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An Approach for Analysis of Protein Toxins Based on Thin Films of Lipid Mixtures in an Optical Biosensor

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Biological membrane-like lipid films were deposited on the sensing surface in an optical biosensor instrument. The membranes were mixtures of biologically occurring lipids. Eight surfaces were prepared, some of which contained various glycolipids as minor components. One was supplemented with membrane proteins. The binding of six protein toxins (cholera toxin, cholera toxin B subunit, diphtheria toxin, ricin, ricin B subunit, staphylococcal enterotoxin B) and of bovine serum albumin at pH 7.4 and pH 5.2 to each of the sensor surfaces was studied. Each of the seven proteins gave a distinct binding pattern. The assay is rapid and simple, with no need for reagents. The lipid sensor surface is readily regenerated after binding and very stable. The concept with mixed lipid layers and assays at different pHs gives numerous combinations and could be applicable for developing a sensor for protein toxins.

Much of the communication in living organisms takes place at and over the cell membranes. A cell membrane consists of a lipid bilayer, composed of many lipid species, and membrane proteins. These can be peripheral or integral, spanning through the bilayer. Both proteins and lipids, not the least glycolipids, can be receptor molecules for circulating molecules. These molecules give a message to the cell binding and in some cases uptake into the cell. This general picture holds not only for endogeneous molecules but also for foreign substances, such as protein toxins. To exert a toxic action, a toxin must at some stage have interaction with the cell membrane in the exposed organism. Some toxins have a direct action on the membrane, for example, by creating pores in the lipid bilayer, and others enter the cell and have their action in the cytoplasm.[1]

The interactions between toxins and cell membranes have been studied mainly with cytological methods, using primarily different cell lines. Simpler model systems such as liposomes and black lipid membranes (BLMs) have also been used. Currently, we see a development toward a new, versatile model system, namely, membranes deposited on solid supports.[2−7] Such structures have several advantages as compared to liposomes and BLMs. They are often stable and have areas large enough to permit different analyses not possible to perform on liposomes and BLM, such as spectroscopy and microscopy. Furthermore, they can be used for biosensor applications.[8] The most widely used method to prepare supported membranes starts from liposomes. Possible mechanisms for the transfer of material from such vesicles to the solid support have been treated both theoretically and experimentally.[9−14] The issue is of practical importance, as it is difficult to obtain perfect membranes on solids. Supported membrane structures should be useful in binding and other studies aiming at a better understanding of the biological membrane as well as in several applications. The method is still in its infancy, and rather simple, well-known membrane reactions have so far been employed for testing it. Binding of the protein cholera toxin, or only its binding subunit B5, to the ganglioside GM1 incorporated in a lipid environment is the favorite model, used by ourselves[15] and other groups in different biosensor configurations.[16−18] With atomic force microscopy (AFM), it is possible to visualize this binding and the resolution is good enough to show the pentameric structure of the B-subunit of the toxin.[19] Recently, supported membranes containing different gangliosides have been used in both optical and quartz crystal microbalance biosensors to study interactions and binding kinetics for cholera,[20,21] tetanus, and pertussis toxins.[21]

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Table 1. Lipid Compositions (mol %) in Liposomes Used for Sensor Surfaces

denotion	DPPC	DPPE	DPPG	cholesterol	glycolipid	brain membrane protein
Lip A1	50	25	20	5		
Lip A2	50	25	20	5		60 μg/μmol of lipid
Lip B	35	25	20	20		
Glucer	40	25	20	5	10	
Laccer	40	25	20	5	10	
Galcer	40	25	20	5	10	
GM1	40	25	20	5	10	
GD1b	40	25	20	5	10	
	POPC					
POPC	70		10	20		

In previous papers, we have focused on developing stable membrane preparations, suitable for biosensor applications.[22−24] Membrane proteins have also been introduced into the lipid layer,[25−27] with the purpose to use them as specific sensing molecules for, for example, some toxins. We have found that membrane proteins retain their biological activity in the supported membrane with acceptable half-lives (usually more than one week).[15,25,27] Membranes with lipids only are however much more stable and furthermore easier to regenerate after exposure to protein analytes. An idea of exploiting a number of lipid surfaces for less specific binding of toxins, followed by pattern analysis, came up. Chang et al.[28] reported an odorant sensor, in which thick lipid membranes were deposited on surface acoustic wave sensors. Four different lipids, and thus four sensors, were used to find binding patterns for seven odorants, mainly alcohols. "Electronic tongues", in which the sensing elements were eight different lipids mixed with polymers to thick films deposited on a multichannel electrode, were reported by Hayashi et al.[29,30]

Mixtures of biologically occurring lipids are used in the approach presented here. This suggests that a huge number of sensor surfaces can be produced, if necessary. As pH effects on binding are also exploited, the number of combinations is almost infinite. The set of toxins used for testing the idea was chosen rather arbitrarily, but came to include both some with known interaction with specific lipid receptors and some with no specificity or unknown lipid interaction. The patterns that evolved in this first evaluation suggest that it should be possible to realize a lipid-based biosensor array for identification of many proteins. The direct measurement with no need for reagents, the ease of regeneration of sensor surfaces, and the rapid response are other attractive features of the sensing principle.

