Anal. Chem. **1997,** *69,* 4808−4813

Antibody Binding to a Functionalized Supported Lipid Layer: A Direct Acoustic Immunosensor

Electra Gizeli,*,† Martha Liley,‡ Christopher R. Lowe,† and Horst Vogel‡

Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QT, U.K., and Laboratoire de Chimie Physique des Polymères et Membranes, Swiss Federal Institute of Technology, Lausanne, CH-1015, Switzerland

A direct immunosensor has been developed using an acoustic wave device as a transducer. The device is based on an acoustic waveguide geometry that supports a Love wave. The biorecognition surface, formed on a gold layer, consisted of a biotinylated supported lipid layer which specifically bound streptavidin and, subsequently, biotinylated goat IgG. The modified surface was used as a model immunosensor and successfully detected rabbit anti-goat IgG in the concentration range $3 \times 10^{-8}-10^{-6}$ M. Using the anti-goat IgG binding isotherm and the time-resolved measurements of antibody binding, both the binding and rate constants of the reaction were determined. The specificity of each binding step was studied with the acoustic wave device, and it was concluded that the phospholipid bilayer showed a good suppression of nonspecific binding. Comparative measurements using surface plasmon resonance allowed the response of the immunosensor to be quantitatively correlated with mass binding to the surface.

Immunosensors have been used extensively during the last few years in a number of applications as an alternative immunoassay technique.[1,2] The detection principle of this new generation of analytical devices is based on the continuous monitoring of one or more physical parameters at a solid/liquid interface during the interaction of a biomolecule either directly with the surface or with another biomolecule immobilized at the surface. Of the immunosensors available, the most promising systems are those that can detect the immunological reaction directly, without using an additional label but still with high sensitivity. Many successful immunosensors are based on the monitoring of optical properties of the interface, specifically, the refractive index change during the process of the binding reaction. Surface plasmon resonance (SPR) and the resonant mirror (RM), the most established optical immunosensors, are both based on the measurement of refractive index change.[3,4] Another class of immunosensors uses acoustic techniques that monitor the acoustic parameters of a solid/liquid interface during immunological reactions.[5,6] Interest in the development of acoustic immunosensors has recently rekindled, mainly because of considerable advances in the fabrication of acoustic devices.

For the production of in situ acoustic immunosensors, capable of operating in liquids with a high sensitivity, two factors are paramount: First, high operating frequencies, up to the megahertz range, are important for obtaining a high sensitivity to surface processes, ideally, combined with confinement of the wave very close to the sensing surface. Second, the use of a pure shear acoustic wave is essential if the device is to be operated in a liquid environment. Of the few shear acoustic wave systems available, the Love wave device has been shown both theoretically and experimentally to be the most promising system for biosensing applications.[7−10]

The Love wave device is a bilayer geometry in which the shear wave propagates in the upper layer of a low shear acoustic velocity material deposited on a substrate with a higher shear acoustic velocity. The major advantage of the Love wave device is that it offers enormous design flexibility, since, at a certain frequency, energy confinement is purely determined by the acoustic properties and thickness of the deposited overlayer and is independent of the design characteristics of the piezoelectric substrate. The most surface sensitive waveguide structure was shown to be that incorporating an inelastic polymer layer on top of the quartz substrate.[11,12] Because of the shear nature of the wave, the device can operate in the presence of liquid without any losses occurring due to mode conversion. The evanescent field of the shear acoustic wave probes mass, viscosity, and electric changes occurring within ∼60 nm from the device surface in the liquid sample.[13] Thus, by monitoring the propagation characteristics of the acoustic wave, i.e., the amplitude and phase or frequency, in real time it is possible to detect the kinetics of the binding of an antigen to the surface-bound antibody and have the acoustic equivalent of an optical immunosensor. During recent years, a number of papers have been published that demonstrate the successful application of shear acoustic waves for the detection of immunological reactions;[7,14−17] however, their full potential as

† University of Cambridge.

‡ Swiss Federal Institute of Technology.

