Bilayer Lipid Membranes as Electrochemical Detectors for Flow Injection Immunoanalysis

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Abstract
This work describes the use of filter-supported stabilized bilayer lipid membranes (BLMs) for the rapid electrochemical monitoring of an immunological reaction in flowing solution streams. BLMs were prepared from egg phosphatidylcholine (egg PC) and dipalmitoyl phosphatidic acid (DPPA) and the ultrafiltration membranes used were composed of glass microfibers. Thyroxin (T4)/anti-rabbit T4 was used as a representative immunological reaction for these studies. Antibody was incorporated into a floating lipid matrix at an air–electrolyte interface, and then a casting procedure was used to deliver the lipid onto the filter supports for BLM formation. Injections of antigen were made into flowing streams of a carrier electrolyte solution. Experiments were done in a stopped-flow mode using lipid mixtures containing 15% (w/w) DPPA to provide only a single transient current signal with a magnitude related to the antigen concentration. Differential scanning calorimetric experiments provided evidence that the antibody-lipid interactions at the BLMs occurred through electrostatic interactions. BLMs containing 35% DPPA were used to examine regeneration of the active sites of antibody after complex formation by washing with the carrier electrolyte solution. Repetitive cycles of injection of antigen followed by regeneration of antibody binding activity have shown that the maximum number of cycles is about 5, followed by a degradation of signal for a larger number of injections. However, the sensor can also be easily regenerated by recasting of the existing lipid/antibody film at the air–electrolyte interface to form fresh BLMs.

Keywords: Immunological reaction, Bilayer lipid membranes, Flow injection, Differential scanning calorimetry, Antibody regeneration

1. Introduction

Natural molecular recognition and transduction systems that are associated with the function of living organisms have been proposed for direct use in the preparation of biosensors. The inherent biological recognition of molecules is realized through noncovalent reversible interactions between complementary structures, leading to a docking of the molecule to be recognized with a receptor site that is associated with a macromolecule. A large number of naturally occurring antibody–antigen interactions involve such recognition and selective binding. Immunogenic responses associated with natural cell membranes have motivated various investigations including studies of artificial lipid membranes [1, 2] either in the form of vesicles [3–6], or as membranes supported on solid substrates for the development of immunosensors [7–12].

A limited number of immunosensors which can be used in flow analysis have recently appeared in the literature [13–19]. Such sensors must be able to regenerate the active reagent after use. Some immunosensors require replacement of the chemistry at the device surface, which is costly and time consuming [16–18]. Efforts to prepare analytical devices with regenerable active antibody sites have made use of acidic [13, 14] or chaotropic media [15]; this however might permanently alter the antibody conformation and result in loss of activity. A few reports have described the regeneration of active sites of antibodies by simply washing with a flowing electrolyte solution (i.e., mass action) [20–22]. Such a reversible immunosensor is limited by the kinetics of the antibody–antigen dissociation, which should be fast to allow regeneration of the antibody in a short period of time.

In a previous article we reported the electrochemical transduction of an immunological interaction by the use of bilayer lipid membranes (BLMs) composed of egg phosphatidylcholine (PC) and dipalmitoyl phosphatidic acid (DPPA) [23]. Antibody-antigen complexation at BLMs caused transient ion current signals due to modulation of surface charge of the membranes. An increase in magnitude of these signals was related to an increase of concentration of the antigen in bulk solution, which could be determined over a range of nM to μM levels in a period of less than two minutes. However, the inherent mechanical instability of these BLMs prevented their use in flow injection experiments. Our recent work has developed a method for the formation of stabilized BLMs on microporous filtering (glass microfiber and polycarbonate) media for flow injection experiments [24].

The aim of the present work was to use such stabilized filter-supported BLMs for flow injection immunoanalysis. The flow injection system was used in a stopped-flow mode. The injected antigen was brought into contact with BLMs containing antibody for a period of less than two minutes. Studies were done with a lipid mixture which gave multiple transient signals [35% (w/w) DPPA] to estimate the time required for regeneration of antibody binding sites by washing with a carrier electrolyte solution. A lipid mixture which gave single transient signals [15% (w/w) DPPA] was investigated to determine the maximum number of injections of antigen which could give ion current transients useful as calibrated analytical signals. Studies using differential scanning calorimetry (DSC) were done to investigate the mechanism of antibody association at the lipid membranes.

2. Experimental

2.1. Materials and Equipment

The lipids that were used throughout this study were egg phosphatidylcholine (PC; lyophilized, Avanti Biochemicals, Birmingham, AL) and dipalmitoyl phosphatidic acid (DPPA;

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Sigma, St.Louis, MO). Other chemicals supplied by Sigma included HEPES (*N*-2-hydroxyethyl-piperazine-*N'*-2-ethanosulfonic acid) which was used for pH adjustment, gramicidin D and thyroxin. Rabbit antiserum to T4 was supplied from UCB Bioproducts (Braine-l'Alleud, Belgium). The filters and (nominal) pore size used were glass microfiber (GF/F; 0.7 μm, Whatman Scientific Ltd., Kent, UK). Water was purified by passage through a Milli-Q cartridge filtering system (Milli-Q, Millipore, El Paso, TX) and had a minimum resistivity of 18 MΩ cm. All other chemicals were of analytical-reagent grade.

