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Modification of $\beta_2$glycoprotein I by Glutardialdehyde

Conformational Changes and Aggregation Accompany Exposure of the Cryptic Autoepitope

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Abstract

Autoantibodies from patients with antiphospholipid syndrome (APS) recognize an epitope on $\beta_2$glycoprotein I (β_2GPI) only when native β_2GPI is adsorbed on surfaces composed of anionic phospholipids or oxidized polystyrene. β_2GPI was modified with the crosslinking agent, glutardialdehyde (GDA), which induced exposure of the anti-β_2GPI epitope at GDA:β_2GPI mol ratios in the range of 500–2000. A second crosslinking agent, dimethylsuberimidate (DMS), did not expose the epitope, which may be a consequence of its having less tendency than GDA to form intermolecular links. SDS-PAGE experiments demonstrate that GDA does promote extensive intermolecular crosslinking of β_2GPI, and DMS does not. Formaldehyde also reacts with the lysine residues of β_2GPI, but does not expose the epitope. The circular dichroism spectra of native and modified β_2GPI confirm that GDA induces changes in conformation that are qualitatively different from those caused by formaldehyde. These data provide evidence that binding of lysine residues is not a sufficient condition for exposure of the autoepitope, and also support the likelihood that anti-β_2GPI antibodies bind only to aggregates of the protein. Thus, by synthesizing an active holoantigen of β_2GPI, conditions were defined that are necessary for binding of human autoantibodies.

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The authors also suggest that treatment of phospholipid-binding proteins with chemical agents might provide a strategy to modify their structure and permit exposure of epitopes, resulting in synthetic antigens for therapeutic and diagnostic use.

Index Entries: Antiphospholipid syndrome; $\beta_2$glycoprotein I; autoimmune diseases; protein crosslinking; circular dichroism; glutardialdehyde.

Introduction

$\beta_2$glycoprotein I (β_2GPI) is one of several plasma proteins against which autoantibodies are produced *(1–5)* in patients with the autoimmune, hypercoagulable state referred to as antiphospholipid syndrome (APS) *(6)*. The anti-β_2GPI autoantibodies from APS patients bind to the protein only when it is attached to certain surfaces, such as anionic phospholipid membranes *(7,8)* and oxidized polystyrene, but not untreated polystyrene *(9,10)*. Binding of β_2GPI to these surfaces is believed to either alter the conformation of the protein in a manner that exposes a normally cryptic autoepitope *(11,12)* or to generate clusters of β_2GPI to which the antibodies bind *(2)*. In vivo, the surface-bound antibody–protein complex presumably interferes with the normal regulation of blood coagulation *(2,13–17)*.

β_2GPI is composed of 326 amino acids with five N-linked glycans that represent approx 20% of its mol wt. It is a member of a family of immunologically important proteins composed of short consensus repeats (SCR). In β_2GPI, these SCRs occur as five sushi domains *(18–20)*, which comprise nearly the entire primary sequence. The first and fifth domains are involved in binding to anionic phospholipids of membranes *(20–24)*, and encrypted epitopes for autoantibodies have been identified in both the fourth and fifth domains *(20–22)*.

There are 30 lysine residues in β_2GPI, 15 of which are located in the fifth domain, with a cluster of four defining the lipid binding site *(19–24)*, i.e., Lys^{282}, Lys^{284}, Lys^{286}, and Lys^{287}. There are also lysines on the ends of the putative epitope for anti-β_2GPI *(20,23)*: Lys^{266}, Lys^{268}, and Lys^{276}. Interaction with some combination of these lysines is probably responsible for epitope exposure, i.e., electrostatic interactions with the anionic lipid surfaces *(7)*, and possibly Schiff base reaction with carbonyl sites on oxidized polystyrene surfaces *(9,10)*.

In this study, the authors covalently modified the lysine residues with formaldehyde and glutardialdehyde (GDA), in order to mimic the lysine chemistry that may occur on the oxidized polystyrene. The intent was to determine whether such modification can effect exposure of autoepitopes on β_2GPI. Formaldehyde was chosen to test whether it is sufficient to simply block individual lysine groups and, in that way, to alter the protein conformation. GDA will likewise block the lysine residues by forming Schiff bases, but it can also form intramolecular and intermolecular crosslinks among lysine residues *(25)*. Exposure of the epitope may require multiple points of attachment to the oxidized polystyrene or phospholipid surface

in what is, effectively, a crosslinking of lysines; GDA was chosen to mimic this type of interaction. A second crosslinking agent, dimethylsuberimidate (DMS), was used to distinguish between the effects of intramolecular and intermolecular crosslinks, because it is less prone to forming intermolecular links *(26)*.

To determine the effectiveness of protein modification in exposing the autoepitope of β_2GPI, the authors took advantage of the observation that native β_2GPI does not react with autoantibodies when adsorbed on untreated polystyrene *(11)*. A standard enzyme-linked immunoassay (ELISA) was used to determine whether the aldehyde or DMS modification of β_2GPI promotes anti-β_2GPI, if untreated polystyrene is used as the solid support. Circular dichroism (CD) was used to detect conformational changes in β_2GPI induced by derivatizing the lysine residues.