EXPERIMENTAL SECTION

Materials. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE), and cholesterol were from Avanti Polar Lipids (Alabaster, AL). Sigma (St. Louis, MO) supplied 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (DPPG), 1-palmitoyl-2-oleoyl-3-phosphocholine (POPC), and the glycolipids. These were glucocerebrosides (glucer) from human spleen, galactocerebrosides type II (galcer), the gangliosides GM1 and GD1b, all three from bovine brain, and lactocerebrosides (laccer). Bovine serum albumin and the toxins (cholera toxin, cholera toxin B subunit, diphtheria toxin, ricin, ricin B subunit, staphylococcal enterotoxin B, abbreviated SEB) also originated from Sigma. A brain membrane protein fraction was prepared from a Triton X-100 extract.[24] *n*-Octyl glycoside was from Boehringer Mannheim GmbH (Germany). Water was taken from a Milli-Q Plus 185 (Millipore, Molsheim, France) ultrapure water system with resistivity of >18 MΩcm.

The optical biosensor IAsys, as a one-well, manual system was from Affinity Sensors, Cambridge, U.K., also supplying cuvettes.

Liposomes: Compositions and Preparation. Liposomes were prepared by a detergent depletion technique, with *n*-octyl glucoside as the detergent.[24,31] Detergent was removed by extensive dialysis, using Spectropor dialysis membrane with a cutoff of 3500. The liposome preparations were generally shortly sonicated with a tip sonicator before use.

The basic composition of lipids were 50 mol % DPPC, 25 mol % DPPE, 20 mol % DPPG, and cholesterol at 5 mol % (Table 1). This composition is denoted Lip A1. All the glycolipid-containing liposomes had 10 mol % of the glycolipid and are symbolized by the acronym for the glycolipid (i.e., glucer for liposomes containing 10 mol % glucocerebroside). The preparation denoted Lip A2 had the same lipid composition as Lip A1 but contained a mixture of brain membrane proteins. The proportion of protein to lipid was 60 μg of protein/μmol of lipid, which theoretically should produce membrane structures in which proteins contribute to 5−10% of the membrane area. Lip B was a cholesterol-rich preparation.

Preparation of Supported Planar Membranes from Liposomes in the IAsys Cuvette. The resonant mirror biosensor instrument IAsys is a cuvette-based system. The measuring principle is described below (Results and Discussion). The sensing surface is at the bottom of the 0.2-mL cuvette. The cuvettes with unmodified sensor surface were cleaned prior to use with acidic

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ethanol (90% ethanol, 10% HCl) for ~1 min, followed by extensive washing with water. The deposition of lipids on the sensor surface was started by adding 20 mM Tris-HCl buffer pH 7.4, 100 mM NaCl, and 0.02% sodium azide to the cuvette at 37 °C. When a stable baseline had been established, liposomes in the same buffer were added to give a concentration corresponding to 0.5 mM lipid. Mostly, a small volume of calcium chloride to give 10 mM was added after a couple of minutes in order to speed up the transfer rate of lipids from the liposomes to the surface.[24] The process was interrupted by washing with buffer when a signal of at least 1000 arc sec had been reached. The value corresponds to ~6 ng of biomolecules/mm^2 according to the user's manual, which in turn is a rough estimate of the mass of a lipid bilayer. The surface was ready for use or for storage in the cold after a short rinse with 20 mM HCl, followed by buffer.

The resonance scan was checked frequently during all steps in order to get an indication of quality. An uneven surface gives an asymmetric resonance curve. Only preparations with a symmetrical resonance curve were used.

Preparation of Test Substances, Including Safety Considerations. Most of the test substances were toxins, and some precautions were taken in order to handle them safely. Ricin and ricin B were delivered as solutions, while cholera toxin, cholera toxin subunit B, diphtheria toxin, and SEB were in dry form including buffer salts. These were all dissolved in water to give 1 mg of protein/mL. All the toxins were then diluted to stock solutions. Protective gloves and a face shield were carried when these steps were performed, with protective gloves also in subsequent steps. A 2 M sodium hydroxide solution was used for decontamination.

Binding Procedure. The proteins were diluted to 2−40 μg/mL in 20 mM sodium phosphate buffer or sodium phosphate/citrate buffer, also containing 100 mM NaCl, at pH 7.4 or 5.2. A cuvette with deposited lipid membrane was equilibrated with buffer of the same composition as used for the toxin under study. After establishing a steady baseline, the buffer was pumped away. Test solutions with increasing concentration of toxin were added and removed sequentially, with no buffer wash between the additions. Cumulative responses were recorded. A slightly different procedure was applied for experiments at pH 4.2 in order to minimize time for exposure to the low pH. The proteins were diluted in water to 50 or 120 μg/mL, from which small aliquots were taken and added to the low pH buffer in the cuvette, without removing the solution between the increasing concentrations.

