Synthetic Peptides as Receptors in Affinity Sensors: A Feasibility Study

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A relatively simple method for immobilizing synthetic peptides as a receptor onto a gold surface using the self-assembling monolayer (SAM) technique has been investigated. A synthetic peptide with an amino acid sequence similar to the 9–21 gD sequence of herpes simplex virus type 1 was modified with an alkylthiol chain and adsorbed in combination with an alkylthiol chain without peptide according to the SAM procedures. The resulting self-assembled receptor layers (SARs) were able to specifically interact with a monoclonal antibody directed against the 9–21 gD peptide. Binding studies monitored with a surface plasmon resonance setup showed that the response to the antibody depended on the composition of the SAR; a maximum response was reached at approximately 3 mol% peptide coverage. The response itself exceeded the value obtained from passive adsorption of the antibodies under similar conditions, indicating that the formed antibody layer is highly oriented. The equilibrium binding properties of the immobilized receptor molecules were found to be identical to those found when the immobilized antibodies interacted with the receptor molecule. The presence of a hydrophobic moiety in the alkylthiol derivates contributes to the ordering of the monolayer: a less specific interaction occurred when SARs without hydrophobic moiety were used. © 1993 Academic Press, Inc.

In recent years much research has been done on the development of immunosensors (1). Whereas the sensor transducer principles may vary widely, the common feature of all these sensors is that they use an immunoreaction to selectively detect an analyte. The majority of these sensors use an antibody as receptor to detect the antigen; generally, these antibodies are bound to a substrate, thus forming a sensor surface.

The use of antibodies as selector in a sensor system is suboptimal because antibodies are complex molecules and therefore their chemical and physical properties are difficult to control. (i) Immobilization procedures of the antibody involve the use of certain chemical groups of the antibody which are situated on different locations on the molecule. A nonuniform immobilization could occur, resulting in a partial obstruction of binding sites. (ii) The actual reacting amino acid residues of an antibody, the contact residues, compose only a relatively small part of the antibody. The nonspecific remainder of the antibody could be involved in aspecific interactions, particularly if, after immobilization, the contact residues are not oriented toward the analyte solution. (iii) Production of antibodies involves biological systems; it often proves laborious to maintain the quality of the antibody over a prolonged period of time. For instance, hybridoma cultures for monoclonal antibody production are subject to viability- and contamination-related problems (2). (iv) The immunoreaction itself is a complex reaction in which the combined interaction of the contact residues of the antibody with the antigen results in the specific binding of the antigen. Generally, the reaction is essentially irreversible; consequently, separation of antigen and antibody therefore often requires harsh conditions. The use of antibodies as receptors therefore usually limits the user to batch-type assays.

It has previously been shown that synthetic parts of an antibody, containing contact residues, can still react with an antigen (3,4). The use of synthetic peptides mimicking the antibody binding site as receptor molecule in a biosensor can significantly assist in the solution of the above-mentioned problems: (i) Peptides can readily be

synthesized without the use of biological intermediates; therefore the quality of the synthetic antibody can easily be maintained. (ii) For a receptor molecule consisting of about 15 amino acid residues the chemical properties are easier to predict and to control than those for intact antibodies. This could lead to a uniform and optimum immobilization of the synthetic antibody. (iii) The aspecific moiety of a synthetic antibody is relatively small, limiting aspecific interactions. (iv) Smaller proteins, and thus synthetic peptides, are usually physically more stable than large, complex molecules.

Small peptides, specific against a predetermined analyte, can be selected using random peptide banks or epitope libraries (5–8). Now, the use of these peptides as an affinity ligand offers new possibilities: in view of the smaller number of contact residues in a peptide ($M_r \sim 2 \times 10^3$) than in an intact antibody ($M_r \sim 150 \times 10^3$) the affinity of such a small peptide to an analyte is probably lower. The reversibility of an affinity reaction with such a peptide is therefore expected to be larger, which presents prospects for the development of reversible sensor surfaces able to monitor changing concentrations of analyte. To maintain selectivity, different peptides with different affinity constants for the analyte can be used. The response pattern of a sample to such an array of peptides can be compared to that of the desired analyte.

In this paper we demonstrate that an ordered layer of synthetic receptor peptides, with controlled surface density, is capable of very specific and sensitive recognition of the partner molecule. We show that such a layer can be conveniently immobilized on a gold substrate in the form of a self-assembling monolayer (SAM)[1] (9–14) composed of a mixture of receptor thiol peptides and alkylthiols. Throughout this paper, a SAM containing a receptor molecule is referred to as a self-assembled receptor layer (SAR).