(1) North, J. R. *Trends Biotechnol.* **1985**, *3* (7), 180−185.
(2) Aizawa, M. *Adv. Clin. Chem.* **1994**, *31*, 247−275.
(3) Stenberg, E.; Persson, B.; Roos, H.; Urbaniczky, C. *J. Colloid Interface Sci.* **1991**, *143*, 513−526.
(4) Buckle, P. E.; Davies, R. J.; Kinning, T.; Yeung, D.; Edwards, P. R.; Pollard-Knight, D.; Lowe, C. R. *Biosens., Bioelectron.* **1993**, *8*, 355−363.
(5) Suleiman, A. A.; Guilbault, G. G.; *Analyst* **1994**, *119*, 279−2282.
(6) Andle, J. C.; Vetelino, J. F. *Sens. Actuators A* **1994**, *44*, 167−176.
(7) Gizeli, E.; Goddard, N. J.; Stevenson, A. C.; Lowe, C. R. *Sens. Actuators B* **1992**, *6*, 131−137.
(8) Kovacs, G.; Venema, A. *Appl. Phys. Lett.* **1992**, *61*, 639−641.
(9) Gizeli, E.; Goddard, N. J.; Stevenson, A. C.; Lowe, C. R. *Sens. Actuators B* **1993**, *13−14*, 638−639.
(10) Du, J.; Harding, G. L.; Ogilvy, J. A.; Dencher, P. R.; Lake, M. *Sens. Actuators A* **1996**, *56*, 211−219.
(11) Gizeli, E. Ph.D Thesis, University of Cambridge, 1992.
(12) Gizeli, E. *Smart Mater. Struct.* **1997**, *6*, 3−7.
(13) Kondoh, J.; Saito, K.; Shiokawa, S.; Suzuki, H. *Jpn. J. Appl. Phys.* **1996**, *35*, 3093−3096.
(14) Dahint, R.; Grunze, M.; Josse, F.; Renken, J. *Anal. Chem.* **1994**, *18*, 2888−2892.

S0003-2700(97)00519-2 CCC: $14.00

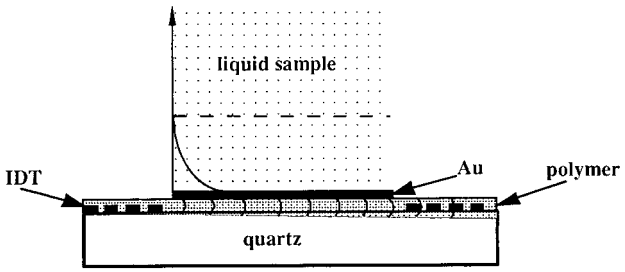

Figure 1. Schematic representation of the acoustic waveguide sensor. A polymer layer (~1 μm) is deposited on the surface of an acoustic device which supports shear horizontal waves. A gold layer (50 nm) is deposited on top of the polymer between the interdigital transducers (IDTs). Loading the gold layer with a liquid sample results in an acoustic evanescent field which extends 60 nm into the aqueous solution. For this acoustic geometry, the wave is guided in the polymer overlayer and that part of the substrate close to the IDTs.

direct immunosensors has not yet been realized. In this application, the waveguide system used consisted of a quartz shear surface acoustic wave device, operating at 110 MHz, coated with a cross-linked polymer layer ~1 μm thick and a thin gold layer of 50 nm deposited on the polymer surface in the area between the interdigitated transducers (IDTs). The phase of the acoustic wave was monitored during binding of proteins or lipids to the device surface. The device geometry is shown in Figure 1.

We built up a model immunosensor for the detection of anti-goat IgG, based on a supported lipid layer and on the subsequent addition of protein monolayers by molecular recognition. This system was chosen for several reasons: (i) the use of supported lipid layers opens up new possibilities for sensor applications since phospholipids with phosphatidylcholine head groups are known to form excellent biocompatible surfaces which suppress nonspecific binding,[18,19] (ii) the lipid layers can be removed easily using a detergent solution; thus, it is possible to regenerate the device surface, and (iii) supported lipid layers provide a membranelike environment suitable for the incorporation of, for example, membrane proteins and receptors.[20] The final gold layer on the Love wave device allowed us to exploit gold surface chemistry for surface modification. A self-assembled monolayer of mercaptoundecanol [$HS(CH_2)_{11}OH$] was formed on the gold layer to give an extremely hydrophilic surface. Exposure of this hydrophilic surface to an aqueous dispersion of phospholipid vesicles resulted in the spontaneous formation of a lipid bilayer. Supported lipid bilayers containing 3 mol % biotinylated phospholipids specifically bound the protein streptavidin to the lipid layer. Subsequent exposure of the layer to biotinylated goat IgG resulted in binding of the IgG to the streptavidin. This surface was then used for the detection of anti-goat IgG. A schematic representation of the surface modification and anti-goat IgG binding is shown in Figure 2.

In this work we demonstrate the use of the acoustic waveguide device as an immunosensor for measuring surface-bound immunological interactions both sensitively and reliably. The Love wave device is used to monitor the various steps involved in the surface modification of the interface for developing an immunosensor and to study nonspecific binding events. Its suitability for detecting the binding of the antibody of interest to the modified surface, producing a binding isotherm, and studying the kinetics of the binding reaction is assessed. Finally, comparisons of the response of the Love wave device with the mass density of the different biological layers, as detected by surface plasmon resonance, allow the acoustic response to be correlated with the mass density at the sensor surface.