The resonance scan was checked frequently as a quality indicator of the surface. Each series was ended by washing with buffer, followed by regeneration of the lipid surface by a wash for 1−2 min with 20 mM HCl. The mean values of responses obtained from measurements at different occasions were calculated and used either as such or for calculating apparent B_{max}/K_d by nonlinear regression (concentration of protein versus response), using software in GraphPad Prism by Graph Pad Software Inc., San Diego, CA.

Calculations for principal component analysis were made by the SIMCA-P software package (Umetrics AB, Umeå, Sweden).

RESULTS AND DISCUSSION

Preparation and Properties of Lipid Sensor Surfaces. Optical evanescent biosensors for direct measurements of molecular interactions in real time have been on the market for a decade. The most common instruments, for example, Biacore, use surface plasmon resonance (SPR) for detecting binding events on a thin metal layer on a dielectric prism. The resonant mirror uses an evanescent field emerging from an optical waveguide structure. The outermost part of the sensing surface is a dielectric resonant layer with high refractive index, for example, titania. Under this, there is a low index coupling layer, for example, silica, on top of a prism. The angle of the incident beam is critical for efficient coupling to occur.[32] As molecules with a refractive index different from the bulk solution bind to the sensor surface, the critical resonant angle is changed. This shift is the signal and expressed in arc seconds (sec).

All sensor surfaces were prepared from liposomes, made from mixtures of lipids. The basic composition, denoted Lip A1 (see Experimental Section), had its origin in a previous work in which its advantages in stability and in ability to form a planar bilayer were shown.[24] It was possible to obtain good coverage with lipids from all liposome preparations used in the present study. Full coverage was considered essential to limit protein binding to lipid-free spots in the subsequent experiments.

By using different mixtures of lipids as the principle for sensing, the number of possible sensor surfaces is almost unlimited. The results presented here are limited to eight surfaces, all of which are variations on the basic composition Lip A1. In five of the surfaces, a glycolipid was introduced at 10 mol %. Lip A2, having the same lipid composition as Lip A1, was supplemented with membrane proteins to occupy 5−10% of the surface area. The rationale was to introduce more charges into the membrane. In Lip B, the cholesterol content was 4 times higher than in Lip A1, which should influence packing density and fluidity of the membrane.[33]

All of these surfaces had favorable features as regards stability. They could be used repeatedly in the binding studies, which included exposition to 20 mM HCl after each cycle. The surfaces could also be stored for prolonged periods, even months, provided they were kept cold, wet, and in the presence of a bacteriostat such as sodium azide. Long-term stability was tested mostly on GM1-containing lipid surfaces. A couple of surfaces were used for four months. They were repeatedly exposed to different analytes and 20 mM HCl, up to ~40 times, during this period and still had unchanged binding properties, tested with cholera toxin B as analyte.

The deposited lipid material could be removed from the cuvette surface by 1−2 min of washing with acidic ethanol (HCl/ethanol 1:9 v/v). This washing procedure was routinely followed in the instrument. The magnitude of the negative signal obtained when a film was removed was generally in the same range as the positive one obtained when the membrane was formed from liposomes, an observation that support the other data on stability. The strong binding of the lipidic material to the cuvette surface was also evident from experiments in which removal by detergent was tested. A 40 mM concentration of *n*-octyl glucoside had no or only partial effect.

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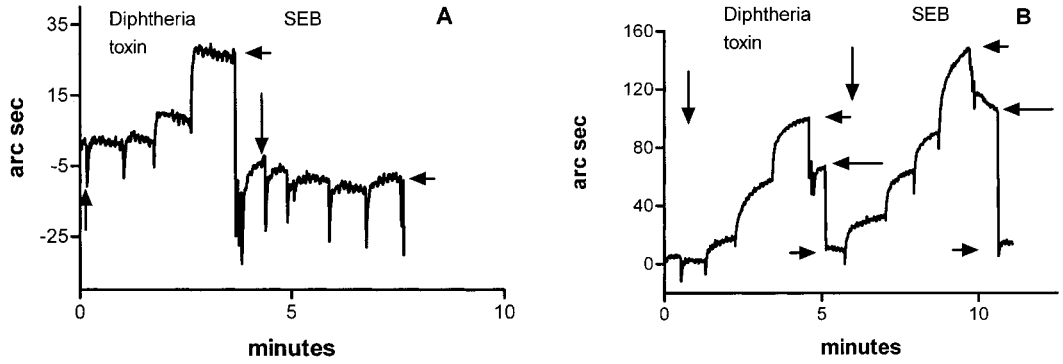

Figure 1. An example of the binding assay in the optical biosensor IAsys, at pH 7.4 (A) and pH 5.2 (B). The sensor surface in the cuvette was in this example covered by a bilayer with the lipid composition denoted Laccer in Table 1. Diphtheria toxin and staphylococcal enterotoxin B, respectively, were added sequentially at concentrations of 5, 10, 20, and 40 μg/mL. Vertical arrows denote the first addition. Short, horizontal arrows represent wash with buffer and long horizontal arrows regeneration of the lipid surface by a wash with 20 mM HCl for 1−2 min. The registration was stopped during the regeneration step. No HCl wash was performed in experiments shown in (A), as no binding took place (note the difference in scale between panels A and B).