As a model system, a synthetic peptide with an amino acid sequence similar to the 9–21 amino acid sequence of the gD protein of herpes simplex virus type 1 (HSV) (15) has been modified with an alkylthiol "tail" in order to be able to incorporate the peptide into a SAM. A monoclonal antibody, Mab A-16, raised against the 9–21 gD peptide has been used as the analyte.

MATERIALS AND METHODS

Reagents

Bovine serum albumin (BSA) and polyclonal antibody directed against human serum albumin (α-hSA)

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[1] Abbreviations used: SAM, self-assembling monolayer; SAR, self-assembled receptor layers; PB, phosphate buffer; PBS, phosphate-buffered saline; hSA, human serum albumin; α-hSA, polyclonal antibody directed against hSA; SPR, surface plasmon resonance; BSA, bovine serum albumin; HSV, herpes simplex virus; TFA, trifluoroacetic acid.
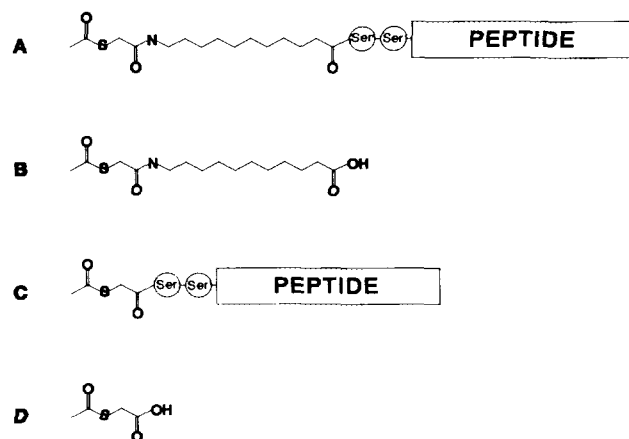

FIG. 1. Synthesized thiol compounds. **A** and **C** contain a spacer of two Ser amino acids and a specific 9–21 HSV I gD peptide. Compounds **B** and **D** are meant to coadsorb with **A** and **C**, respectively, to provide a spatial separation of the peptide moieties in the SAR.

were obtained from Sigma. The phosphate buffer (PB) used contained 0.1 M phosphate, pH 7.2. The phosphate-buffered saline solution (PBS) used contained 0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 6.5 mM Na_2HPO_4, pH 7.3.

A sample of Mab A-16 was provided by Dr. A. J. Scheffer (University of Groningen).

Thiol compounds. Gold has a strong interaction with sulfur (9), which makes the formation of adsorbed monolayers of functionalized alkylthiols, SAMs, possible. To obtain a SAR, the thiol adsorbates can be functionalized with a receptor molecule. Figure 1 shows the structural formulas of the thiol adsorbates which have been synthesized as described below. The lipopeptides **A** (M_r 2003) and **C** (M_r 1820) contain a synthetic peptide part, consisting of a spacer of two Ser residues, and a ligand part. This ligand part has an amino acid sequence similar to the 9–21 sequence of the gD protein of HSV type 1 (15). In the synthesized peptides methionine was replaced by norleucine to avoid side reactions during synthesis and isolation of the peptides. The dilutors **B** (M_r 317.5) and **D** (M_r 118) are intended to coimmobilize with **A** and **C**, respectively, and thus provide a lateral separation of the surface-bound peptides. In the following both lipopeptides and dilutors are referred to as adsorbates.

Peptides. Peptides were synthesized by solid-phase strategies on an automated multiple peptide synthesizer (Abimed AMS 422) on a 10-μmol scale (16). Tentagel S AC (0.21–0.23 meq/g peptide loading) was used as a resin (17,18). Coupling of the appropriate protected Fmoc amino acids was performed by BOP/NMM (1/2) in NMP using sixfold excess, 0.3 M, acetylating species (19–21). For introduction of the lipophillic spacer in **A** and **C** Fmoc undecanoic acid was coupled in the same
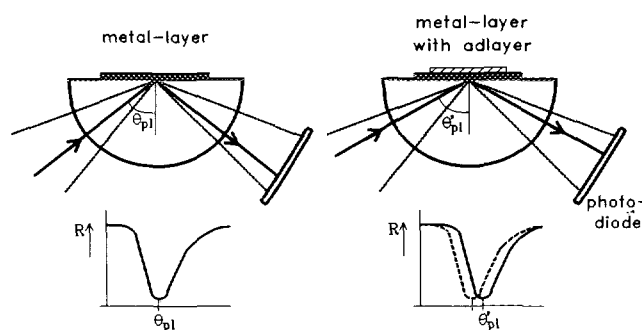
FIG. 2. Principle of SPR experiment: For a bare layer of gold an angle of reflection minimum (θ_{pl}) is found. The presence of a protein adlayer on top of the gold will result in a θ'_{pl}.