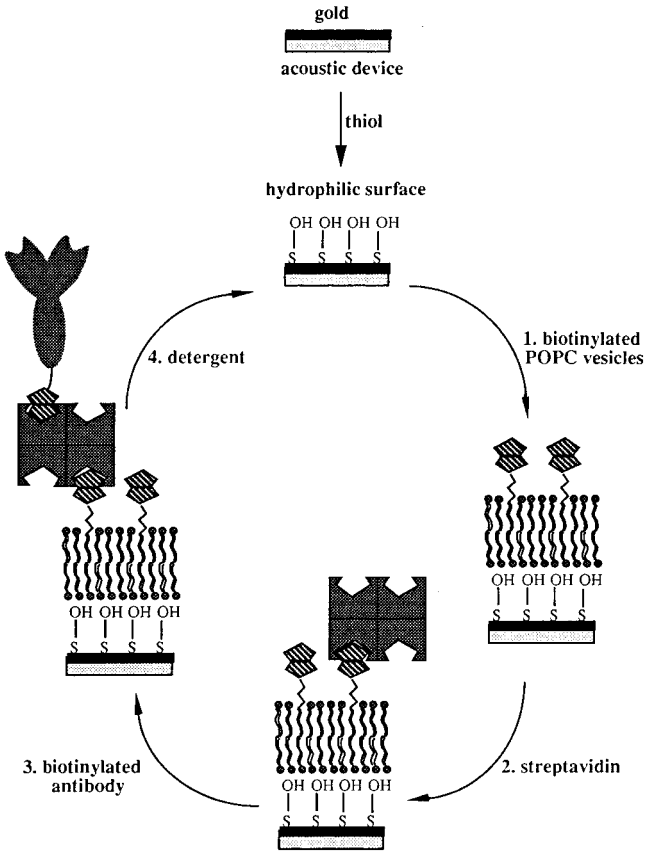

Figure 2. Schematic representation of the modification of the surface. The gold-coated device surface is rendered hydrophilic by self-assembly of a thiol monolayer. (1) Addition of biotinylated vesicles of POPC results in the spontaneous formation of a lipid bilayer on the hydrophilic surface, (2) streptavidin is attached to the surface through binding to biotin molecules, (3) a biotinylated antibody is coupled to streptavidin via one of the remaining streptavidin binding sites, and (4) the surface is regenerated on addition of detergent.

EXPERIMENTAL SECTION

(i) Device and Instrumentation. Single-crystal Y-cut (42.5°), *z*-propagating, 0.5 mm thick quartz was used as a piezoelectric substrate. The input and output interdigital transducers comprised a 10 nm chromium flash followed by a 200 nm gold overlayer. The IDTs consisted of 80 pairs with a periodicity of 45 μm. Devices were patterned by conventional photolithographic techniques in the Southampton Microelectronics Centre. The uncoated device supports a surface-skimming bulk wave (SSBW), which is a shear horizontal wave largely dispersed in the bulk of the crystal as it propagates from the input to the output IDT.[21] A Hewlett-Packard 4195A network analyzer was used to monitor the phase and frequency of the wave. The temperature was held

(15) Moy, M. T.; Baer, R. L.; Spira-Solomon, D.; Doherty, T. P. *Anal. Chem.* **1995**, *67*, 1510−1516.
(16) Gizeli, E.; Lowe, C. R.; Liley, M.; Vogel, H. *Sens. Actuators B* **1996**, *34*, 295−300.
(17) Welsch, W.; Klein, C.; von Schickfus, M.; Hunklinger, S. *Anal. Chem.* **1996**, *68*, 2000−2004.
(18) D. Chapman, *Langmuir* **1993**, *9*, 39−45.
(19) Terretaz, S.; Stora, T.; Duschl, C.; Vogel, H. *Langmuir* **1993**, *9*, 1361−1369.
(20) E. Sackmann, *Science* **1996**, *271*, 43−48.

constant at 22.0 ± 0.1 °C by a Peltier plate. A Perspex flow cell, a peristaltic pump, and poly(vinyl chloride) (PVC) tubing were used to pump through liquid samples at a flow rate of 1 μL/s. A rubber sleeve exposing an area of 23 mm^2 was used to seal the cell to the acoustic device.

(ii) Polymer Coating−Gold Deposition. The polymer Novolac, provided by Plessey GEC Marconi, was diluted 50% (w/w) in 2-ethoxyethyl acetate (Sigma). A Novolac layer was applied over the entire surface of the device by spin-coating with the polymer solution at 4000 rpm for 40 s. The film was further cross-linked by heating the device in a conventional oven for 2 h at 180 °C. After the polymer layer was prepared, the acoustic waveguide device was placed in the vacuum evaporator chamber (Edwards) where a 50 nm gold layer was deposited on top of the polymer in the area between the IDTs by thermal evaporation at a pressure of 5×10^{-6} mbar.