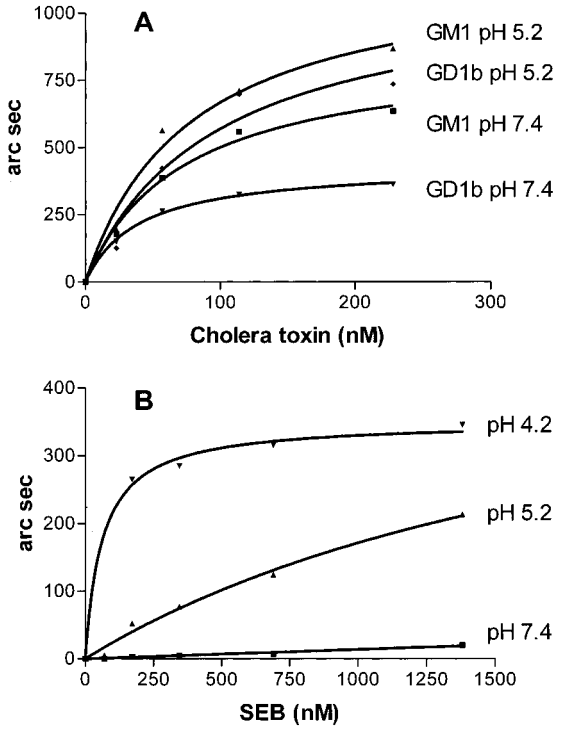

Figure 2. Some examples of binding curves, obtained from nonlinear regression of signal versus protein concentration data. The examples are chosen to illustrate specific binding, cholera toxin toward ganglioside-containing membranes in (A), and unspecific binding, staphylococcal enterotoxin B toward laccer-containing membranes in (B).

Binding of Proteins to Lipid Sensor Surfaces at pH 7.4 and 5.2. The binding experiments are illustrated in Figure 1. An analyte was added in increasing concentrations to a lipid-modified cuvette. If no binding had taken place, as in Figure 1A, the series was ended by a buffer wash only. If protein had bound, as in Figure 1B, the buffer wash was followed by 20 mM HCl for 1 min. The registration was stopped during that period because of the large difference in refractive index of the solutions. Finally, the system was washed again with buffer and registration restarted. The figure is limited to two test substances. Usually, all the test substances were run in sequence at one pH, after which the procedure was repeated at a new, lower pH.

The figure also illustrates the difference in signals from a change in bulk refractive index due to ingredients in the protein solution (at higher concentrations of diphtheria toxin in Figure 1A) and the slower changes resulting from binding as in Figure 1B. Bulk refractive index changes, when present, were subtracted from binding data in the evaluation. The wash with buffer, before regeneration with hydrochloric acid, was also used as a check on binding. Addition of buffer resulted in instantaneous drop in signal, when only bulk changes had taken place. In cases of true binding, one could notice no change in signal or a dissociative phase.

Binding was rapid for all proteins in the test series and typical kinetics shown in Figure 1B for two toxins. Plateau values were not reached in all cases within the time frame given (1 min), which however was insignificant for the signal pattern. The supported lipid membrane is ideally a two-dimensional structure. If the sensor surface is filled by binding proteins, we should expect a resonant mirror signal of the same range as obtained during membrane formation, that is ∼1000 arc sec. At pH 7.4 and 5.2, we obtained in some cases protein binding signals of 800−900 arc sec, but never a value exceeding 1000, which indicates that proteins do not stack in the present system.

Binding data were collected for the various proteins in the concentration range 2−40 μg/mL and at two pHs, 7.4 and 5.2. The series were in general performed at sensor surfaces prepared at different occasions, in some cases also prepared from liposomes of different batches, with satisfying reproducibility. For example, the binding of ricin at 20 μg/mL to the GM1 surface resulted in a signal of 61 ± 7 arc sec at pH 7.4 and 64 ± 17 are sec at pH 5.2 (mean ± sd; $n = 4$ for both), while cholera B also at 20 μg/mL to the A2 surface gave 89 ± 9 ($n = 3$) arc sec.