way. For Fmoc deprotection piperidine/DMA (1/4) was used. The N-terminal S-acetylmercaptoacetyl moiety was introduced by coupling with an equimolar mixture of S-acetylmercaptoacetic acid pentafluorophenyl ester and HOBt (sixfold excess, 0.3 M). After synthesis the peptides were cleaved from the resin and side chain deprotected with TFA/water (19/1, 2.5 h). Isolation and purification of the peptides were done by several ether precipitations. The purity of the peptides and lipopeptides was determined by reversed-phase HPLC; an aliquot of about 50 nmol was dissolved in 100 μl 30 vol% acetic acid. Of this solution 30 μl was applied to the HPLC system and analyzed on a RP select B column (Merck) using an acetonitrile/water gradient containing 0.1% TFA. The products were found to contain at least 90% of the target peptide, as deduced from the relative uv absorptions at 214 nm.

SPR Setup

To study the binding events occurring at the substrate/solution interface we used a surface plasmon resonance (SPR) sensor system developed for monitoring surface affinity reactions (23,24). As it is not within the scope of this paper to discuss the principles of SPR and its application as a sensor, we refer to Liedberg et al. (25) and previously published works (26,27). For the present purposes it is sufficient to mention that the angle θ_{pl}, under which a light beam exhibits a minimum in reflectance when reflected at a glass/gold interface (see Fig. 2), is a very sensitive measure of the layer thickness that is present on top of the metal layer. Obviously, the presence of the gold layer, necessary for the excitation of surface plasmons, makes the SPR technique ideally compatible with the SAM immobilization procedure. We have previously calculated (27) that for protein adsorption in a water environment the sensitivity is approximately an angular shift of 0.2 degree/nm of layer growth. For the present SPR setup the experimental angular resolution is 10^{-3} degrees, whereas the error in

the angular shift is $\sim 2 \times 10^{-3}$ degree. In the remainder of this paper it can be assumed that the θ_{pl} shift ($\Delta\theta_{pl}$) is proportional to the average thickness of an immobilized layer on the gold surface (27).

Preparation of Gold Substrates

Gold substrates were prepared by resistive evaporation of high-purity gold onto glass slides with a deposition rate of 10 Å/s under a 10^{-6} mbar vacuum to a final thickness of approx 46 nm. The glass slides we used were optically polished coverglasses for a Bürker-type cytometer (Tamson Holland, Catalog No. 995K41). Prior to evaporation the glass slides were thoroughly cleaned by immersing them into a 1:4 teepol:H_2O solution. This solution with immersed slides was first heated to approximately 100°C with a ramp of 10°/min and then placed in an ultrasonic bath for about 30 min. Subsequently the slides were rinsed with distilled water and dried with nitrogen before loading them into the vacuum chamber. The use of this cleaning procedure resulted in a good adhesion of the gold layer to the underlying glass substrate.

Preparation of SARs

In order to be able to form a SAM, thiol groups are necessary. The sulfur groups in both lipopeptides and dilutors are protected by an acetyl group to prevent side reactions such as S–S bond formation during storage. The S-acetyl group can be removed by reacting the thiol compounds with a nucleophilic agent, such as hydroxylamine. To obtain the free thiol groups, solutions of adsorbates in PB were incubated for 1 h at room temperature with a 100-fold molar excess of hydroxylamine · HCl. SARs were prepared by either of the following methods:

1. Sequential adsorption of constituent thiol compounds. Sequential adsorption of thiol adsorbates **A** and **B** from 10^{-5} M deacetylated adsorbate solutions onto freshly evaporated gold slides was monitored using the SPR setup. This procedure permitted us to determine the actual surface coverage of the two adsorbates in each SAR (cf. below). Moreover, we were able to prepare a series of SARs with known surface coverages ranging from 0 to 100% peptide.

2. SARs adsorbed from mixed solutions of thiol compounds. SARs were also prepared by adsorption from a solution mixture of adsorbates. These mixtures of thiols (e.g., **A** + **B**) with concentration ratios ranging from 0 to 100% peptide, to a final concentration of 10^{-5} M in PB, were deacetylated as described. Freshly evaporated gold slides were immersed in these solutions and incubated at 4°C overnight.

