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13. ABSTRACT (Maximum 200 words)
Ligand-Receptor binding is important to the development of biosensors for CBW defense. In the case of antigen (Ag)-antibody(Ab) binding, the binding constant is often so high that the response time of a continuous sensor is very long. It is desirable to regulate or control the binding constant in order to optimize the response time and sensitivity of an immunosensor. Two methodes were studied:

1) Use of a thermal pulse at the interface to raise the interfacial temperature, which for many Ag-Ab systems--results in decreased binding.
2) Direct modifications of IgG antibody with a photo isomerizable compound, to enable binding constant modulation under external optical control.

The role of immobilization of Ab on its Ag binding properties was also studied.

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Regeneration of Immunologically-active Surfaces

by

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1. Problem Statement

The project was initiated on October 1, 1987. The key objective is to develop means to regulate antigen (Ag)-antibody (Ab) binding constants to facilitate the development of biosensors based on antibody recognition and receptor-ligand binding principles. Of particular interest and importance is the off-rate constant ($Ag\text{-}Ab \overset{k_{off}}{} Ag+Ab$). The off-rate constant is an inverse measure of the response time of a sensor (1). Three major approaches were proposed to regulate the off-rate:

1. Use of photoresponsive polymers to change the local solution environment, thereby changing (decreasing) the Ag-Ab binding constant.

2. Use of a thermal pulse at the interface to raise the interfacial temperature, which-for many Ag-Ab systems-results in a decreased binding constant.

3. Direct modification of IgG antibody with a photo-isomerizable compound, to enable the off rate constant to be modulated under external optical control.

2. Major Results

10/1/87 - 12/31/88:

There were two major findings and conclusions in this reporting period:

1. We have concluded that the photo-isomerizable synthetic polymers we have studied, particularly methacrylate polymers with photo active azobenzene side chains, do not appear practical as IgG binding constant "switches". We have discussed the concepts and our work to date with Dr. M. Irie, an expert on photoresponsive polymers (1), and J. Kopecek, our co-investigator who is an expert on water soluble synthetic polymers. The results of the discussions were that it is unlikely that we could ever achieve a polymer dimensional change sufficient to provide the binding constant switching envisioned and proposed in the original proposal.

   Although the problem is one of considerable scientific interest, we have decided to focus our efforts and resources on the other two approaches to binding constant regulation. These approaches have a far greater probability of practical success for Army biosensor application.

2. We have learned that practically all common methods of IgG immobilization on biosensors result in highly irreversible binding of Ag--ie. it is extremely difficult to remove bound Ag by any reasonable means (2). We now feel it is necessary to immobilize IgG via a highly hydrophilic water soluble polymer or gel and to keep the IgG away from interactions with any rigid, solid surface, such as the optical fiber surface. Thus, we are directing our IgG immobilization efforts to the use of a gel, such as agarose and polyhydroxypropyl methacrylamide, on the sensor surface. We are also studying the grafting of polyethylene oxide (PEO) and other neutral hydrophilic polymers to the sensor surfaces, followed by IgG immobilization (4).

1/1/89 - 6/30/89:

During the past six months we have focused on two approaches:

1. Use of a thermal pulse at the interface to raise the interfacial temperature, which for many Ag-Ab systems results in a decreased binding constant.

2. Direct modification of IgG antibody with a photo-isomerizable compound, to enable the off rate constant to be modulated under external optical control.

One is generally overwhelmed by the large number of coupling chemistries available for the immobilization of Abs. Many different functional groups have been derivatized on various types of solid surfaces to match functional groups on biomolecules. However, the effects of the adsorption properties of solid supports and biomolecules on their surface activity have not been discussed. We have demonstrated that on silica surfaces the adsorption effects cannot be ignored (2). The orientation of the Ab on such surfaces will be partly determined by its adsorption properties instead of exclusively by matching of reactive functional groups (2).

Dissociation of Ab-Ag complexes on solid surfaces has been investigated using different combinations of Abs, Ags, and substrates. It has been observed that most Ab-Ag binding on solid surfaces is virtually irreversible. Several hypotheses have been suggested (3) to explain the slow dissociation process: 1) change in solution microenvironment at interfaces, 2) non-specific adsorption, 3) lateral interactions of bound antibody, 4) conformational change of immobilized antibody, 5) multivalent binding, and 6) diffusion limitations.