Visual inspection of simple plots of biosensor responses versus concentration of toxins for the various sensor surfaces suggested that each protein exhibited an individual binding pattern. To make use of all signals obtained in different series, the mean values of the responses for the various proteins were calculated and used for nonlinear regression of the binding curve. Some examples with cholera toxin binding to ganglioside-containing surfaces at two pHs, and SEB binding to laccer-containing lipid surface at three pHs, are shown in Figure 2. The initial slope of each binding curve, corresponding to B_{max}/K_d, which was obtained from nonlinear

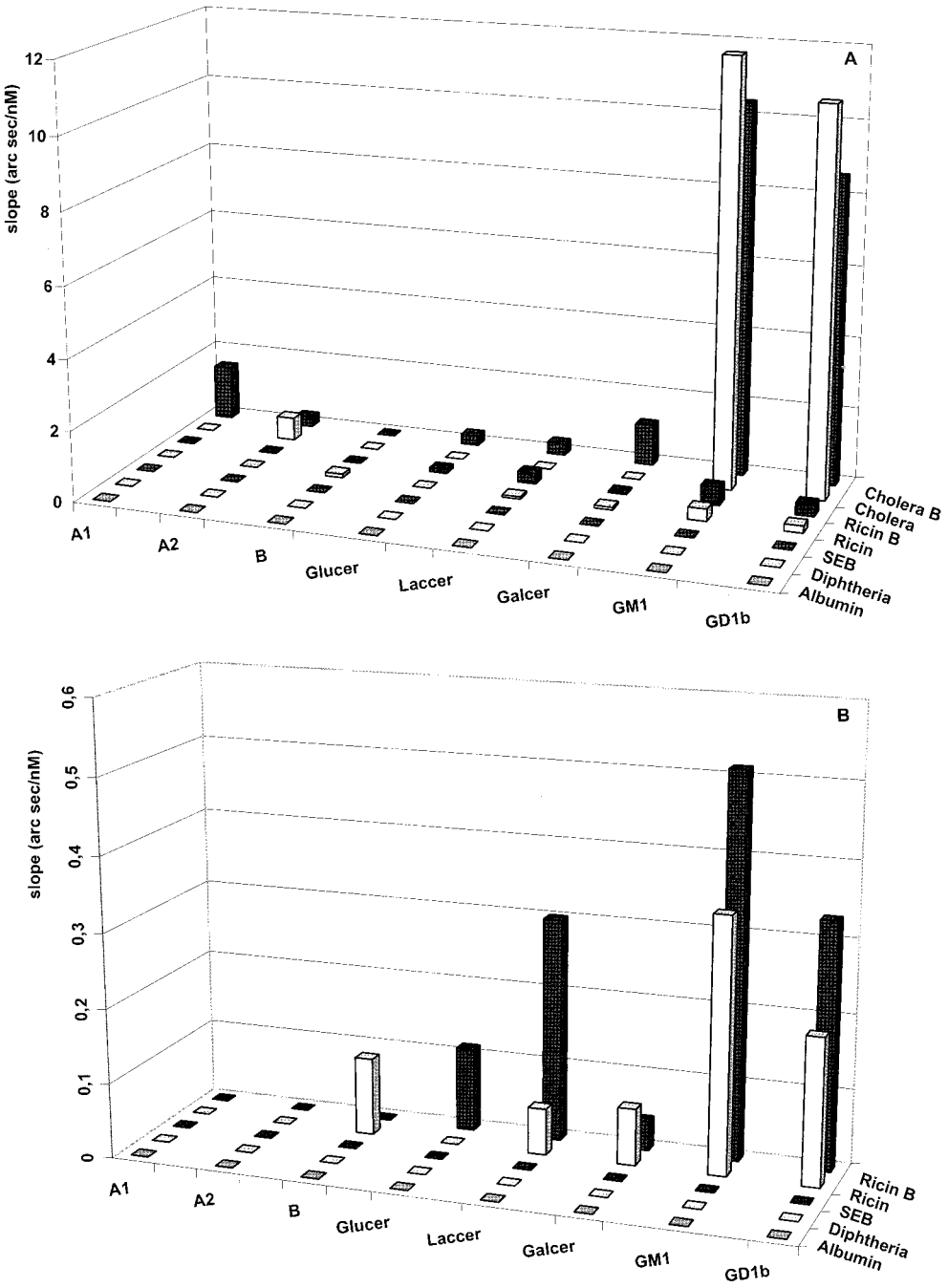

Figure 3. Binding patterns at pH 7.4. An overview is given in (A), while (B) is a magnification of data for proteins with no or moderate binding.

regression, was chosen as the measure for comparing the overall binding properties in the various combinations of protein and surface interactions.

The pattern obtained for experiments performed at pH 7.4 are shown in Figure 3. Three of the proteins—albumin, diphtheria toxin, and SEB—were devoid of any affinity to all the surfaces. Ricin is composed of two glycoproteins A and B of about the same size (mw of 32 000 and 34 000, respectively). Ricin B is known to interact preferentially with galactosides. In the lipid mixtures used in the present study, it is obvious that both ricin AB and ricin B show preference for galactose in more complex structures and especially for the ganglioside-containing mixtures, while binding to galactosylcerebroside is low. Finally, the figure shows the well-known, strong affinity of cholera toxin (mw 87 000) and its pentameric B-chain (mw 60 000) to gangliosides. Thus, the experiments performed at pH 7.4 and at protein concentrations between 2 and 40 μg/mL (20−1400 nM) show that "nonspecific" binding of proteins to various lipid surfaces is negligible, while introducing "specific" lipid receptors into the membrane permits detection of the corresponding analytes.