Materials and Methods

Materials

The β_2GPI used in this study was obtained from Crystal Chemical (Chicago, IL); GDA was purchased as a 50% solution in water (Aldrich, Milwaukee, WI); formaldehyde was purchased as a 37% solution (Sigma, St. Louis, MO); and DMS was obtained as dihydrochloride salt (Sigma).

The source of the probe autoantibody was a reference calibrator, HRM-01, described previously *(27)*. This is a recalcified plasma containing IgG anti-β_2GPI, and was used in this study with no further modification other than dilution, as described below. The control probe (with no appreciable anti-β_2GPI antibody activity) was LRM-01 *(27)*.

Untreated polystyrene microtiter plates were obtained from Xenopore (Hawthorne, NJ). These plates have a hydrophobic surface that binds β_2GPI, but does not permit exposure of the autoepitope on the native protein *(11)*.

Incubation Protocol

β_2GPI and stock solutions of the aldehydes were prepared with 0.15 *M* NaCl and 0.015 *M* phosphate, pH 7.4 (phosphate-buffered saline, PBS). These solutions were chilled in an ice bath before and during mixing, to inhibit reaction between β_2GPI and the aldehydes until they were thoroughly mixed. After the appropriate concentrations of reagents were added to 10 µg/mL β_2GPI solutions to achieve the final molar ratio, the mixtures were brought to 37°C and incubated for 1 h before binding, electrophoretic, or spectroscopic studies were performed.

Binding Studies

Epitope exposure and subsequent binding by a reactive autoantibody was assayed by ELISA on polystyrene microtiter plates. As mentioned, the hydrophobic surface of this material does not alter the conformation of the protein in a manner that exposes the epitope. The incubation mixtures

(100 µL) described in the preceding subheading were added to the microtiter plate wells and incubated 2 h at 37°C. The wells were then washed thrice with 3% bovine serum albumin (BSA) in PBS (BSA–PBS). The same BSA–PBS solution was used to block unbound sites on the wells by incubating the wells with BSA–PBS 2 h at 37°C.

After blocking, the wells were incubated with 1:100 dilutions of HRM-01 or, in the case of the matrix control study, LRM-01. These incubations were carried out for 15 min at room temperature (RT, 25 ± 1°C), after which the wells were washed four times with BSA–PBS. They were then incubated (15 min, RT) with 100 µL horseradish peroxidase-conjugated goat anti-human IgG (1:500 dilution of stock affinity-purified antibody containing 1 mg/mL total protein, obtained from Cappel Research, Division of Organon Teknika, Durham, NC). The wells were again washed with BSA–PBS and incubated with 100 µL substrate solution prepared by mixing a tetramethylbenzidine solution with an equal volume of H_2O_2 solution, both obtained from Kirkegaard and Perry, Gaithersburg, MD. After 10 min at RT, the reaction was stopped by the addition of 100 µL 2.5 *N* sulfuric acid (reagent grade). Absorbance was measured at 450 nm in a Bio-Tek (Winooski, VT) model EL307C microtiter plate reader vs a reagent blank.

Electrophoretic Methods

All electrophoretic procedures were carried out on mini-gels using the PhastSystem™ (Pharmacia, Piscataway, NJ). Isoelectric focusing (IEF) was performed using p*I* 3–9 gels, as previously described *(28)*. The protein (8 µg) was applied using 4 µL capillary combs. Polyacrylamide gel electrophoresis (PAGE) was carried out after treatment of the protein with β-mercaptoethanol (5% w/v) and sodium dodecyl sulfate (SDS 2% w/v) in PBS containing 1 m*M* ethylenediaminetetraacetate (EDTA) for 5 min at 100°C. SDS-PAGE was run on 4–15 gradient gels (4.5% T, 3% C) using buffer strips containing SDS, as supplied by the manufacturer, for 63 Vh. For SDS-PAGE, 1.7 µg protein was applied. Silver staining was used to visualize the bands.

CD Studies

CD spectra were collected on an Aviv model 62ADS CD Spectrometer (Aviv Associates, Inc., Lakewood, NJ) at 37°C (thermostated turret) using a 1-nm band pass and a 5-mm path-length cell. The spectra were collected using 2 m*M* protein, following the incubation protocol described above.

Results

IEF: Evidence of Covalent Modification by Aldehydes

Richter and Cleve *(29)* have reported six phenotypes for β_2GPI in a pool of 400 donors and observed 11 unique isoforms by IEF. All six phenotypes show a single band for the most anionic isoform, and several of the

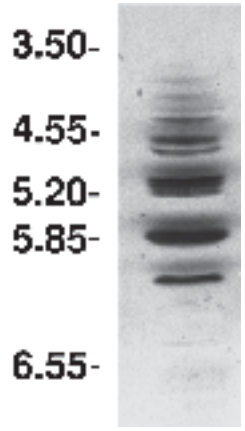

Fig. 1. IEF of native β_2GPI. In the p*I* range of 3.5–6.5, there appear to be 15 or more bands for native β_2GPI; 11 bands were expected on the basis of the reported isoforms *(29)*. Some of these may be the result of partially desialylated protein, impurities, or even unreported isoforms.

bands are closely spaced, giving the appearance of doublets *(29)*. IEF of the β_2GPI used in this work shows more than 11 discrete IEF bands between pH 3.5 and 6.5 (Fig. 1), some of which may be impurities, additional isoforms, or partially sialylated species.