Each mechanism plays a different role in different situations. On flat and nonporous surfaces, such as silica and synthetic polymers, for example, non-specific adsorption probably dominates the overall dissociation rate when protein antigens are used, whereas translational diffusion is expected to have a small effect. This is one of the obstacles to the development of truly on-line and remote immunosensors. One approach to overcome this problem is to recreate the solution environment for the immobilized Ab. Thus we are studying immobilization of Abs through long hydrophilic polyethylene oxide (PEO) tethers (4). We are also looking into some very novel and easy to apply surface modification chemistry using a photo-initiated PEO binding to surfaces (5).

We have studied three heating processes for thermal regulation of Ag-Ab binding: 1) electrically resistive heating, 2) radiative heating, 3) and evanescent wave.

We have investigated the use of an evanescent wave heated surface inside of a flow cell by using computer simulation. The equations developed by Hansen (6) have been used. In this work we estimate the energy flux that can transmit through a 1mm thick quartz slide into the water. Since the absorption coefficients of water are high enough in the near infrared the analysis model has assumed all transmitted energy is absorbed by the water medium.

As the energy flux of the evanescent wave is very small compared to the incident energy flux, it is impractical to use the evanescent wave to heat the flow cell. The evanescent wave energy flux is on the order 10^{-6} to 10^{-7} of the incident energy flux.

7/1/89 - 12/30/89:

During the past six months we have focused on two main tasks:

1. Ab immobilization resulting in a solution-like environment so as to maintain the reversibility of Ag-Ab binding.

2. Ab binding constant modulation utilizing a photo-isomerizable compound bound in the Ab's deep pocket.

Effort has been devoted to immobilization of Abs through long hydrophilic polyethylene glycol (PEG) tethers. The purpose of using PEG tethers is to recreate the solution environment for the immobilized Abs and in turn minimize the nonspecific adsorption of bound Ag. PEG with molecular weights of 2000, 3400, 5000, and 8000 have been derivatized with hydrazine (PEG-Hz). The PEG-Hz was then immobilized on glutaraldehyde-treated silica surfaces in different conditions. X-ray photo-electron spectroscopy was used to determine the relative PEG surface concentrations by monitoring carbon 1S peaks. It was found that PEG-Hz immobilized in acetate buffer (pH 5.2) containing 11% K_2SO_4 at 60° C gave the highest ratio of ether carbon over alkyl carbon. The next step is to immobilize oxidized Abs on the PEG-Hz-coated surfaces or to further modify the PEG-Hz surfaces for immobilization of native Abs.

We also continue the study of the reversibility of Ab-Ag binding at solid-liquid interfaces. Murine monoclonal antifluorescyl Ab has been used as the model Ab-Ag system in the recent experiments. Various types of substrates have been used to immobilize the Ab or Ag. Although it is difficult to prove if there are any changes in intrinsic binding properties, results indicated that apparent binding is in part affected by the types and geometries of substrates used, as well as procedures for separation of free and bound analytes. We are currently looking into the possibility of using fluorescence spectroscopy for in situ monitoring of this behavior.

We have been investigating the possibility of modulating an antibody's (Ab) affinity for its antigen (Ag) through light-induced conformational changes of the Ab-Ag complex. Our goal is to bind a small photo-isomerizable compound inside a dead space beneath the Ab's binding site, then allow the Ag to bind normally to the Ab. We expect that subsequent light irradiation will cause the small photo-isomerizable compound to change its conformation, thereby inducing physical changes in the Ab-Ag complex, which are expected to decrease the affinity of the complex.

We have been studying a model system that has been extensively characterized crystallographically by Allen Edmundson and colleagues (8). Our immunoglobulin protein is a Bence-Jones light chain dimer from the patient termed Mcg. The Mcg dimer acts as a primitive Ab by binding haptens in a defined truncated cone-shaped binding site. The structure resembles an Ag binding (Fab) fragment of an Ab in that one of the light chains simulates the role of a heavy chain (9).