The apparatus for the formation of stabilized BLMs was essentially identical to the one described previously [24]. The apparatus consisted of two Plexiglass chambers separated by a Saran-Wrap (PVDC; DowBrands L. P., Indianapolis, IN) partition of a thickness of ca. 10 μm. This plastic sheet was cut to more than twice the size of the contact area of the faces of the chambers with a paper cutter and folded in half; an orifice of 0.32 mm diameter was made through the double layer of the plastic film by punching with a perforation tool as previously described [25]. A microporous glass fiber ultrafiltration disk (diameter of about 0.9 cm) was placed between the two plastic layers with the filter centered on the 0.32 mm orifice. The partition with the filter in place was then clamped between the Plexiglass chambers. One of the chambers was machined to contain an electrochemical cell with circular shape (diameter 1.0 cm and depth 0.5 cm) connected with plastic tubing for the flow of the carrier solution; an Ag/AgCl reference electrode was immersed in the waste of the carrier electrolyte solution. The second chamber was machined to contain a cell with a cylindrical shape having its longitudinal axis perpendicular to the flow of the carrier solution of the opposing cell. The upper hole of this cell was circular (diameter 0.5 cm) and the lower was elliptical (with diameters 0.5 and 1.4 cm parallel and vertical respectively to the flow of the carrier electrolyte solution) facing the opposing cell. The microporous filter disk was positioned approximately at the center of the cells. An Ag/AgCl reference electrode was placed into the cylindrical cell and an external 25 mV d.c. voltage was applied across the filter membrane between the two reference electrodes. A digital electrometer (Model 614, Keithley Instruments, Cleveland, OH) was used as a current-to-voltage converter. A peristaltic pump (Masterflex with SRC Model 7020 pump head) was used for the flow of the carrier electrolyte. Injections of antigen were made with a Hamilton repeating dispenser with a disposable tip (Hamilton Co., Nevada). The electrochemical cell and electronic equipment were isolated in a grounded Faraday cage.

2.2. Procedures

The dilute lipid solution used for the formation of the solventless stabilized BLMs contained 0.04 mg/mL total lipid and was composed of 15 and 35% (w/w) DPPA; these solutions were prepared daily from stock solutions of PC (2.5 mg/mL) and DPPA (2.5 mg/mL) in *n*-hexane-absolute ethanol (80 + 20). The stock lipid solutions were stored in the dark in a nitrogen atmosphere at −4 °C. The BLMs were supported in a 0.1 M KCl electrolyte solution which contained 10 mM HEPES as a buffer. A pH value of 6.0 was used for all experiments containing no calcium (for the calibration graph) and 1.0 mM of calcium ions for studies of regeneration time of the binding sites of antibody.

The antiserum solution from the supplier had a volume of 1.6 mL (protein concentration 17.5 mg/mL) and was diluted to 10.0 mL with a 0.02 M phosphate buffer at pH 7.4. Samples of 20 μL volume were further diluted on a daily basis to 90 μL with the same phosphate buffer to give a protein concentration of 0.62 mg/mL. These diluted solutions were used for the deposition of the protein onto the air–electrolyte interface in the electrochemical cell. The stock solution of thyroxin (0.1 mg/mL) was prepared daily just before use and the injected dilute antigen solutions were buffered identically as the carrier electrolyte solution.

The process of formation of stabilized BLM for flow injection experiments was described recently [24]. Lipid solution (10 μL) was added dropwise from a microliter syringe to the water surface in the cylidrical cell near the partition (surface area of about 0.2 cm^2). A volume of 3 μL of the protein solution was applied to the same air–electrolyte interface subsequent to the deposition of lipid. The level of the electrolyte solution was dropped below the aperture and then raised again within a few seconds. The formation of solventless BLMs was verified by the magnitude of the ion current obtained, and by electrochemical characterization using gramicidin D. When the ion current stabilized (over a period of 20 min) and no more transient current changes occurred, the antigen solution (75 μL) was injected in carrier electrolyte solution. The experiments for the calibration plot were done using BLMs composed of 15% DPPA in a stopped-flow mode. A flow rate of 1.0 mL/min was initially used, and the flow of the carrier electrolyte solution was stopped 15 s after antigen injection and was again initiated after a single transient signal was obtained. A 5-min period of washing out with carrier electrolyte solution was found adequate to regenerate the antibody binding sites for multiple repetitive injections of antigen. This period of time was determined by use of BLMs composed of 35% DPPA, and experiments were initiated using a similar stopped-flow mode of operation. The flow was though reinitiated after about 3 min (sufficient time for appearance of the first transient signal), and the time required for complete disappearance of transient signals was then obtained. All experiments were done at 25 ± 1 °C.