(iii) Self-Assembly of Thiols on Gold. The first step in the biochemical modification of the waveguide was the self-assembly of a thiol monolayer on the gold surface. The quality of the self-assembled layer was found to be critical for the subsequent deposition of supported lipid layers. Therefore, particular attention was paid to the reproducibility of this step; only high-purity solvents, very clean glassware, and freshly prepared thiol solutions were used. On removal from the vacuum chamber of the coating unit, the acoustic device was immediately incubated in a 10^{-4} M solution of $HS(CH_2)_{11}OH$, synthesized as described in ref 22, in cyclohexane for 2 h at room temperature. Cyclohexane was used since it appeared to have minimal effects on the chemical stability of the polymer layer.

(iv) Vesicle Adsorption. Vesicle dispersions were prepared by adding 50 μL of water to 1 mg of lipid and sonicating the resultant mixture in a bath sonifier to produce a clear solution. Either pure palmitoyloleoylphosphatidylcholine (POPC, purchased from Fluka) or a mixture of 1 mg of POPC and 0.03 mg of *N*-(biotinoyl)dipalmitoyl-L-α-phosphatidylethanolamine (Biotin-DPPE, from Pierce) was used. The vesicle dispersion was diluted with water to 0.5 mg/mL and was applied to the device surface using a flow-through cell. Lipid layers were removed from the sensor surface by rinsing in a 40 mM solution of octyl β-D-glucopyranoside (purchased from Aldrich).

(v) Protein Binding. Streptavidin (Pierce) was bound to the biotinylated supported bilayer from a 5×10^{-6} M solution of streptavidin in water (0.3 mg/mL). After adsorption of the streptavidin, the surface was washed with water followed by 0.1 M phosphate buffer, pH 6. Biotinylated goat IgG (purchased from Pierce) was applied in the same buffer at a concentration of 10^{-6} M (150 mg/mL), and the surface was washed with buffer. Finally, affinity-purified polyclonal anti-goat IgG (purchased from Sigma) was incubated with the resulting multilayer system in 0.1 M phosphate buffer, pH 6.5. The Love wave devices used to measure the IgG binding isotherm were prepared simultaneously to ensure maximum reproducibility of the measurements. All the devices were placed on a Teflon holder and each was sealed with a Teflon static cell with a geometry very similar to that of the flow cell used on the acoustic setup. An area between the IDTs slightly larger than that of the flow-through cell was exposed to each solution. The thiol SAM, lipid bilayer, streptavidin, and biotinylated IgG layers were built up on the device surfaces as described above. All steps were performed at room temperature and each incubation step was 2 h long. In between adsorption steps, the devices were carefully rinsed with ultrapure water or buffer when protein solutions were applied. After the preparation of the multilayer, each device was placed in the acoustic setup and connected to the flow cell; the binding of the anti-goat IgG at different concentrations was detected acoustically.

(vi) SPR Measurements. The surface plasmon technique used for comparison with acoustic measurements has been described elsewhere.[23] We used the Kretschmann configuration with a 50 nm gold film evaporated onto glass slides which were optically matched to the base of a 60° SF10 prism ($n = 1.725$) via an index-matching oil. This allowed the excitation of plasmon surface polaritons at the gold/dielectric interface upon total internal reflection of a p-polarized laser beam (HeNe, $\lambda = 633$ nm) at the prism base. The intensity of the reflected light was measured as a function of the angle of incidence, θ. The reflected intensity shows a sharp minimum in intensity at the resonance angle θ_0 which depends upon the precise architecture of the metal/dielectric interface. From the Fresnel fit to the resonance curve for a bare gold surface, it is possible to obtain the dielectric function and thickness of the gold layer. Addition of a thin organic layer to the gold surface results in a shift in the angle of resonance which depends on the optical thickness Δnd of the organic layer. The mass coverage of the surface can be deduced if the relation between mass and refractive index (the refractive index increment) of the organic monolayer is known. The refractive index increment for a variety of proteins has been shown to be constant up to concentrations as high as 0.4 g/mL. We used the value 0.184 mL/g for the refractive index increment of both streptavidin and antibody.[24]

RESULTS AND DISCUSSION

Bilayer Formation. The gold surface of the acoustic devices was first modified by the self-assembly of a monolayer of mercaptoundecanol to produce an extremely hydrophilic surface. The exposure of this surface to phospholipid vesicle solutions resulted in spontaneous fusion of vesicles to the surface to give a lipid bilayer.[25] The formation of the bilayer was observed acoustically by monitoring the phase of the wave. Figure 3 shows the phase change observed on addition of a 0.5 mg/mL POPC vesicle aqueous dispersion to the hydrophilic surface of the device for three different experiments. The bilayer formation was found to be independent of the vesicle concentration for concentrations up to 1 mg/mL. The formation of the phospholipid layer was normally completed within 5 min of the vesicle addition. The overshoot behavior observed in some cases can be explained by a small temperature difference between the POPC vesicle dispersion and the device surface. The mean phase change of 17 measurements was 2.35 ± 0.14 deg per POPC bilayer deposition. The scattering of the results can be attributed to problems in reproducing the sensing surface area and should not be confused with the phase noise of the system, which is below ±0.025 deg. The lipid bilayer could be removed on addition of a detergent solution, and the regenerated hydrophilic thiol surface could be used for depositing a new lipid bilayer (data not shown).[16]