By changing the pH during binding, it should be possible to manipulate the interactions between membrane components and the proteins under study, primarily by changing the charges of ligands and analytes. Lowering the pH by 2 units, to pH 5.2, had drastic effects on both binding ability and binding patterns, as shown in Figure 4. Albumin was the only test substance for which there still was no binding to most surfaces. Some affinity was observed to the two Lip A compositions (Figure 4A). At the other

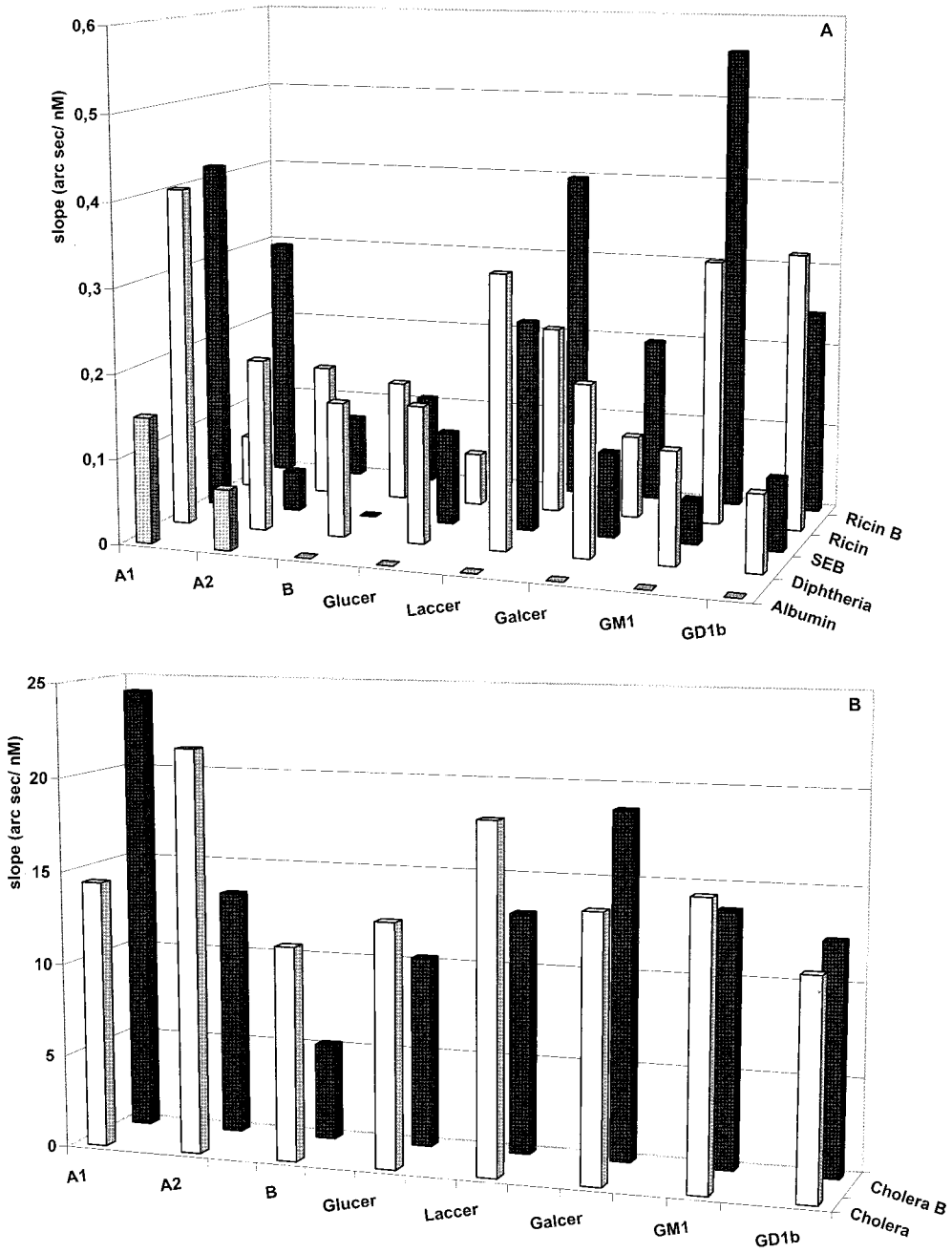

Figure 4. Binding patterns at pH 5.2, in (A) for proteins with no, low, or moderate binding and in (B) for cholera toxin and cholera toxin B with high binding.

extreme, cholera toxin and its B-chain seem to stick to all the lipid compositions (Figure 4B). The overall binding activity (B_{max}/K_d) is 10−50 times higher than obtained for any other protein on any other sensor surface (Figure 4A). The specificity toward gangliosides is no longer evident and the binding to such surfaces was not drastically changed by lowering the pH (compare with Figure 2A). We can also notice that there are differences in the binding pattern between cholera toxin and its B-chain. This suggests that properties in the A-chain also contribute to binding at an acidic pH.