Vesicles composed of 15% (w/w) of DPPA were prepared and used for the DSC experiments. The organic solvent of the stock lipid solution (2.5 mg/mL of total lipid) was evaporated under a stream of nitrogen. The lipid was resuspended by sonication with an equal volume (with respect to the initially evaporated organic solvent) of a buffer of pH 6.0 (0.1 M KCl and 10 mM HEPES). This solution was left refrigerated overnight. An aliquot was withdrawn using a calibrated syringe and hermetically sealed in an aluminum pan. An amount of 20 μL of lipid suspension was mixed thoroughly with 10 μL of protein solution containing 2.8 mg of protein/mL about 20 min before initiation of DSC experiments. The lipid suspension was replaced by the buffer solution at pH 6.0 for experiments which studied the thermal destabilization of the protein. Vesicles were scanned from 10 to 70 °C with 10 °C/min scanning rate using a Perkin-Elmer DSC-4 differential scanning calorimeter (buffer solution was used as the control). The DSC curves were analyzed using the Thermal Analysis Data Station (TADS) of the DSC-4.

3. Results and Discussion

The electrochemical transduction of an immunological interaction by the use of BLMs which were prepared from mixtures of egg PC and DPPA has recently been reported [23]. Thyroxin/anti-rabbit T4 was used as a representative immunological reaction for these studies, and was selected because of the clinical significance of T4. Antibody–antigen complexation

caused transient ion current signals due to dynamic changes of the electrostatic fields at the surface of the BLMs. The transient charging signals occurred as singular or multiple events, where each transient lasted for a period of seconds [23]. The use of lipid mixtures containing 15% DPPA produced a single current transient with a magnitude that was logarithmically related to the concentration of the antigen in bulk solution. The use of lipid mixtures containing 35% DPPA resulted in the appearance of multiple transient events of the form that have been shown to relate frequency to concentration [26, 27] with an average delay time to the first signal of about 3 min.

The same immunological interaction was used in combination with stabilized filter-supported BLMs for flow injection experiments. To maximize protein loading, antibody in solution was deposited directly onto egg PC-DPPA mixtures at the air–electrolyte interface prior to casting of the lipid–protein mixture onto the filter supports. The maximum protein loading that could be incorporated in the BLM structure without inducing permanent permeability alterations was found to be 3 μL of solution containing 0.62 mg of protein/mL. This value corresponds to a lipid:protein w/w ratio of 0.22 (equivalent to about 45 mol of lipid to 1 mol of protein given that the molecular mass of the anti-rabbit T4 is 150000 [28]) and is similar to the previously reported value of 0.25 [23]. This volume of antibody solution resulted in the appearance of random transient ion current signals (in the absence of antigen) as the BLMs stabilized. The transients occurred on a timescale of seconds and were not of discrete pulse height as would be associated with ion channel gating events [29]. An instability of ion current with time was noticed for concentrations of antibody solution larger than 0.62 mg/mL; e.g., the ion current was initially in the order of a few pA when the protein concentration was 1.4 mg/mL, but it increased over a period of 4 min after membrane stabilization to 40 pA and after 7 min to 150 pA. These results suggest that the protein molecules do not span the membrane to form conductive pores since permanent alterations of ion current were not observed when using concentrations of antibody less than or equal to 0.62 mg/mL. Aggregation of charged protein molecules, and interactions with the charged lipid component of the BLMs (electrostatic or protein binding to hydrogen bond accepting sites of DPPA [30]) can induce electrostatic field gradients at a BLM surface [31]. These processes should reduce in frequency and then terminate as an equilibrium concentration of protein and subsequent equilibrium of aggregation at the membrane solution interface was reached. Stabilization of BLMs which contained 0.62 mg/mL or less of antibody occurred within 20 min, and injection of antigen into the carrier electrolyte solution was done after this delay time.

Figure 1 shows DSC thermograms of vesicles composed of 15 % DPPA in the absence and presence of anti-rabbit T4. The phase transition temperature (T_m) was 52.0 ± 1.6 °C ($N = 5$) in the absence of the protein (endothermic transition) as shown in Figure 1A. Heating scans of vesicles with antibody exhibited an exothermic transition at 59.9 °C (Fig. 1B). DSC scans of solutions containing only protein molecules have shown similar exothermic transitions at 58.0 °C (Fig. 1C) which is characteristic of heat denaturation of anti-rabbit T4 [32]. Denaturation of the protein is accompanied by release of heat where the position of the maximum of the exothermic peak and its form strongly depends on the concentration of protein [32]. The results indicate that the antibody is not thermally stabilized by incorporation onto lipid membranes as both of the observed exothermic transitions occur at about the same temperature. Lipid-protein associations occur in the gel phase structure of BLMs composed from 15% DPPA, in which the charged lipid component serves as a nonspecific receptor for proteins [8,33]. A second cycle of heating of vesicles with antibody resulted in the appearance of the endothermic transition of the lipid vesicles at 59.0 °C (Fig. 1D). This modest increase in T_m can be attributed to the fact that the protein and phospholipid molecules interact mainly by electrostatic forces, and an increased availability of electrostatic sites provided by transition to a more fluid state after the first DSC scan leads to a permanent increase of electrostatic stabilization that is reflected by the elevated phase transition temperature [34].