A schematic drawing of a SAR supposedly formed using adsorbates **A** and **B** is shown in Fig. 3. In this figure
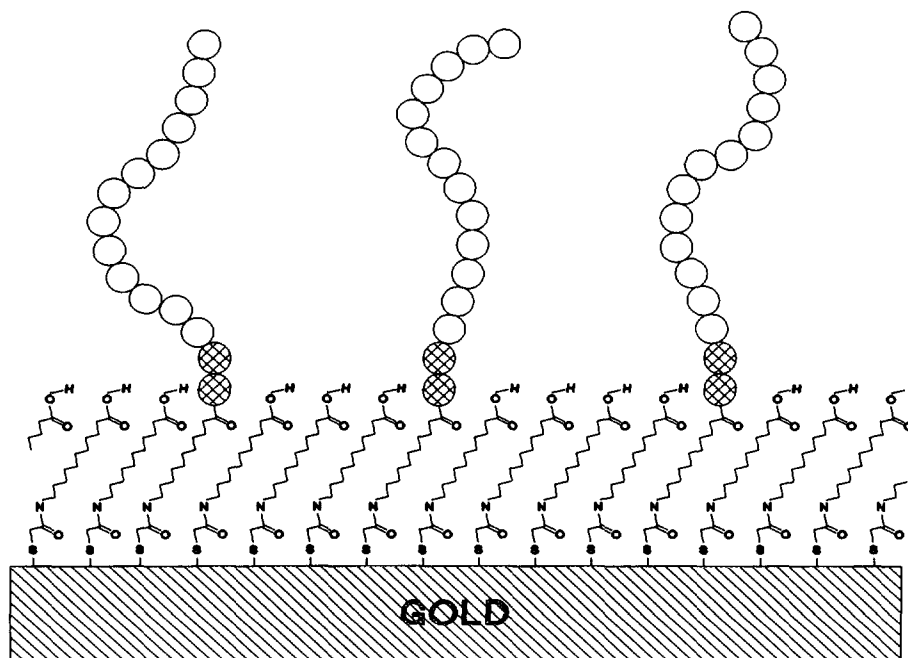
FIG. 3. Schematic depiction of a SAR formed from adsorbates **A** and **B**. The peptide is scaled to the hydrophobic moiety. Lateral scaling has not been considered.

the assumption has been made that the adsorbates are not formed as single-component domains. At least for procedure 2 this has previously been demonstrated (13).

RESULTS AND DISCUSSION

Intrinsic Affinity of Thiol Peptides

When used for a SAR, the 9–21 gD peptide has been modified with an alkylthiol "tail" resulting in either adsorbate **A** or **C**. To check whether the presence of the tail resulted in a decreased affinity of the peptide to Mab A-16, the intrinsic affinity of the modified peptide was tested using SPR. To this end a monolayer of Mab A-16 was prepared on the gold sensor surface by passive antibody adsorption from a 4×10^{-8} M solution of Mab A-16 in PBS. After addition of a solution of approximately 1.5×10^{-4} M BSA in PBS, to block any sites not covered by Mab A-16, and rinsing, a series of different concentrations of compound **C** in PBS was added. From the θ_{pl} shifts of all assay steps the binding characteristics of the adsorbate were determined. The results are shown in Fig. 4, using an adsorbed BSA coat as reference signal.

It is well known that for a first-order surface reaction the affinity constant (K_{aff}) can be estimated by taking the inverse analyte concentration at half the plateau response found at high analyte concentration (28). Thus for the present case, depicted in Fig. 4, we find K_{aff} ~3×10^8 M^{-1}. A K_{aff} of that order is not unusual for an immunoreaction involving an antibody and suffices for

our purposes. The maximum response is about 13 mdeg, which corresponds to an average layer growth of about 0.7 Å.

Coverage-Related Response

Sequentially adsorbed SARs. Formation of a series of SARs containing adsorbates **A** and **B** was monitored in the SPR setup. The adsorbates were adsorbed sequentially, resulting in θ_{pl} shifts of the two adsorbates. A
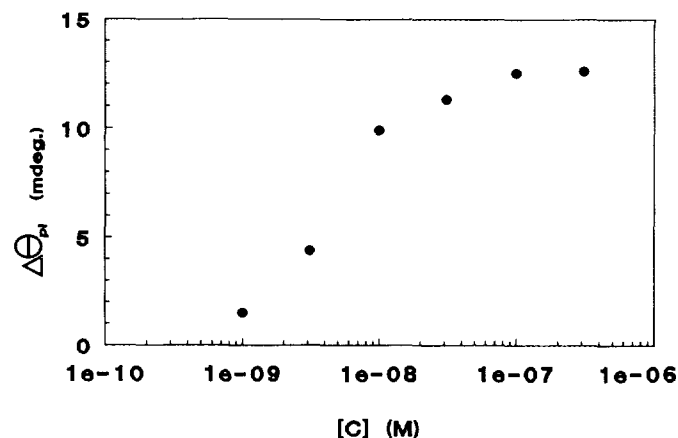