For the remaining four proteins, binding patterns that are distinct from the ones obtained at pH 7.4 and distinct from each other have emerged. When comparing SEB and diphtheria toxin, we can notice that the latter has some binding to all the surfaces, with the lowest to the ganglioside-containing membranes. SEB displays a more varied pattern, with negligible binding to the cholesterol-rich Lip B surface and preferential binding to the Lip A1 surface (like the diphtheria toxin) and to the laccer-containing lipid surface (compare with Figure 2B). Ricin and ricin B both bind to more surfaces at the lower pH. Like cholera toxin and its B-chain, binding to GM1 and GD1b surfaces is unaffected by the decrease in pH; the only exception was that ricin AB binds more to the last-mentioned surface. For the B-chain, binding to laccer is also essentially unaffected, while the holotoxin interacts more strongly to this surface at the lower pH.

Figures 3 and 4 give a visual impression of patterns. A multivariate analysis was performed on the data, using principal component analysis (PCA). The data from assays at both pHs were

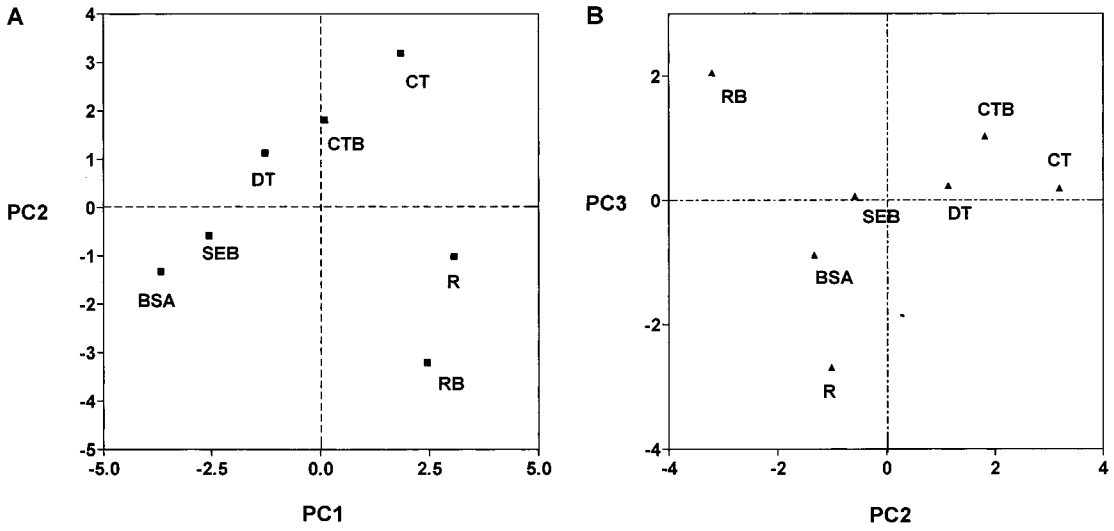

Figure 5. Score plots for the first principal component versus the second (A) and for the second PC versus the third (B). The abbreviations are BSA for albumin, DT for diphtheria toxin, SEB for staphylococcal enterotoxin, R for ricin, RB for ricin subunit B, CT for cholera toxin, and CTB for cholera toxin subunit B.

used as input variables ($n = 16$), for each of the seven toxins recalculated as percentage of the highest binding (e.g., for ricin, the binding to GM1 at pH 7.4 was 100%). Three significant principal components were identified. The first one accounted for 42% of the variance, the second one for 29%, and the third one for 14%. Thus, in total, the model explains 85% of the variance in data. The score plots for PC1 vs PC2 and for PC2 vs PC3 are shown in Figure 5. The loading plots (not shown) indicated that, for a planned, extended analysis on binding patterns of various toxins to lipid surfaces, one of the ganglioside-containing membranes (GM1 and GD1b) might be deleted.

It is well known that proteins are sensitive to extreme pHs and denature, that is, lose the three-dimensional structure and expose normally hidden amino acid sequences, often with aggregation as a consequence. It is also well known that proteins have a tendency to aggregate at a pH corresponding to the isoelectric point (p*I*) of the protein. Could precipitation of aggregated proteins be the simple explanation to the increased signals obtained at pH 5.2? A first argument against this hypothesis is that, for most proteins, pH 5.2 is not low enough to give denaturation. The p*I*-related precipitation hypothesis is also difficult to believe as the proteins have p*I* values in a broad pH range, from 4.8 for ricin B to ~9 for SEB. No correlation between signal and p*I* was apparent. If the precipitation hypothesis is correct, we should expect for each protein about the same signal for all the surfaces. This was not the case. Furthermore, we should see signals higher than the one corresponding to a protein monolayer, when increasing the protein concentration. Again, we never did. Regular checks on symmetry in the resonance scans could also be indicative of a smooth surface and absence of precipitated aggregates.