Figure 2A shows recordings of the signals obtained at pH 6.0 (in the absence of calcium ions) with BLMs composed of 15% DPPA when different concentrations of thyroxin were injected in the carrier electrolyte solution (using stopped-flow mode). It was necessary to stop the flow of the carrier electrolyte solution 15 s after injection of antigen because continuous flow injection experiments gave no detectable electrochemical signals. By selecting the 15 s period (at a flow rate of 1.0 mL/min) which elapsed between injection and commencement of the stop flow period, the centroid of the dispersed zone of antigen could be held in contact with the BLM surface (as observed with injections of the indicator methyl orange). The delay time before the appearance of a transient signal shown in Figure 2A indicates the time in seconds after flow was stopped. The flow of the carrier solution was initiated again after the appearance of the single transient.

The lipid composition of 15% DPPA used in the present studies was the only one (using the stated lipids and salts) to provide single transient responses as were previously reported [23]. The magnitudes of the transient signals were in direct proportion to the logarithmic concentrations of the injected antigen in the carrier electrolyte solution, as can be seen in Figure 2B. Control experiments involving the use of antigen alone, nonselective protein interactions (i.e. triiodothyronine, T3) and injections of equal volumes of solutions with identical composition to the carrier solution were completed to demonstrate that the transient ion current signals were due to selective interactions.

We have previously speculated on a mechanism responsible for signal generation from selective antibody–antigen complexation at BLMs [23]; the transient signals obtained from membranes supported in filters look similar to those reported previously for planar BLMs, take about the same time to appear after exposure to antigen, and have magnitudes of signal that vary directly with the logarithm of antigen concentration. The data in Figure 2 indicates that for all antigen concentrations that were tested, it takes about the same delay time (60 to 90 s) for the signal to be observed after the flow has been stopped. The relatively invariable delay time does not appear to be related to antigen concentration, and suggests a mechanism of response that may occur in two steps; the immunoreaction occurs to provide surface loading of complex, which may then associate as aggregates to provide electrostatic pertubation of the lipid membrane. This second step (aggregation) may be relatively independent of the rate of the first step (complex formation). At any one temperature (our data has only been collected at room temperature) the process of aggregation should be relatively constant, and is expected to be slow as based on movement across the surface of membranes. Such slow aggregative events have been observed by fluorescence microscopy for antibody–antigen complexation at lipid monolayers on an air–water interface [35]. The aggregative process could be responsible for the delay time, where the process could lead to a subsequent rapid reorganization of electrostatics and phase structure at the surface of a membrane [36] in a process analogous to nucleation