(21) Gizeli, E.; Stevenson, A. C.; Goddard, N. J.; Lowe, C. R. *IEEE Trans. Ultrason. Ferroel. Freq. Contr.* **1992**, *9*, 657−659.
(22) Bain, C. D.; Troughton, E. B.; Tao, Y. T.; Ewall, J.; Whitesides, G.; Nuzzo, R. G. *J. Am. Chem. Soc.*, **1989**, *111* 321−335.
(23) Spinke, J.; Liley, M.; Schmitt, F.-J.; Guder, H-J.; Angermaier, L.; Knoll, W. *J. Chem. Phys.* **1993**, *99*, 7021−7019.
(24) Feijter, J. A.; Benjamins, J.; Veer, F. A. *Biopolymers* **1978**, *17*, 1759−1772.
(25) Duschl, C.; Liley, M.; Corradin, G.; Vogel, H. *Bioph. J.* **1994**, 67, 1−9.

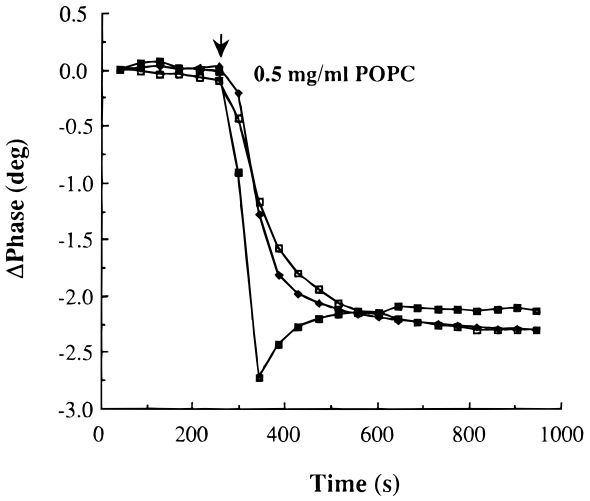

Figure 3. Acoustic detection (ΔPhase) of the formation of a phospholipid bilayer on addition of a 0.5 mg/mL POPC vesicle dispersion to the hydrophilic gold surface for three different experiments. The baseline corresponds to the phase response when surface is in contact with Nanopure water.

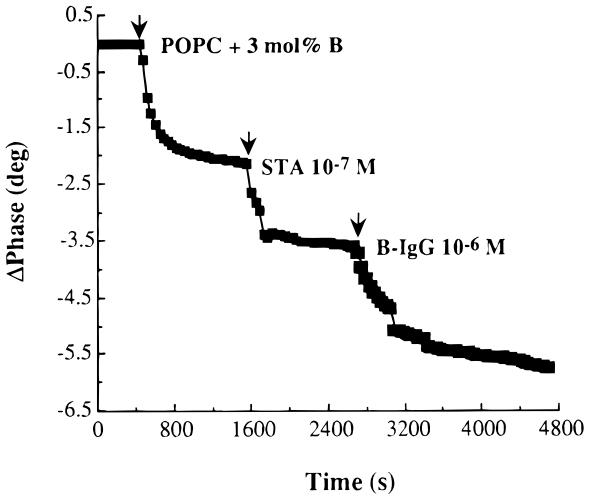

Figure 4. Detection of the modification of the acoustic waveguide sensor surface by monitoring the phase of the wave in real time. The first phase drop corresponds to the formation of a biotinylated lipid bilayer on addition of an aqueous vesicle dispersion (0.5 mg/mL) of POPC with 3 mol % Biotin-DPPE. Addition of 10^{-7} M streptavidin (STA) resulted in a further phase drop due to its binding to the biotinylated lipids. Further addition of a 10^{-6} M solution of biotinylated IgG to the surface-immobilized streptavidin resulted in binding of the IgG.

SPR measurements were used to evaluate the thickness of the lipid layer formed on POPC vesicle adsorption under similar conditions. The shift in the angle of resonance observed (0.7°) corresponded to the formation of a lipid bilayer at the surface.[25] The mass density of the lipid bilayer was calculated using the average area of a POPC molecule in a lipid bilayer[26] and was found to be 4.2 ng/mm^2.