The floor of the binding site is made of Tyr residues which form a barrier to a deep pocket beneath. Debra Harris has studied the binding of various peptides to Mcg crystals (10). She found that the compound known as Wünsch's substrate, made of the five amino acid chain Pro-Leu-Gly-Pro-D-Arg, derivatized with azobenzene (PZ) at the N-terminus, bound irreversibly in the crystal with the PZ moiety located in the deep pocket, and the amino acid chain trailing in the main binding site. PZ was believed to be the major factor in Wünsch's binding. PZ was also found to be in about a 1:1 ratio for the trans and cis conformations. The energy for the conformational change came from the X-rays used to collect the crystallographic data.

We have been studying the behavior of the model system of Mcg and Wünsch in solution. We have determined an average equilibrium binding constant (Ka) of 10^4 M^{-1} for Mcg versus Wünsch in the trans conformation through the methods of fluorescent quenching and equilibrium dialysis. This value is much lower than the irreversible condition of the crystal. Thus we have not yet conclusively determined if we have realized our goal of binding the photo-isomerizable PZ moiety in the deep pocket. Preliminary results indicate that both the on and off rates are probably quite slow, on the order of days.

To enable another antigenic compound to bind in the main cavity of Mcg, it is necessary to bind a smaller PZ compound in the deep pocket that does not fill the main cavity as Wünsch does. We have performed binding experiments with the smaller compounds PZ-Pro and PZ-NH_2, but in neither case has the binding been as strong as with Wünsch. The fluorescent pressure method may enable a smaller compound to bind in the deep pocket. Rhodamine 123 can then be used as a substrate to bind in the main cavity. Rhodamine 123's binding has also been crystallographically characterized by Edmundson and colleagues (11). Upon binding Rhodamine123, irradiation experiments should convert PZ from trans to cis.

1/1/90 - 6/30/90

During the past six months we have focused on two main tasks:

1. Ab immobilization result in a solution-like environment so as to maintain the reversibility of Ag-Ab binding.

2. Ab binding constant modulation utilizing a photo-isomerizable compound in the Ab's deep pocket.

1. Immobilization:

Dissociation of Ag from immobilized Abs on agarose beads, porous silica beads, and flat silica chips was investigated. Two model Ab-Ag systems were used: polyclonal anti-human serum albumin (anti-HSA) Ab and monoclonal anti-fluorescyl (anti-Fl) Ab. After binding of iodinated HSA by the immobilized anti-HSA, three conditions were used to dissociate the bound HSA: 1) soaked in buffer, 2) exchanged with unlabeled HSA, and 3) soaked in 6M GuHCl to completely denature the immobilized anti-HSA. Percentages of the bound Ag dissociated are summarized in Table 1 and dissociation time is indicated in parenthesis. It is clear that the types and geometries of substrates have a significant influence on the dissociation of Ag-Ag complexes.

For the monoclonal anti-Fl system, because it is not possible to monitor fluorescence from bound fluorescein on agarose beads, we synthesized iodinated tyrosin-fluorescein (Tyr-FI) conjugates for use as the Ag (specific activity of 10^{13} CPM/mole). A series of dissociation experiment was performed with the anti-Fl Ab immobilized on agarose gels. Unfortunately, we observed that the fluorescein or its radiolabeled conjugates nonspecifically adsorbed to the agarose gel. We are currently immobilizing the anti-Fl Ab to flat silica chips. Due to the small surface area of the chips, a fluorescein conjugate with a specific activity of 10^{15} CPM/mole is required. We are synthesizing fluorescein and Bolton-Hunter reagent conjugates, which can be radiolabeled in organic solvents. So far, a specific activity of 10^{14} CPM/mole has been obtained and more conditions will be investigated to further increase the activity in the near future.

We are also immobilizing anti-HSA on silica surfaces via PEG long hydrophilic spacers. Our preliminary results indicated that HSA dissociated from the anti-HSA immobilized via PEG spacers is faster than that from the anti-HSA directly immobilized on silica surfaces. However, the amount of anti-HSA immobilized on the PEG-coated surfaces is low. Current effort focused on the determination of surface concentration of PEG by using radiolabeling techniques.