FIG. 4. Binding isotherm of peptide compound **C** toward a passively adsorbed monolayer of specific monoclonal antibody Mab A-16. A maximum response of approximately 13 mdeg θ_{pl} shift is exhibited.
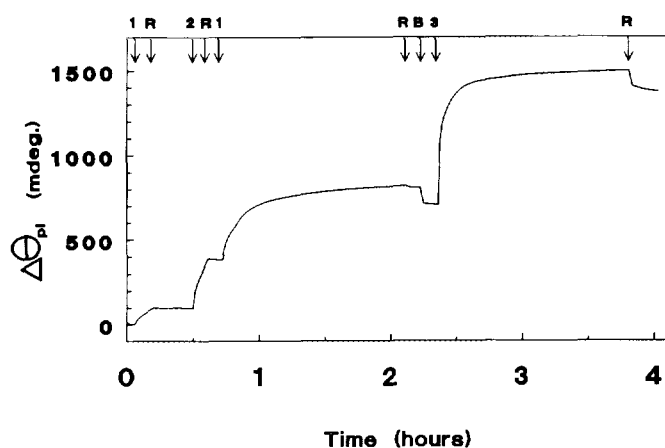
FIG. 5. A typical experiment: SAR formation from sequential adsorption of SAR compounds and subsequent immunoreaction monitored with SPR. 1, Addition of adsorbate **B**; 2, addition of adsorbate **A** (with specific peptide); 3, addition of Mab A-16, start of the immunoreaction; R, rinsing of the sensor surface with buffer; B, buffer change.

representative example of such an experiment is shown in Fig. 5 (for experimental details see the corresponding legends). From such an experiment the composition of the resulting SAR was determined as follows: for both adsorbates **A** and **B** the net $\Delta\theta_{pl,A}$ and $\Delta\theta_{pl,B}$ were determined. For a SPR experiment of this type, in which the refractive indices of the adsorbates are not too different, $\Delta\theta_{pl}$ is proportional to the adsorbed mass per unit of surface. Then, $\Delta\theta_{pl,A}/\Delta\theta_{pl,B}$ weighted by the respective molar masses equals the molar ratio of adsorbed molecules **A** and **B**.

From Fig. 5 it is seen that the interaction of adsorbates **A** and **B** with the surface is irreversible: rinsing the surface does not remove the adsorbates from the surface. The change in $\Delta\theta_{pl}$ at the event marked "B" is due solely to a change in refractive index as a result of a change in buffer composition. The adsorption kinetics showed the establishment of an equilibrium within 1 h for pure monolayers of either **A** or **B**, indicating that the overnight incubation time mentioned in preparation procedure 2 of SARs from mixed solutions should be sufficient. Subsequently the response to 6.7 × 10⁻⁸ M Mab A-16 was monitored. From Fig. 6a it is seen that the system response, expressed by $\Delta\theta_{pl}$, is highly dependent on the surface coverage. A maximum response value is found at approximately 3 mol% peptide coverage. The numeric value of the response at 3 mol%, a $\Delta\theta_{pl}$ of 850 mdeg, indicates that the nature of the binding of the antibodies to the peptides in the SAR is quite different from the binding that occurs when the same antibody is adsorbed to a bare gold layer; for this latter case we find a $\Delta\theta_{pl}$ ∼450 mdeg (data not shown). Apparently the orientation of the SAR-bound antibodies is such that the average area per bound antibody is much

smaller than that of an antibody adsorbed to a bare gold surface. This 850-mdeg θ_{pl} shift corresponds to a layer growth of approximately 5 nm, assuming a refractive index of 1.45 for the antibody (29).

Figure 6b provides a possible explanation for this response pattern. In this figure the response of Mab A-16 is normalized to the number of available receptor molecules **A**. This curve can be divided into two parts. At peptide surface coverages higher than approximately 10 mol%, individual peptide molecules have too little lateral freedom of movement due to steric hindrance of neighboring peptides to specifically interact with the antibody (cf. Fig. 3). The antibody therefore can react only nonspecifically with the available parts of the peptide, resulting in a low response per mole of peptide. Furthermore, at these high surface coverages, one peptide-bound antibody will severely limit the availability of binding sites of neighboring peptides. For peptide surface coverages below 10%, an increasing proportion of peptides in the SAR have enough lateral space to react specifically with the antibody. Below approximately 0.2 mol% of peptide coverage, the normalized response seems to reach a plateau value. This same trend, albeit starting at a somewhat larger coverage of 1 mol%, is found in experiments in which the SARs were prepared according to procedure 2 (cf. below). A rough estimate indicates that with an occupied area of 0.21 nm² per bound thiol derivate (12) the average nearest neighbor distance between peptides is ∼10 nm.