Formation of Multilayers on the POPC Membrane. The formation of a protein multilayer on the hydrophilic gold surface is shown in Figure 4. Formation of the biotinylated lipid bilayer gave a signal change which was indistinguishable from that observed for the pure POPC bilayer. This is to be expected since biotin is a relatively small molecule (M_w = 244.31) and should not have any significant effect on the mass of the bilayer or its organization at the hydrophilic surface. Addition of a solution of streptavidin (10^{-7} M) to the cell resulted in a further phase change of 1.42 ± 0.10 deg caused by the binding of the streptavidin to the biotin groups exposed at the lipid bilayer. The presence of four biotin sites in the streptavidin molecule, and their arrangement on opposite faces of the protein, meant that, after the binding of the streptavidin to the lipid layer, each streptavidin molecule still had two free biotin binding sites, facing away from the sensor surface. Finally, incubation of the device with a solution of biotinylated goat IgG gave a phase change of 2.45 ± 0.25 deg on binding of the IgG to the free biotin binding sites of the streptavidin.

(26) Nollert, P.; Kiefer, H.; Jahning, F. *Biophys. J.* **1995**, *69*, 1447−1455.

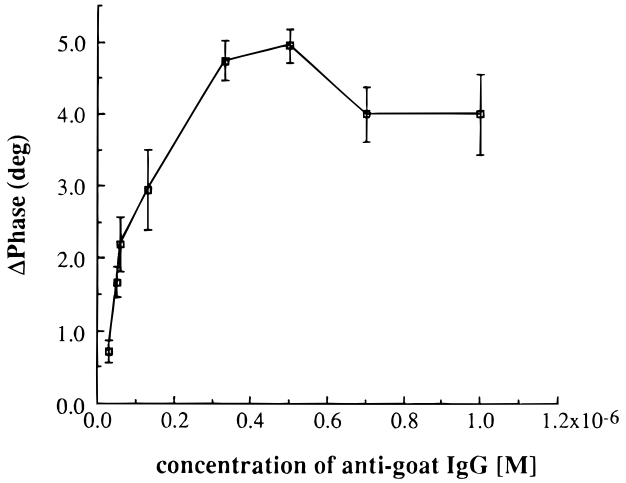

Figure 5. Phase detection of the binding isotherm of anti-goat IgG. IgG was detected within the concentration range of 3 × 10^{-8}−10^{-6} M in a phosphate buffer solution, pH 6.5. All points correspond to the equilibrium state of the binding reaction.

SPR measurements were also performed in order to measure the mass density of the two protein layers. The angle shifts of 0.47 and 0.77° observed on adsorption of streptavidin and biotinylated IgG, respectively, correspond to mass loadings of 1.9 and 3.1 ng/mm^2, assuming a refractive index increment of 0.184 mL/g.

IgG Binding Isotherm. The binding of the final anti-goat IgG to the protein multilayer system was investigated as a function of solution concentration of the anti-goat IgG. Figure 5 shows the binding isotherm of the anti-goat IgG, with phase change plotted against solution concentration for concentrations 3 × 10^{-8}−10^{-6} M. From the isotherm, it is clear that the detection limit for anti-goat-IgG is below 3 × 10^{-8} M. An apparent binding constant of the anti-goat IgG/biotinylated IgG pair can also be estimated from the binding isotherm: the most simple estimate is the inverse of the concentration at which half-maximal binding occurs. This is ~1.5 × 10^7 M^{-1}. More sophisticated methods for determining the binding constant from a binding isotherm involve fitting to a model of the binding process, the most commonly used being the Langmuir model. The Langmuir model makes a variety of assumptions about the binding process which are almost certainly not fulfilled in our case (e.g., no interaction between proteins on the surface, interaction via only one binding site), but it does have the advantages of being simple and allowing easy comparison with literature results. A Langmuir fit to the data in Figure 5 gives a very similar value for the binding constant, K = 1.1 × 10^7 M^{-1}.

Specificity of the Binding. A number of control experiments were carried out to ensure the specificity of protein binding. The

Table 1. Specificity of the Binding of the Different Layers to the Device Surface

binding of	to	Δphase (deg)
10^{-6} M anti-goat IgG	POPC	0.0
complexed streptavidin	biotinylated POPC	0.0
10^{-6} M anti-goat IgG	streptavidin	0.0
10^{-6} M anti-goat IgG	biotinylated POPC	0.2

results are summarized in Table 1. No phase change was observed on incubation of a pure POPC layer with protein (anti-goat IgG), demonstrating that POPC is a very good substrate for suppressing nonspecific binding. The specificity of streptavidin binding to the lipid layer was tested using streptavidin that had been incubated with free biotin. This blocked the biotin binding sites of the streptavidin, and subsequent incubation of the biotinylated lipid layer with the streptavidin gave no phase change. The specificity of the biotinylated anti-goat IgG binding to the surface through biotin was tested by incubating the streptavidin-modified surfaces with 10^{-6} M (nonbiotinylated) anti-goat IgG, which resulted in no phase change. Finally, a 0.2 deg phase change was detected when 10^{-6} M anti-goat IgG was added to the biotinylated phospholipid surface, indicating nonspecific binding of anti-goat IgG to biotin. Since the goat IgG is randomly biotinylated, with an average of eight biotin groups per antibody, it is possible that a fraction of the anti-goat IgG binding to the surface binds nonspecifically to biotin groups exposed on the goat-IgG. Optimization of the immunosensor would require site-directed biotinylation of the goat-IgG.