Table 1. Percent dissociation of bound HSA from the anti-HSA immobilized on various substrates

Sub-strate	Soaked in buffer	Exchanged with HSA	6M GuHCl
Agarose (high [Ab])	5 (20 hrs)	70 (20)	95 (1)
Agarose (low [Ab])	5 (20)	65 (20)	-
Silica bead	2 (20)	30 (20)	40 (1)
Silica chip (high [Ab])	40 (87)	65 (87)	90 (2)
Silica chip (low [Ab])	65 (87)	70 (87)	85 (2)

2. Binding Constant Modulation

Pressures of 4-8 kbar have been shown to denature proteins, while pressures up to 3kbar reversibly dissociate protein domains without denaturation (12). The pressure-induced binding of various fluorescent compounds to Mcg was previously reported by Herron et al. (11).

We used pressures up to 3kbar to study the binding of various PZ ligands with Mcg. Mcg's binding cavity is located at the interface of the two monomer variable domains. We thought that these pressures would be sufficient to dissociate the monomers enough so that the PZ moiety of the ligands would be forced into Mcg's deep pocket, and would remain there upon release of the pressure. If the PZ moiety did indeed lodge there, we expected a significant decrease in Mcg's Tryptophan fluorescent intensity from non-radiative energy transfer to PZ. Our first experiments compared Mcg alone to Mcg vs. Wunsch. We saw no significant difference between the two cases. In both cases the relative fluorescent intensity of Mcg increased by about a factor of two. This indicated that high pressure was not enough to force Wuncsh's PZ moiety into the deep pocket. The low affinity was due to either PZ, or the peptide tail. We next tried a series of smaller ligands.

We have done initial pressure studies with the following ligands: PZ-Pro-Leu, PZ-Pro-Phe, PZ-Pro-OH, and aminoazobenzene. In the studies with Mcg alone, Mcg vs. PZ-Pro-Leu, PZ-Pro-Phe, and PZ-Pro-OH the relative fluorescent intensity increase of Mcg was again about a factor of two for each case. With Mcg vs aminoazobenzene, however, the relative increase was about a factor of eleven. Herron reported (11) a similar large intensity increase for Bis-ANS when it bound to Mcg under pressure. These results with aminoazobenzene should be due to its binding in Mcg's deep pocket. If this is true then the PZ moiety is probably the major factor in promoting binding of the discussed series of compounds.

7/1/90 - 10/14/90:

1. IgG Immobilization

We have previously demonstrated that the covalent immobilization of the partially denatured Abs (low pH treatment) on pre-treated silica surfaces resulted in higher antigen (Ag) binding capacity and higher Ab surface concentration than for the native Ab. These results suggest that noncovalent interactions (physical adsorption) play an important role in covalent coupling.

In order to understand the surface behavior of the partially denatured Abs mechanistically, four hypotheses have been formulated to guide experiments. The first hypothesis was that the increase in the Ag binding capacity of the immobilized partially denatured Abs was due to the increase in nonspecific binding resulting from the acid denaturation. Thus, we determined the solution activities of the native and partially denatured Abs. The results showed that the activity of the denatured Abs is approximately 25% lower than the native Ab in solution (Table 2). This means that the increase in surface Ag binding capacity must be a surface induced phenomenon, and is not due to changes in the specificity of the Abs.

Table 2 The antigen binding activity of native and partially denatured goat polyclonal anti-(human serum albumin, HSA) IgG (acid treatment for 1 hour) in solution

Anti-HSA*	Maximum bound HSA ($\times 10^{-7}$ M)
Native	1.4 ± 0.0
Denatured	1.0 ± 0.0

Table 3 Surface affinities of native and partially denatured anti-HSA Abs on DDS-treated silica surfaces

Antibody	Surface affinity (10^6 M^{-1})
Native	4.7
Denatured (1 min)	5.2
D (20 min)	8.9
D (60 min)	9.0
D (300 min)	6.4

Table 4 Competitive adsorption of the native and whole or monomer IgG fraction of the partially denatured anti-HSA on DDS-treated silica surfaces. Data for whole IgG fraction were taken from Figure 2.1 (D(1:1) and N(1:1) at 3 hour adsorption time)

Partially Denatured Ab	5.0 ± 0.4 (whole IgG fraction)	4.9 ± 0.7 (monomer IgG fraction)
Native Ab	0.8 ± 0.2	0.7 ± 0.4

Unit: 10^{-12} mole/cm^2

Table 5 Binding of the antigen (iodinated fl-BSA-I-125) by the partially denatured anti-fluorescyl 9-40 monoclonal Ab immobilized on DDS treated-silica surfaces as a function of Ab storage time in buffer