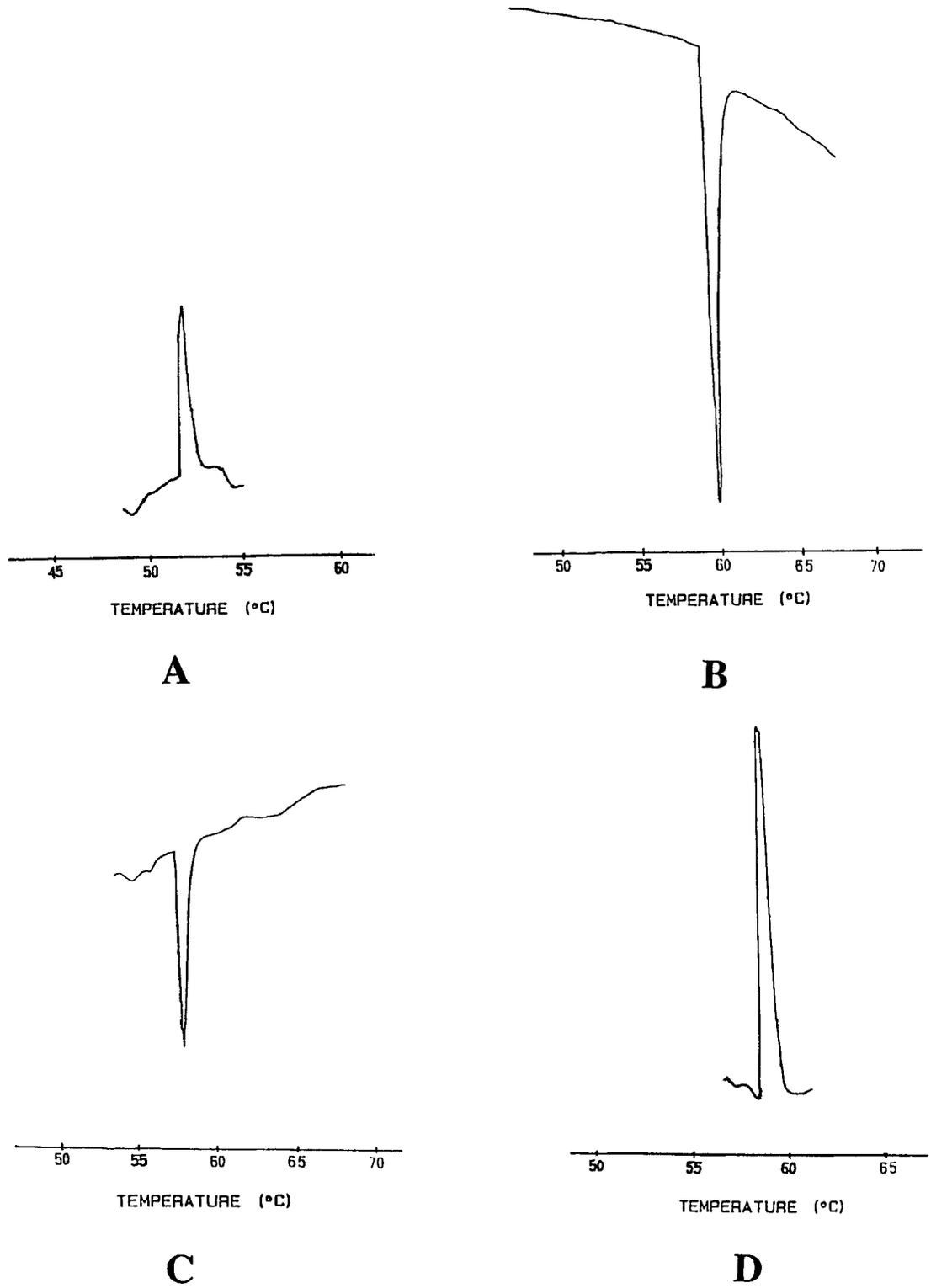
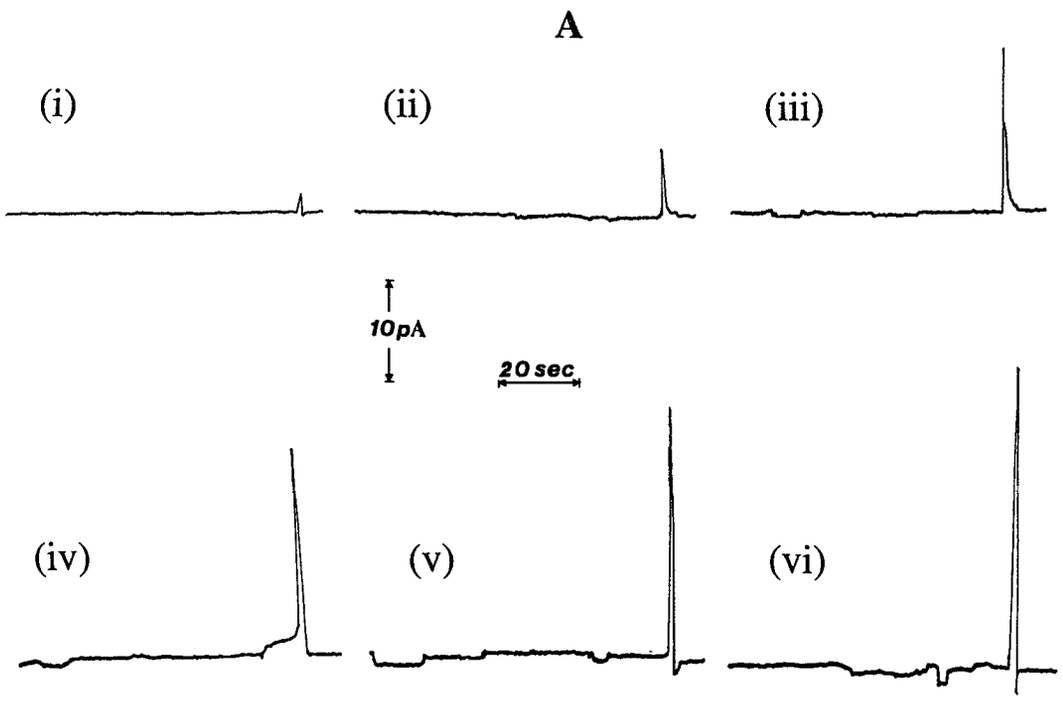


Fig. 1. A) Differential scanning calorimetry thermogram of vesicles composed from 15% DPPA at pH 6.0 (0.1 M KCl, 10 mM HEPES and in the absence of calcium ions). B) Differential scanning thermogram obtained during first scan of vesicle solution containing protein. C) Calorimetric scan of aqueous suspension of rabbit anti-T4. D) Phase transition during second scan of vesicle solution containing rabbit anti-T4.

in a supersaturated solution. At higher concentrations of antigen, aggregates may be larger and cause a greater effect; therefore the signal would be related to antigen concentration. If the ion current (measured signal) is driven by electrostatic (voltage) changes at the surface of a membrane, then the voltage (and measured ion current) should be logarithmically related to the concentration of antigen by the Nernst equation. Further experimental work investigating the mechanism of response is presently underway.