The patterns illustrated in Figures 3−5 indicate that the approach with mixed lipid membranes as sensor surface array and analysis run at a couple of pHs seems to be adequate for distinguishing a number of proteins. The choice of proteins in the present study was rather arbitrary but related to our interests in toxin detection. Commercial availability and price were the main factors in the selection. The choice of serum albumin as an example of nontoxic proteins was obvious because of its use in blocking "unspecific" binding in many biochemical methods. The list of proteins came to include both toxins with "specific" binding to some of the glycolipids incorporated in the sensor membrane and a couple devoid of specific interactions with glycolipid receptors. The important point to make here is that specificity is not the prime feature explored in the present biosensor approach. We are only interested in total binding to the various surfaces, regardless of its biological significance.

Binding at pH 4.2. The interaction between diphtheria toxin and membrane is known to be highly pH-dependent.[1,34] It is negligible at neutral pH and optimal at pH 4−4.5. Binding is followed by uptake into the membrane and ultimately into the cell interior. The heparin binding-epidermal growth factor precursor has been recognized as the target molecule for binding in the cell membrane. Still, protein-free lipid bilayer model systems such as liposomes and black lipid membranes work well to study the interaction. The presence of negatively charged phospholipids promotes incorporation of the toxin in the model systems.

The binding of diphtheria toxin at pH 4.2 to some of the sensor surfaces was tested. The protocol was designed to minimize the time during which the toxin was exposed to the low pH (see the Experimental Section). A new lipid composition was also introduced, with POPC, DPPG, and cholesterol (Table 1). POPC has a lower transition temperature than DPPC and might result in a membrane with more fluid properties, which in turn might facilitate insertion of the protein into the membrane. As before, all the sensor surfaces contained an acidic phospholipid, DPPG. Figure 6 shows that the change from pH 5.2 to 4.2 had a drastic effect on diphtheria toxin binding to all the surfaces. A concentration as low as 5 μg/mL (86 nM) resulted in resonant mirror signals almost corresponding to a protein monolayer. Higher concentrations, up to 40 μg/mL, resulted in only marginally higher signals on the GM1 surface. Higher values, up to 1400 arc sec, were observed on the glycolipid-free sensor surfaces Lip A2, Lip B, and POPC. The signals were very similar for these three surfaces for

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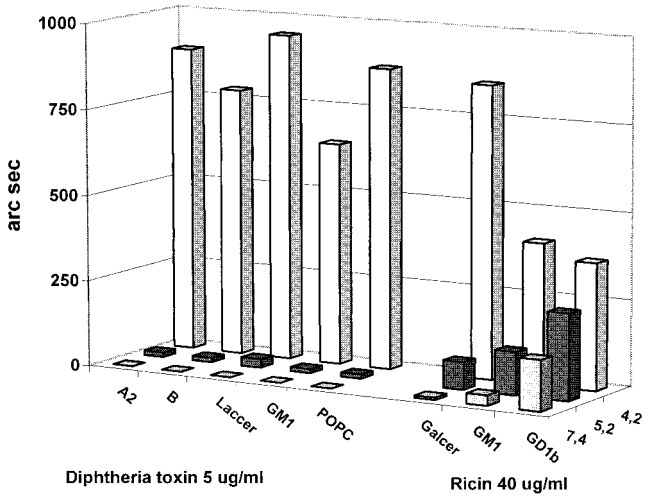

Figure 6. Binding data at pH 7.4, 5.2, and 4.2 for diphtheria toxin (5 μg/mL) and ricin (40 μg/mL) to a selection of lipid surfaces.

each concentration, the POPC-containing membrane not being exceptional. One interpretation of these relatively high signals is that the protein did not only bind but also penetrate into the membrane, resulting in a more compact proteolipid layer close to the solid sensor surface. There was no dissociation in the buffer wash, and surface regeneration with HCl was not efficient.

There are a few reports on increased membrane interactions at lower pHs for ricin.[35,36] Ricin, at 40 μg/mL, showed a ~10-fold higher affinity for the galcer membrane when pH was decresed from 5.2 to 4.2, while the changes were less drastic for ganglioside-containing membranes (Figure 6). The signal increases upon shifts to more acidic environment were more moderate for ricin B and even less for SEB and albumin. These experiments show that an assay at pH 4.2 has advantages in clearly distinguishing diphtheria toxin from other proteins and in being sensitive. The lack, so far, of a general and gentle regeneration procedure limits its use.

CONCLUSIONS

The mixed lipid layer-based biosensor approach has several advantageous properties, which are encouraging for continued research and development. The binding data obtained at two pHs for seven proteins suggest that it might be possible to design tailor-made surfaces/pHs for any set of protein toxins of interest, as the number of possible combinations is almost unlimited.

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