Storage time (hrs)	FI-BSA-125I-bound ($\times 10^{-13}$ mole/cm^2) Native	Denatured (60 min)
0	5.3 ± 0.71	22.2 ± 1.10
3	4.6 ± 0.28	17.3 ± 0.64
6	-	16.5 ± 0.42
20	4.8 ± 0.55	14.7 ± 0.57

The second hypothesis was that the increase in the surface concentrations of the immobilized partially denatured Abs was due to the changes in the affinity of the Abs to the surfaces. To demonstrate this point, two types of experiments were performed. First, the surface affinities of the native and partially denatured Abs on dimethyldichlorosilane (DDS)-treated silica surfaces were determined by radiolabeling technique. Values of 10^{-6} M^{-1} for all Abs were obtained (Table 3). One to two factor differences in affinity constants are not significant because affinity measurements are subject to large experimental uncertainty. The second experiment was the competitive adsorption of the native and partially denatured Abs as a function of adsorption time. It was found that the partially denatured Abs adsorbed to the surfaces much more effectively than the native Ab (Figure 1). For example, after three hours adsorption, Ab surface concentrations of 4.5×10^{-12} and 0.7×10^{-12} mole/cm^2 for the denatured and native Abs were obtained, respectively.

It is well known that denatured proteins tend to form aggregates in solution. Therefore, the enhanced surface concentrations of the partially denatured Abs may be caused by the preferential adsorption of the aggregates (Third hypothesis). Two techniques were used to study the aggregation of the partially denatured Abs: light scattering and gel filtration chromatography. The results from light scattering experiments showed that the size of the partially denatured Abs at very low concentration is approximately 20% larger than the native Ab and their size increases linearly with increasing concentration, indicating the presence of the Ab aggregates. The aggregates were then separated from the monomer fraction by using Fast Performance Liquid Chromatography. A competitive adsorption experiment was then performed by mixing equal amounts of the native and monomer fraction of the partially denatured Ab. The results still showed strong preferential adsorption of the denatured Ab (Table 4).

The fourth hypothesis was that, although they were monomers in solution, the partially denatured Abs formed aggragates on surfaces, resulting in multilayer adsorption. Therefore, the thickness of the adsorbed native and partially denatured Abs was determined by ellipsometry. Ellipsometry is an optical method in which light undergoes changes in polarization when it is reflected at a surface. From these changes, it is possible to determine the refractive index and thickness of the thin layer, provided that the optical parameters of the surface and the environment are known. Values of approximately 50 Å and 70 Å for the thickness of adsorbed native and the partially denatured Ab was not present in multilayers. The difference in thickness was due to different orientations of the Abs on the surfaces.

Based on the results discussed above, we have come to the conclusion that by changing conformation of certain portion of IgG molecules (the ideal case would be when changes occur only in Fc domain), the surface packing efficiency and orientation of an immobilized Ab are dramatically affected. It also means that physical adsorption dominates the orientation of immobilized Abs on silica surfaces. Although chemical bonds may be useful in providing stable linkages between the Ab and surface, they do not affect orientation of Ab on the surface. This concept is applicable to all materials that have high nonspecific protein adsorption properties.

So far, all Abs used were polyclonal anti-human serum albumin (HSA) Ab (IgG fraction purchased from Cappel Laboratories). We have also