The use of stabilized filter-supported BLMs for flow immunoanalysis provides for a detection system where the antibody bound onto the lipid membrane should not be limited to a single



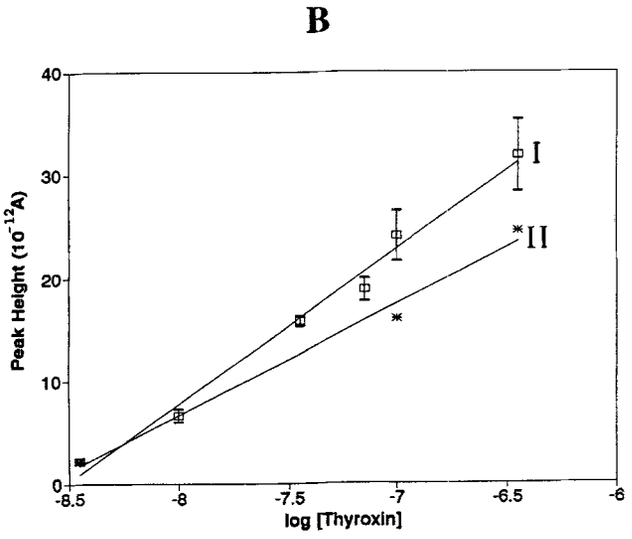


Fig. 2. A) Experimental results obtained at pH 6.0 (0.1 M KCl, 10 mM HEPES and in the absence of Ca^{2+}) with BLMs composed of 15% (w/w) DPPA when 3 μL of antibody stock solution was co-deposited onto the air–electrolyte interface. Thyroxine concentrations [M]: (i) 3.50×10^{-9}; (ii) 1.00×10^{-8}; (iii) 3.50×10^{-8}; (iv) 7.00×10^{-8}; (v) 1.00×10^{-7}; (vi) 3.50×10^{-7}. Each recording starts when the flow of the carrier solution stops (15 s after injection of antigen). B) Calibration of the analytical signal from the experimental results that are shown in Fig. 2A; I): Mean of five repetitive determinations. II): Calibration obtained using only results of a sixth injection.

use. An immunosensor based on the BLM transduction scheme should be regenerable and capable of multiple analyses. A number of immunosensors have been regenerated by placing the device in acidic [13, 14] or in chaotropic media [15]. Regeneration of BLMs that contain antibody by using acidic conditions can not be done as this can disrupt membrane structure and can induce the formation of ion channels [37]. Chaotropic agents generally act to disrupt antibody–antigen complexes by inducing a change in the antibody structure that results in weakening of the interaction with the antigen [20]. Depending on the chaotropic agent used, the protein may become denaturated and can lose activity if the denaturation is irreversible, as was noticed in previous experiments using liposomes [20]. Recent studies using chaotropic anion-phospholipid membrane interactions have furthermore shown that the water structure in the headgroup region of membranes can be altered by the anions [38].

An alternative approach to the regeneration of the binding sites of the antibody in BLMs is by infinite dilution allowing dissociation to occur by mass action [20–22]. This method is easily implemented by use of a continuous flow system where dissociated antigen is continuously removed and prevented from reassociating with antibody. In this case, the dissociation rate constant determines the time required for regeneration of the active antibody binding sites. For a low or moderate affinity interaction, bound antibody can be regenerated in a reasonable time under very mild non-denaturing conditions [20].

The time required for dissociation of antibody and antigen to regenerate the binding sites of the antibody was estimated by the use of BLMs containing 35% DPPA. This lipid composition was selected based on our previous studies which demonstrated that multiple transient signals which continue to appear over periods of an hour are obtained from thyroxin–antirabbit-T4 interactions at BLMs containing 35% DPPA in the presence of calcium ions [23]. This lipid mixture assured the occurrence of a continual series of transient signals that could be used to determine whether the antibody–antigen complex was present. Experiments were done using such membranes in a stopped-flow mode. A 15 s delay time was also used between injection of antigen and stopping of the flow of the carrier solution so that the injected antigen volume would come in contact with BLMs containing antibody. Figure 3 was obtained during experiments which served as controls, where the antigen was in continuous contact with membranes. The results were similar to those previously reported in which the immunological interactions at BLMs produced multiple transient events [23]. The time delay between antigen injection and appearance of the first signal was about 3 min. It was therefore decided that the flow of the carrier solution would be re-initiated after this period of time (after the appearance of this first signal), so that the loss