The plateau value itself seems to be reached at a Mab A-16/**A** ratio of approximately 0.4. In calculating the values, we assumed similar refractive indices for all compounds; $\Delta\theta_{pl}$'s were considered to be proportional to mass growth on the sensor surface. This suggests that not every peptide is able to react with an antibody, although the distinctive peptides in the SAR should have sufficient lateral space. Apparently, within the investigated coverage span, some of the peptides are still inactive. This finding can have two explanations: (i) a certain fraction of the peptides could be inactive, as suggested by the HPLC results, and (ii) there is a distribution of nearest-neighbor distances around the average. Consequently, a nonnegligible fraction of peptides could suffer from lack of lateral space required for antibody binding.

SARs from mixed solutions. SARs with different coverages of peptide were also prepared by incubating a series of gold substrates with mixed solutions of different concentration ratios **A** and **B** as described above. Subsequently the gold substrates with SARs were applied in the SPR sensor and the θ_{pl} shifts as a result of interactions with 6.7 × 10⁻⁸ M Mab A-16 were monitored. Polyclonal antibody α-hSA was used as a reference.

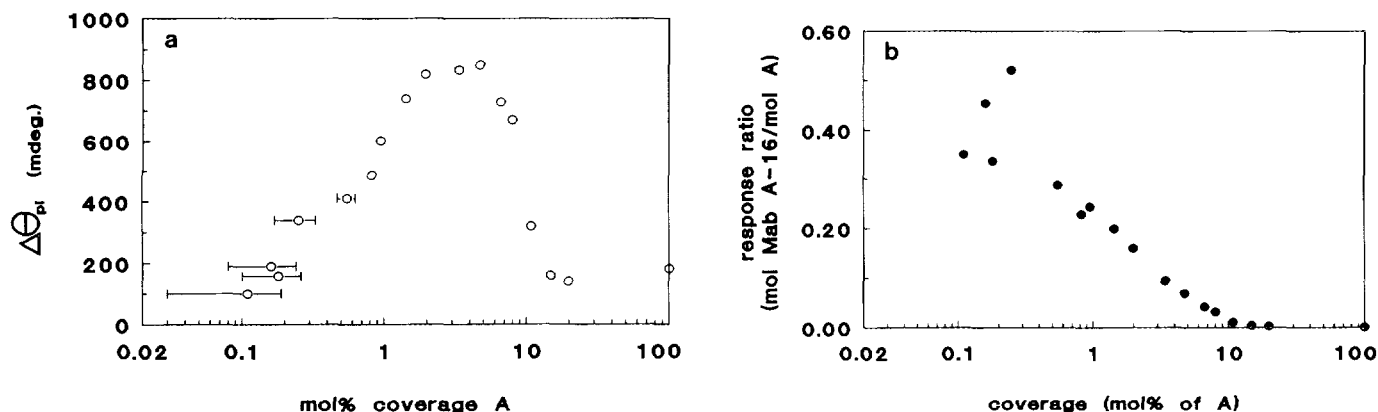Figure 7 depicts the responses of Mab A-16 for different series of prepared SAR layers. In this case the speci-

FIG. 6. (a) Response to 6.7×10^{-8} M Mab A-16 for different coverages of adsorbate A in a A/B-SAR obtained from sequentially adsorbed compounds. The error bars result from the angular resolution limit of the SPR setup during monitoring of the SAR formation. (b) The response of Mab A-16 normalized to the number of available receptor molecules A (see text).

ficity of the A/Mab A-16 reaction is also demonstrated by the low response to the nonspecific α-hSA (▲) over the whole concentration-ratio span.

