control group is shown in S.I. (Fig. S.6) The deflection signals (volts) were converted to force (Newton) using the settings of: (a) deflection sensitivity in the range of 40~100 nm/volts, (b) spring constant of tips of 0.058~0.065 N/m (from the thermal-tune method^[31]) and (c) applying correction for offset deflection. The thermal noise associated with the experimental set-up involving the cantilever (DNP-10), instrumentation (MFP-3D-Bio) and the data acquisition mode (force scan rate: 0.99 Hz, approach scan rate: 1 Hz) used in our current study with PPB or PBS, were kept minimal (< 2 pN) and was verified with the “amplitude spectral density” function provided with the instrumentation.

Complete descriptions for $\Delta G^\circ_{\text{ads}}$ from SPR and F_{des} measurements from AFM methods, surface characterization results and raw data for all figures here were also included in Supporting Information (S.I.).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Plot of $\Delta G^{\circ}_{\text{ads}}$ under PPB versus PBS solution conditions for 64 different peptide-SAM systems. The solid line represents a linear regression of the data points (regression equation in black text). The dotted line represents what the linear regression should be for perfect agreement between the two data sets (regression line in purple text with slope = 1.0 and y-intercept = 0.0).

Figure 2.

Correlation between $\Delta G^{\circ}_{\text{ads}}$ by SPR and F_{des} by AFM for an equivalent set of 64 peptide-SAM systems in PBS (lower dashed trend line with $R^2=0.89$; red triangle data points and red regression equation) and 10mM PPB (upper solid trend line with $R^2=0.88$; blue diamond data points and blue regression equation); pH = 7.4, 25 °C. Raw data points were shown in Table S.6 & S.7.