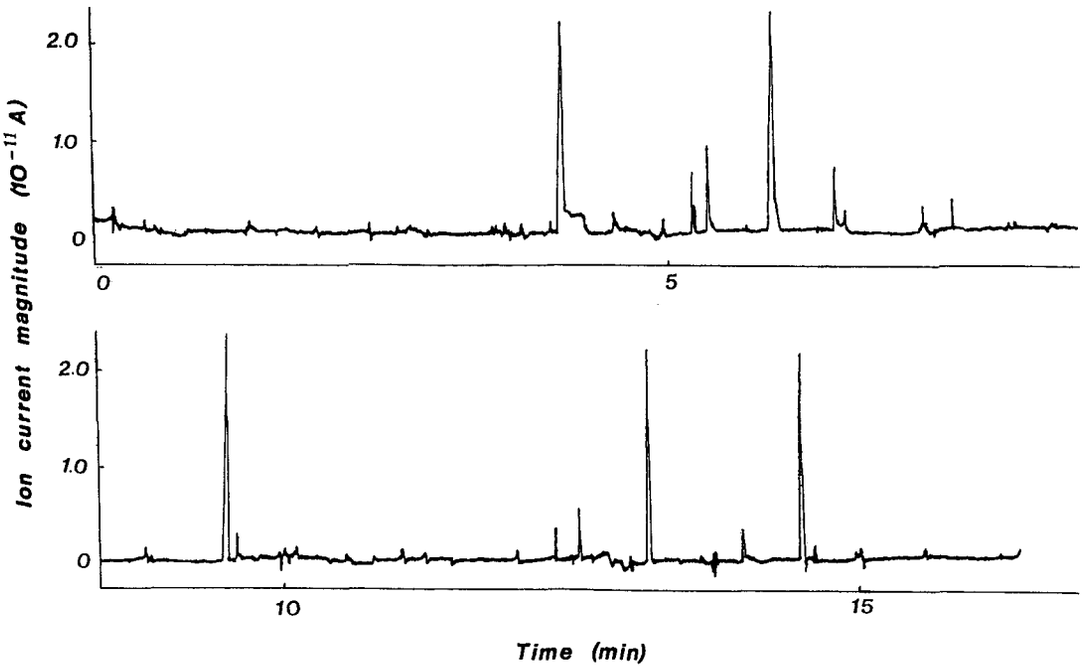


Fig. 3. Experimental results obtained at pH 6.0 (0.1 M KCl, 10 mM HEPES and 1.0 mM Ca^{2+}) with membranes consisting of 35% (w/w) DPPA when a volume of 3 μL of antibody solution was co-deposited onto the air–electrolyte interface and the concentration of T4 in the bulk solution was 1×10^{-6} M.

of the antibody–antigen complex could be observed as a reduction of the frequency and the magnitude of the transient signals.

Figure 4 shows the reduction and then disappearance of the transient signals obtained by re-initiating continuous flow of the carrier electrolyte solution after the appearance of the first transient signal (flow re-initiated at time marked by arrow in Figure 4). A time of approximately 5 min was required to eliminate the appearance of the transient signals when using 1 μM of antigen. Shorter times were required for lower concentrations of antigen; e.g., 3.4 min when the antigen concentration used was 0.1 μM. It was therefore concluded that a time of 5 min would be sufficient between experiments involving repetitive injections of antigen. Similar times for regeneration of the binding sites of antibody were previously reported by other researchers [20].

Figure 5 shows results of experiments that used repetitive injections of antigen. The maximum number of cycles of injection which could be achieved with retention of calibration of the analytical signal was about five. The variability of response of the BLMs to five repetitive antigen injections is between 3 to 11%, $N = 5$ (Figs. 2B and 5); given that this precision is sufficient for five measurements, the calibration graph drifts for larger number of injections (graph II in Fig. 2B)

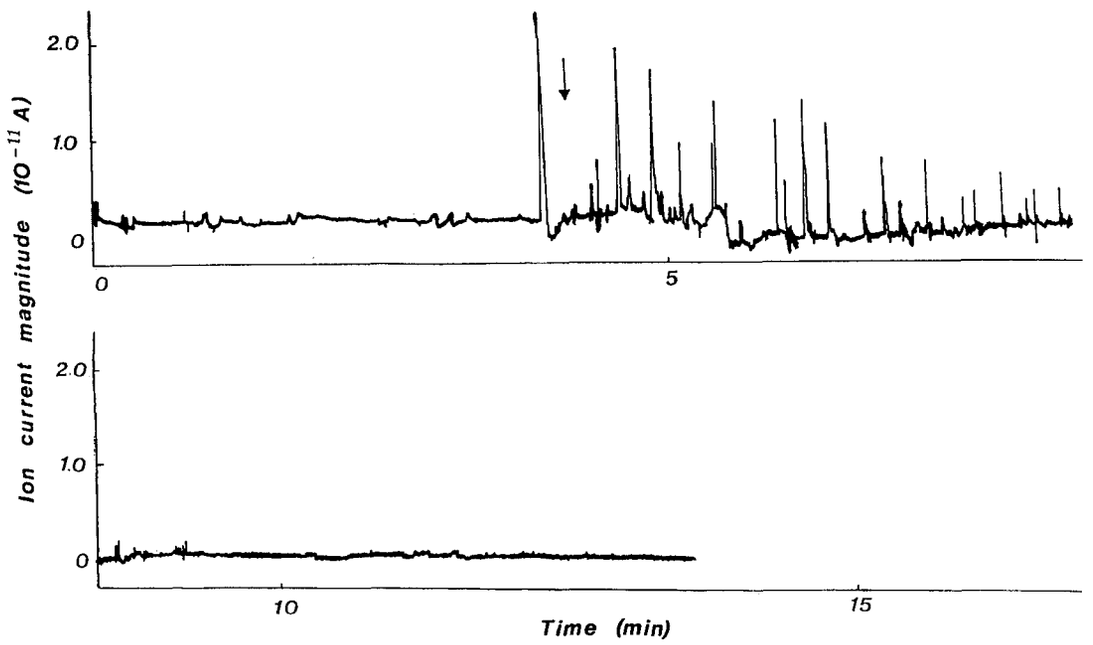


Fig. 4. Recording showing degradation of the first signal of Figure 3 when the flow is initiated again immediately after its appearance. Arrow indicates when the flow of carrier solution starts.