By comparison to Fig. 6a it is seen that for each series in Fig. 7 the overall pattern of the response is very similar. For all series we found a maximum response between 4 and 8 mol% peptide coverage with an average θ_{pl} shift of 847 mdeg within an error range of about 3%. This is also the value found in Fig. 6a. When the data of each series in Fig. 7 are treated as described above to obtain the normalized Mab A-16 response, we find, as already mentioned, a pattern similar to that displayed in Fig. 6b (data not shown). As is also clear from Fig. 7, below approximately 4 mol% of peptide coverage the response to the Mab A-16 is not very reproducible. We believe that the main reason for this must be found in the construction of the horizontal scale in Fig. 7: contrary to SAR preparation procedure 1, the surface cover-

age in Fig. 7 was not measured, but rather assumed to be equal to the solution concentration from which the SARs were formed. Although weighing errors in the preparation of the stock solutions and differences in the diffusion rates of adsorbates A and B contribute significantly to the uncertainty in the quoted surface coverage values, there must be additional adsorption-determining factors that are currently not under control. Indeed, previous work on thiol coadsorption has pointed to the presence of adsorption driving forces that are not fully understood (30). In particular, it was found that the nature of the solvent from which the SAM is adsorbed is an important parameter.

It is clear that SAR preparation procedure 1 provides a much better definition of the SAR layer, and is the only choice if one wants to study the physicochemical characteristics of the SAR layer. However, from a pragmatic point of view, when the aim is to prepare a sensitive receptor surface, procedure 2 is obviously much more convenient, particularly because it is seen from both Figs. 6a and 7 that the region of maximum response lies within fairly wide boundaries and can be easily reproduced, even with procedure 2, without knowing the exact coverage.

Determination of Binding Isotherms

Gold substrates with a SAR adsorbed from a mixed solution with a bulk concentration ratio of adsorbates A:B and C:D of 1:25 were prepared as described previously. Subsequently the θ_{pl} shifts due to the response of a series of different concentrations of Mab A-16 were monitored. This particular 1:25 coverage was chosen in view of the high response value obtained in the coverage-related response experiments.

In Fig. 8a the binding isotherm of Mab A-16 to the A/B-SAR is shown. The plotted results are considered specific, in view of the negligible response to a high con-
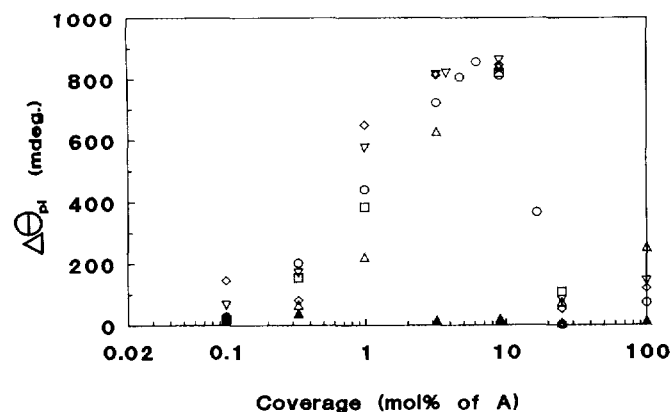

FIG. 7. Response to 6.7×10^{-8} M Mab A-16 for series with different coverages of A in A/B-SARs obtained from adsorption of mixed solutions of A and B. The different markers correspond to different coverage series. The response to nonspecific polyclonal antibody α-hSA is also plotted (▲).
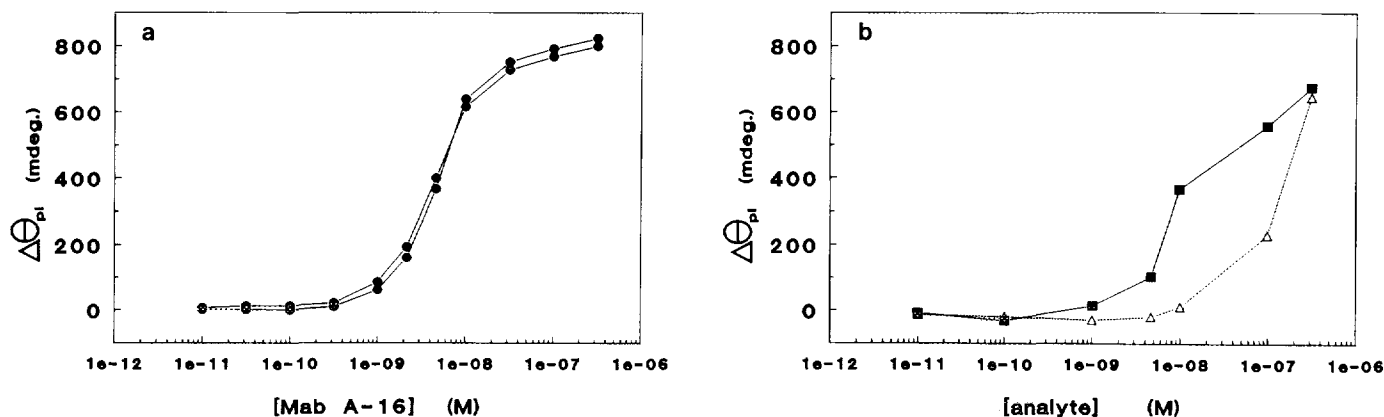
FIG. 8. (a) Binding isotherm of Mab A-16 to a SAR adsorbed from a mixed solution A/B with bulk concentration ratio A:B = 1:25. The lines are intended to guide the eye. (b) Binding isotherms of Mab A-16 (■) and nonspecific polyclonal antibody α-hSA (△) to a SAR adsorbed from a mixed solution C/D with a bulk concentration ratio C:D = 1:25.