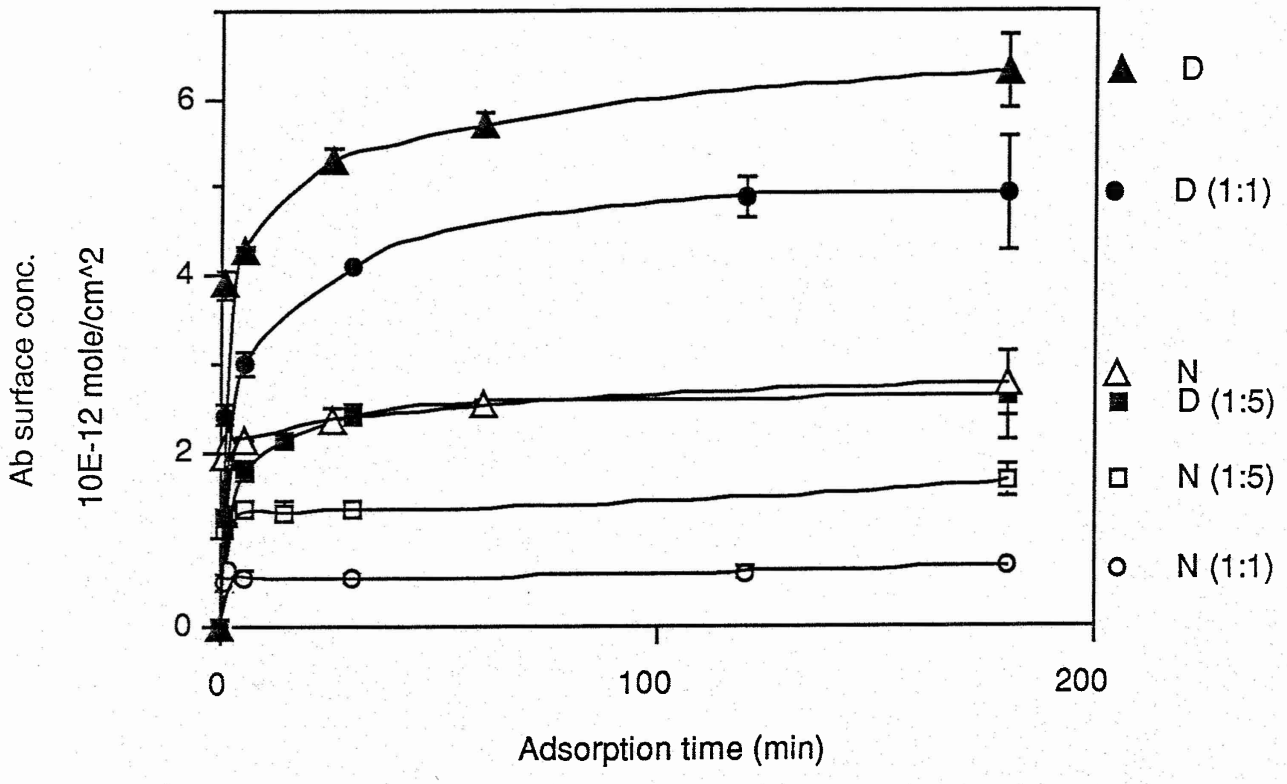

Figure 1 Adsorption kinetics of single or two (native and partially denatured anti-HSA Abs) component on DDS-treated silica surfaces. D: partially denatured Ab (acid treatment for 1 hour at pH 2.8); N: native Ab; D(1:1), N(1:1): mixture of equal amounts of the partially denatured and native Abs; D(1:5), N(1:5): mixture of 5 times more the native Ab than the partially denatured Ab.