Kinetics of IgG Binding. The association kinetics of the binding of anti-goat IgG to the lipid/protein multilayer system were analyzed to obtain information about both the on and off rates of the anti-goat IgG/biotinylated IgG binding and about the binding constant. For binding processes where diffusion is not rate-determining, the binding kinetics can be described by the rate equation:[27,28]

$$\mathrm{d}R/\mathrm{d}t = k_{\mathrm{on}}C_{\mathrm{IgG}}(R_{\mathrm{max}} - R) - k_{\mathrm{off}}R$$

where R is the response of the detector, proportional to the amount of adsorbed IgG, C_{IgG} is the concentration of IgG in solution, k_{on} is the association rate constant, and k_{off} is the dissociation rate constant. Plotting $(\mathrm{d}R/\mathrm{d}t)$ against R gives a straight line with a gradient of

$$\Delta(\mathrm{d}R/\mathrm{d}t)/\Delta R = k_{\mathrm{on}}C_{\mathrm{IgG}} + k_{\mathrm{off}}$$

Plotting this gradient vs IgG concentration for the individual measurements at different concentrations, we should thus obtain a straight line with a gradient of k_{on} and an intercept of k_{off}.

In order to apply this analysis to our data, we first fitted the IgG binding at each concentration to a single-exponential curve as shown in Figure 6. This fit was then used in the subsequent kinetic analysis. The plot of $\Delta(\mathrm{d}R/\mathrm{d}t)/\Delta R$ against C_{IgG} is shown in Figure 7. A straight line fit to the data gives $k_{\mathrm{off}} = 6.0 \times 10^{-4}$ s^{-1} and $k_{\mathrm{on}} = 6.2 \times 10^{3}$ M^{-1} s^{-1}. We thus obtain the apparent

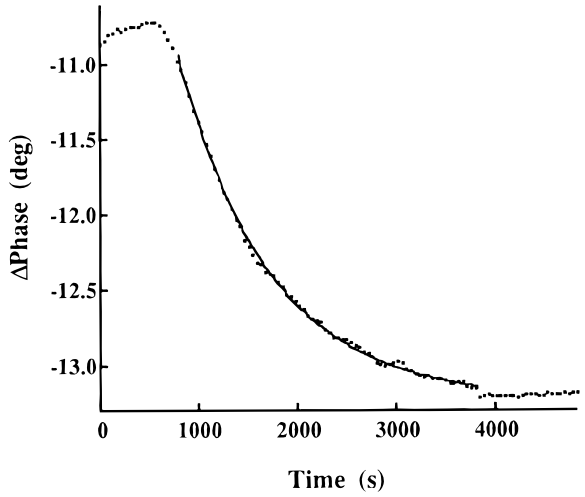

Figure 6. Phase detection of the binding of 0.6×10^{-7} M anti-goat IgG. The points corresponds to experimental data. The solid line is a single-exponential fit to the data.

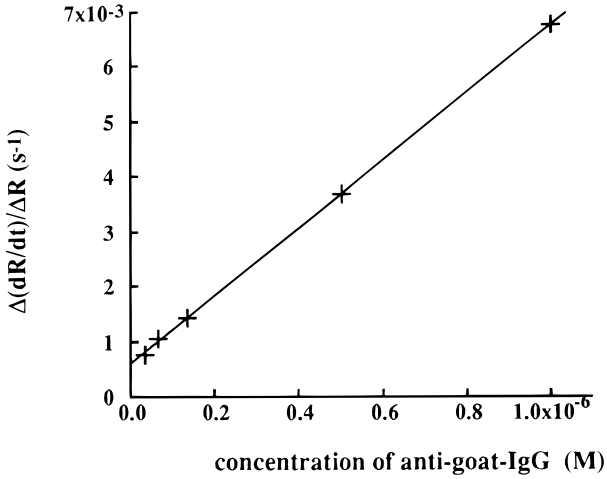

Figure 7. Plot of $\Delta(\mathrm{d}R/\mathrm{d}t)/\Delta R$ against the anti-goat IgG concentration, where R is acoustic phase. The slope of the line is 6.2×10^3 M^{-1} s^{-1} and corresponds to the association rate constant (k_{on}) of the binding of the anti-goat IgG to the surface. The dissociation rate constant of the reaction (k_{off}) is given by the intercept of the line with the y axis and is 6.0×10^{-4} s^{-1}.