centration of polyclonal α-hSA (cf. Fig. 7, ▲). Note the excellent reproducibility of the binding curves. From the value of the angle response at half its saturation value an affinity constant $K_{aff} = 2 \times 10^8$ M^{-1} can be estimated. It appears that the *intrinsic* affinity constant derived from Fig. 4 is equal to the value found in the present experiments, indicating that the incorporation of the 9–21 gD peptide in the SAR did not affect the affinity of the peptide toward Mab A-16.

In Fig. 8b the binding isotherms of both Mab A-16 (■) and α-hSA (△) to the C/D-SAR are shown. Apparently, in this case the (nonspecific) interaction of α-hSA cannot be neglected: the C/D-SAR seems to exhibit a higher nonspecific affinity than the A/B-SAR. The response to α-hSA at high concentrations is eventually as strong as that to the Mab A-16, indicating that these responses could originate from interactions of similar, thus probably nonspecific, moieties of the antibody with the SAR. With this assumption, the Mab A-16 response can be interpreted as made up from the sum of at least two binding isotherms; the first part, the specific part, has a maximum response of approximately 450 mdeg θ_{pl} shift with, not unexpectedly, a $K_{aff} \sim 3 \times 10^8$ M^{-1}, whereas the second part has a response similar to the α-hSA response, with a nonspecific $K_{aff} \sim 5 \times 10^6$ M^{-1}. Considering the maximum specific response ($\Delta\theta_{pl} \sim 450$ mdeg), the coverage of reactive affinity peptides in the C/D-SAR is less than that of the A/B-SAR although the same bulk concentration ratio has been used. Whether the overall ratio of peptide–compound adsorption in a C/D-SAR is less than the A/B-SAR or whether some of the peptides in the C/D-SAR are inactivated after monolayer formation remains undetermined.

CONCLUSIONS

From the results presented, it is obvious that small, synthetic peptides can be used as a receptor molecule to specifically detect a much larger protein. SARs provide the user of synthetic peptides as receptors with a relatively simple tool to immobilize those peptides to a gold surface with an easily adjustable coverage. With gold as a sensor surface the technique is extremely suitable for use in an SPR setup or any other transducer principle using a gold surface.

SARs can be prepared by either adsorption from mixed solutions or sequential adsorption of the components of the SAR. In both cases similar affinity response patterns were found. However, a lack of reproducibility in the lower concentration ratios of the SARs adsorbed from mixed solutions was noted and is not understood. It was shown, however, that a SAR exhibiting a maximum response can be obtained from a relatively large concentration ratio span when the SAR is adsorbed from a mixed solution. Quantitation of the coverage of components in a SAR and a SAM in general, passively adsorbed from a mixed solution of adsorbates, is in our opinion paramount to understanding the physical and chemical behavior of the formed monolayer. We have demonstrated that SPR measurements can be useful in monitoring the formation of the different monolayers.

The maximum response found in the affinity reactions, at approximately 3 mol% peptide coverage, suggests a highly oriented layer formation of the antibody compared to, for instance, passive adsorption of the antibody from a similar solution. This can be the case only when the receptor layer also is macroscopically ordered. This large response results in a sensitivity enhancement of nearly two orders of magnitude, compared with randomly adsorbed receptor layers.

Our experiments show that, when a SAR with a hydrophobic moiety is being used, the binding equilibrium properties of the immobilized peptide molecule are identical to those for the case where the immobilized antibody interacts with the receptor molecule. Appar-

ently the binding capability of the receptor molecule is not affected by its immobilization in a SAR. The SARs without a hydrophobic moiety exhibit a relatively large nonspecific interaction, indicating that nonspecific parts of the constituent thiol compounds are accessible to the antibody. This indicates that a SAR without a hydrophobic moiety is less ordered than one with a hydrophobic moiety.

The application of synthetic parts of antibodies as a receptor incorporated in a SAR is currently being investigated.

ACKNOWLEDGMENTS

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