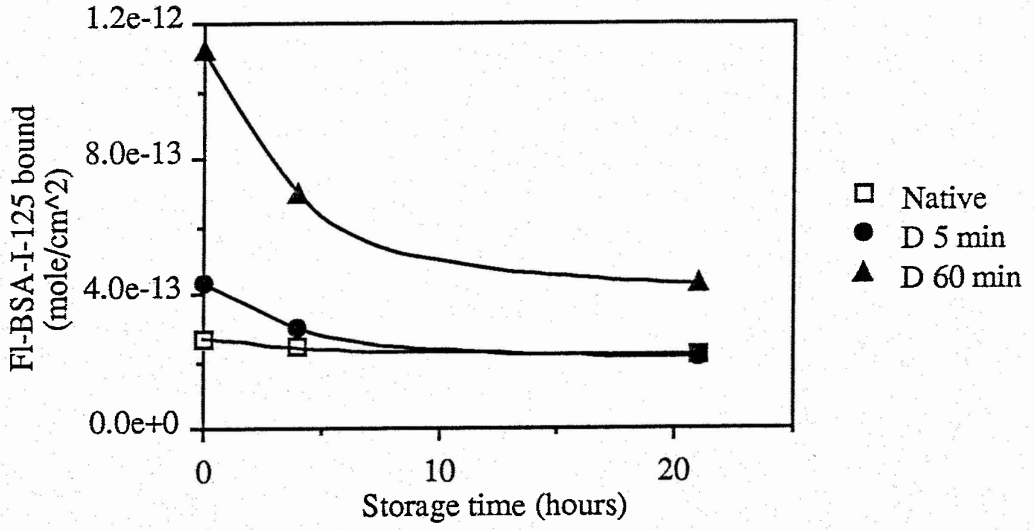

Figure 2 Binding of antigen (iodinated fl-BSA-I-125) by anti-fluorescyl 4-4-20 monoclonal antibody immobilized on DDS treated-silica surfaces as a function of storage time of the partially denatured 4-4-20 antibody. 0 min storage time means that the partially denatured antibody was immobilized immediately after denaturation process. Otherwise, the antibody solution was stored at room temperature for various lengths of time before immobilization.

performed preliminary experiments using monoclonal anti-fluorescyl 4-4-20 (IgG2) and 9-40 (IgG1) Ab. The Ag used in this case was FITC-labeled BSA (degree of labeling was about 10) followed by iodination of the FITC-BSA. It was observed that both partially denatured 4-4-20 (Figure 2) and 9-40 (Table 5) Abs (1 hour acid treatment) had four times higher Ag binding capacity than the native Abs on the silica surfaces if the denatured Abs were immobilized immediately after denaturation. However, the Ag binding capacity decreased rapidly with increasing storage time, suggesting that the partially denatured 4-4-20 and 9-40 Abs renatured back to their original conformation. We also studied the dissociation of the bound FITC-BSA from the immobilized Ab by soaking the samples in a buffered solution or a solution containing excess fluorescein. The results showed that the dissociation of the monoclonal anti-fluorescyl Abs on silica surfaces is very similar to the case of the polyclonal anti-HSA Ab. The rate of dissociation is still very slow (only about 30% bound FITC-BSA dissociated over 24 hours soaking period). This slow dissociation may be due to the high degree of labeling of FITC-BSA, resulting in multivalent binding. Recently, we have synthesized iodinated monovalent Ag conjugates by reacting aminated fluorescein with Bolten-Hunter reagent. The multivalent binding should be minimized by using this conjugated Ag, the work is ongoing.

2. Binding Constant Modulation

We have investigated the solution binding phenomena between various photo-isomerizable ligands with a Bence-Jones immunoglobulin light chain dimer (Mcg). The ligands are derivatized with an azobenzene moiety (PZ) which can photo-isomerize from trans to cis with irradiation at 325 nm. The process is totally reversible with irradiation at 425 nm. We have described our system as being an excellent model to study the possibility of modulating an antibodies' (Ab) affinity for its antigen (Ag) through light-induced conformational changes of the Ab-Ag complex.

We have studied the solution binding behavior of a series of PZ derivatized ligands to the Mcg protein. We have used methods of fluorescent quenching titrations, equilibrium dialysis, chromatographic methods, and fluorescent pressure techniques to elucidate the magnitude and location of the ligands' binding to Mcg. The Mcg binding site is composed of an outer main cavity with a deep pocket beneath. The crystallographic location of PZ was in the deep pocket, making Wünsch's binding to Mcg irreversible.

We initially performed fluorescent quenching titrations and equilibrium dialysis to determine the magnitude of PZ ligand binding to Mcg, and a couple of related Bence-Jones proteins. The PZ ligands we have used are Wünsch, PZ-Pro-Leu, PZ-Pro-Phe, PZ-Pro-OH, and aminoazobenzene. The proteins we have used are Mcg, Hud, and a hybrid protein composed of one light chain each from Mcg and Hud. In all experiments to date the maximum equilibrium association constant (Ka) observed for these ligands with each of the three proteins has been in the range of 10^4 M^{-1}. These two techniques have verified one another in placing Ka in this range. This value is much lower than the irreversible condition of crystalline Mcg with Wünsch. From this it was determined that the PZ ligands were probably binding in the outer main cavity of Mcg under solution equilibrium conditions.