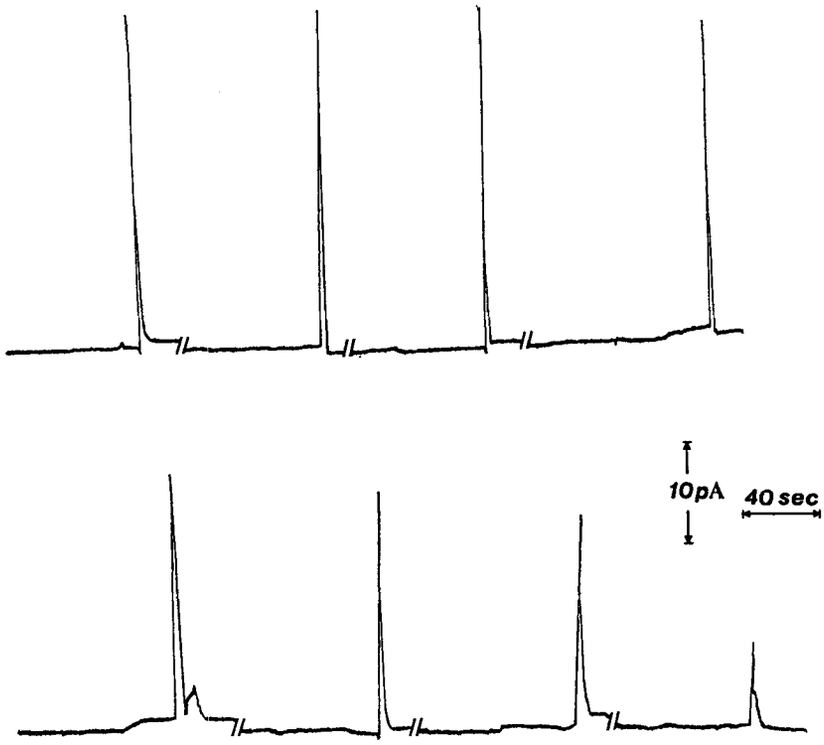


Fig. 5. Experimental results of repetitive injections of T4 (3.50×10^{-7} M) obtained at pH 6.0 (0.1 M KCl, 10 mM HEPES and in the absence of Ca^{2+}) with BLMs composed of 15% (w/w) DPPA when 3 μL of antibody stock solution was co-deposited onto the air–electrolyte interface. The time delay between the repetitive injections (marked as // in figure) was 5 min.

and therefore the accuracy of antigen determination is decreased. This reduction of the signal magnitude, which was observed for a larger number of injections than five, was likely due to removal of antibody from BLMs by the flowing solution. Protein denaturation was not the cause of the loss of the signal, since preparation of fresh BLMs from the original lipid–protein mixture at the air–electrolyte interface provided full electrochemical activity, and regeneration of the sensor which could be used for another 5 cycles of injection. This conclusion is further reinforced by our previous results in which no protein denaturation occurred within the time course of some experiments that lasted an hour or longer [23], and by our present DSC experiments which show no protein denaturation at room temperature for this period of time.

The dissociation of antibody from BLMs due to the flow of the carrier electrolyte solution was dependent on the number of injections made and on the time that membranes containing antibody were exposed to a flowing solution (e.g., 1.0 mL/min). Signal magnitude dramatically decreased after 4 injections made within an hour, or after 3 injections within 2 h (all injections sequential, at equal time intervals). These results are consistent with those shown in Figure 5, which indicates a gradual decrease of signal magnitude. The time to obtain a signal of a magnitude ca. 4 pA is similar in both experiments. These results suggest that the dissociation of antibody from membranes occurs through charge neutralization due to antibody–antigen complex formation and subsequent disruption of the lipid–antibody electrostatic bonds.

Further experiments were done to ascertain the effect of the carrier flow rate on the number of injection cycles which could be done. It was found that the use of a flow rate of 2.0 mL/min limited the number of injections which could provide a calibrated transient signal to two. These results are consistent with the above hypothesis of antibody dissociation from membranes.

4. Conclusions

The results demonstrate the potential of BLMs for applications in flow injection immunoanalysis when using a stopped-flow mode for a limited number of repetitive antigen injections. Stabilized BLMs containing antibody can be used to analyze a number of injections of antigen, and regeneration by washing is fast, simple and inexpensive in contrast to other previously described methods [16–18]. The technique of preparation of fresh BLMs containing antibody is also fast and simple (another film casting step), and permits preparation of membranes useful for a further series of antigen injections without disassembly and cleaning of the detection chamber.

5. References

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