binding constant, $K = 1.0 \times 10^7$ M^{-1}, for the anti-goat IgG/biotinylated IgG binding. This value is in very good agreement with the value of $K = 1.5 \times 10^7$ M^{-1} estimated from the binding isotherm. The excellent fit of the experimental data with the simple theory described may be surprising, since the anti-goat IgG used was an affinity-purified polyclonal antibody and should, therefore, consist of a number of antibody populations, each with its own k_{on}, k_{off} and binding constant. We conclude that one population of the anti-goat IgG must dominate and that only the binding properties of this single population are observed in our studies. In addition, we note that all calculations are based on the assumption that phase change is proportional to the mass of bound anti-goat IgG.

Comparison of SPR and Acoustic Measurements. For the formation of the lipid layer, the binding of streptavidin to the layer, and the binding of the biotinylated antibody to the streptavidin, both acoustic and surface plasmon resonance measurements were made. The results are summarized in Table 2, where the sensitivity of the device is expressed as the specific acoustic response (i.e., the acoustic response divided by the mass density

(27) Karlsson, R.; Michaelsson, A.; Mattsson, L. *J. Immunol. Methods* **1991**, *145*, 229−240.
(28) Duschl, C.; Sévin-Landais, A-F.; Vogel, H. *Bioph. J.* **1996**, *70*, 1985.

Table 2. Calibration of Phase Change to Surface Mass Density, Measured Using SPR

sample	Δphase (deg)	surface density (ng/mm^2)	specific phase change (deg mm^2/ng)
POPC	2.35 ± 0.14	4.2 ± 0.4	0.56 ± 0.06
streptavidin	1.42 ± 0.10	1.9 ± 0.2	0.75 ± 0.09
Goat-IgG	2.45 ± 0.25	3.1 ± 0.3	0.79 ± 0.11

at the surface). From this limited data set we conclude that mass loading appears to be the dominant parameter in the determination of the acoustic response for biological layers but other parameters such as viscosity also seem to influence the response. For the two protein layers, where one might expect similar viscosities and electrical properties, the two values for the specific acoustic response are the same. This suggests that it may be appropriate to model the acoustic response of this Love wave immunosensor as being entirely due to mass density for protein layers, using a mean specific acoustic response of 0.76 deg mm^2/ng. Further improvement of the sensitivity of the acoustic immunosensor can be achieved by applying waveguide layers with higher coupling efficiency of the Love wave.

CONCLUSIONS

An acoustic Love wave device with a biorecognition surface based on a phospholipid/protein multilayer was successfully used for detecting anti-goat IgG in solution in the concentration range 3×10^{-8}–10^{-6} M. The biochemical modification of the surface was carried out using simple chemistry under mild conditions. The phospholipid bilayer was coupled, but not covalently bound, to the surface, allowing regeneration of the sensor on addition of detergent. Further protein layers were built up by molecular recognition. The acoustic sensor was used for the detection of all binding steps in the buildup of the biorecognition surface and for studying the specificity of the binding. It was found that the phospholipid layer provided an excellent matrix for protein attachement and for the suppression of nonspecific binding. This is in agreement with previous studies.

The binding isotherm of the anti-goat IgG was used to calculate an apparent binding constant of 10^7 M^{-1}. The real-time binding curves for different concentrations of anti-goat IgG were used to derive the rate and equilibrium constants of the binding. The association and dissociation rate constants were calculated to be 6.2×10^3 M^{-1} s^{-1} and 6.0×10^{-4} s^{-1}, respectively, and the apparent binding constant was 1.5×10^7 M^{-1}. From the excellent agreement of kinetic and steady-state measurements, we deduce that the polyclonal anti-goat IgG must be dominated by a single antibody population.

Surface plasmon resonance was used to measure the surface mass loading of the different lipid and protein layers. Comparison of the mass loading with the acoustic-phase response allowed a specific phase response to be calculated for each layer as a function of the surface mass density. The similarity of the values obtained for the different layers indicates that the mass density of the deposited layer is the dominant but not the sole parameter in the acoustic response. In the case of protein layers, the Love wave immunosensor's response can be entirely related to the mass density of the deposited layer with a phase sensitivity of 0.76 deg/ng mm^{-2}.

ACKNOWLEDGMENT

E.G. acknowledges Biotechnology and Biological Sciences Research Council (BBSRC), and M.L. and H.V. acknowledge the Swiss National Science Foundation (SPP Biotechnology, 5002-235180) for supporting this work. The authors thank Dr. C. Duschl for many helpful and stimulating discussions.

Received for review May 20, 1997. Accepted July 10, 1997.[⊗]

AC970519M

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1997.