We next performed chromatographic and fluorescent pressure experiments to determine if a stronger driving force for binding could facilitate PZ

binding in the deep pocket. In chromatographic experiments we either combined Mcg with a large excess of ligand for long times (4 weeks), or pressurized a sample of Mcg with excess ligand to 2.75 kbar for ~one hour, before passing the mixture over a sephadex column. The column was intended to separate the free from bound ligand. Previous dialysis experiments had indicated that the ligand kinetic off-rate was possibly quite long, on the order of days, such that the time required to elute from the column would not significantly interfere with the off-rate. In all cases to date, however, the column has completely separated the protein from all ligand. Thus this method did not give enough driving force to bind the PZ moiety in Mcg's deep pocket.

Fluorescent pressure methods were also thought to increase the driving force for ligand binding to Mcg. Pressures of 4-8 kbar have been shown to denature proteins, while pressures up to 3 kbar reversibly dissociate protein domains without denaturation (12). Mcg's binding site lies at the interface between the two variable domains of the immunoglobulin light chains. Pressures around 3 kbar have been shown to cause relaxation between Mcg's variable domains enabling the binding of various fluorescent compounds (11).

We have performed pressure studies up to 2.75 kbar for the same PZ ligands listed previously, and Mcg. A protein intensity increase with pressure has been observed for all ligands. Initially the increase was similar for Mcg alone, and Mcg with the ligands except aminoazobenzene. In some experiments with Mcg versus aminoazobenzene a large intensity increase of about ten fold was observed. This was similar to results reported by Herron (11) for Mcg versus Bis-ANS, which we also repeated as a positive control. These results, however, have not yet been consistently reproducible due to unresolved complications with the pressure instrumentation. Further, our results have indicated the necessity of pushing the pressures up to between 3 and 4 kbar before association constants will be able to be accurately determined.

In order to investigate the possibility of modulating Mcg's affinity for a ligand in its outer main cavity, it is necessary to have a small photo-isomerizable PZ ligand stably bound inside Mcg's deep pocket. Aminoazobenzene is the best PZ ligand candidate for binding in the pocket: because of its small size it can be completely accommodated in the pocket leaving the main cavity free to associate with other ligands. Our original intention was to study affinity modulation with Rhodamine 123 in the main cavity once a PZ ligand was unequivocally bound in the deep pocket. Currently the work with aminoazobenzene looks the most promising, and we will be pursuing results to clearly determine if aminoazobenzene can be irreversibly bound inside Mcg's deep pocket (13).

Summary

The original proposal concepts are still valid. Ab immobilization must be performed with full cognizance of the role of adsorption. If low molecular weight polyethylene oxide tethers are used, and if the surface is otherwise passivated (via protein or PEO binding) to minimize further adsorption of Ab, then immobilized Ab may indeed be induced to release bound Ag.

The original thermal pulse approach developed herein could then be applied for those Ab-Ag systems for which the thermodynamics are suitable.

The question of the possibility for the use of deep-pocket photo-isomerizable ligands to help modulate Ab-Ag binding is still open. The completion of Ms. Turcotte's MSc thesis should enable an assessment to be made(13).

3. List of Publications

"Comparison of Site-specific Coupling Chemistry for Antibody Immobilization on Different Solid Supports," J.N. Lin, I-N Chang, J.D. Andrade, J.N. Herron, D.A. Christensen, J. Chromatography, in press (1991).

"Immuno-Biosensors: The Clinical Chemistry and Coagulation Laboratory on a Chip," J.D. Andrade, J-N Lin. V. Hlady, J.Herron in Y. Sezai, eds., Biomation in the 21st Century, Saunders, 1990, in press.

"Spatially Resolved Detection of Antibody-Antigen Reaction on Solid/Liquid Interface Using Total Internal Reflection Excited Antigen Fluorescence and Charge-Coupled Device Detection," V. Hlady, J.N. Lin, J.D. Andrade, Biosensors, 5 (1990) 291.

"Immunosensors: Remaining Problems in the Development of Remote, Continuous, Multi-Channel Devices," J. D. Andrade, J-N Lin, V. Hlady, J. Herron, D. Christensen, and J. Kopecek, chapter in R. B. Buck, et al., eds. Biosensor Technology, Dekker, Publ., (1990) pp. 219-239.

"The Influence of Poly(ethylene oxide) Spacers on the Covalent and Non-specific Binding of Immunoglobulin G to Silica Surfaces," P. Kopeckova, J. Kopecek, and J. D. Andrade, New Polymeric Materials 1 (1990) 289-297.

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5. Inventions

None.

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