Figure 2A shows changes in the IEF of β_2GPI induced by reaction with formaldehyde, which should form Schiff bases with exposed lysine residues, removing one positive charge from the protein for each point of reaction. At 1:1 mol ratio of formaldehyde:β_2GPI (lane 2), there is little evidence of reaction, but, by 200:1 (lane 4), the most cationic band is clearly vanishing, and more anionic bands are appearing or increasing in intensity. At 1000:1 (lane 7), virtually no native β_2GPI remains, and increasing the level of formaldehyde beyond this results in a general anodic shift in the poorly resolved derivatives. There are only 30 lysines in the protein, and the bands should shift if any one lysine is blocked by formaldehyde. The requirement for relatively high levels of formaldehyde to induce a change in the IEF is probably a consequence of slow kinetics for the reaction.

GDA appears to be much more reactive (Fig. 2B), with a clear increase in the intensity of the anodic bands, even at 1:1 GDA:β_2GPI. The fact that some native β_2GPI remains at 1:1 (lane 2) is probably a consequence of some protein getting more than one adduct, depleting the GDA before all β_2GPI is reacted. This is likely to occur during the initial mixing of the two solutions, if the reaction is relatively fast. By 10:1 GDA:β_2GPI (lane 3), no native protein remains, although discrete bands are still visible closer to the anode. At 100:1 (lane 4) and higher levels of GDA, definition of the individual bands is lost, and a single broad band appears, with some smearing throughout the gel.

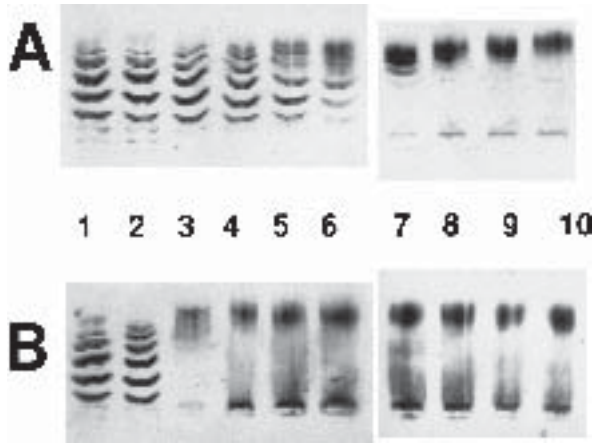

Fig. 2. IEF of modified β_2GPI. The protein was incubated with the aldehydes for 1 h at 37°C before being applied to the electrophoretic gel; p*I* increases from top (anode) to bottom (cathode). **(A)** Formaldehyde does react with β_2GPI, causing a decrease in p*I* probably as a result of blocking lysine residues. Lanes 1–6 are on a single gel, as are lanes 7–10. Both gels were run on the same day. Lanes 1–10 represent, respectively, the incubation products for formaldehyde:β_2GPI mol ratios of 0, 1, 100, 200, 300, 400, 1000, 2500, 5000, and 7500. The more cathodic bands are gradually replaced by a merged set of anodic bands as the aldehyde concentration increases. **(B)** GDA reacts even more readily than formaldehyde. Lanes 1–10 represent GDA:β_2GPI mol ratios of 0, 1, 10, 100, 200, 300, 400, 1000, 2500, and 5000, respectively. The cathodic bands are almost completely gone after 1:1 GDA:β_2GPI. In some of the lanes, there appears to be a general smearing of bands throughout the gel.

GDA-Induced Epitope Exposure on Polystyrene

GDA was added to β_2GPI in mole ratios ranging from 100:1 up to 10,000:1, and was incubated as described in Methods. The GDA-modified β_2GPI was adsorbed onto polystyrene, rinsed, and exposed to sera with either high anti-β_2GPI (HRM-01) or no anti-β_2GPI (LRM-01). Binding of IgG-class autoantibodies to the GDA-modified β_2GPI was then determined.

Both trials using high anti-β_2GPI demonstrate biphasic behavior in epitope exposure with increasing level of GDA (Fig. 3). In the absence of GDA, the assays for the two trials match that for the control experiment with LRM-01, which demonstrates virtually no anti-β_2GPI activity. Binding of anti-β_2GPI to the adsorbed β_2GPI increases with GDA level, until it reaches a maximum at 1000:1 GDA:β_2GPI. Increasing the amount of GDA beyond this reduces binding (Fig. 3). All three data sets were run in duplicate, and the error bars are the standard deviations of the duplicate measurements.

Neither Formaldehyde or DMS Induces Epitope Exposure on Polystyrene

To test whether the epitope is exposed as a result of crosslinking or simply modification of lysine residues, β_2GPI was incubated with

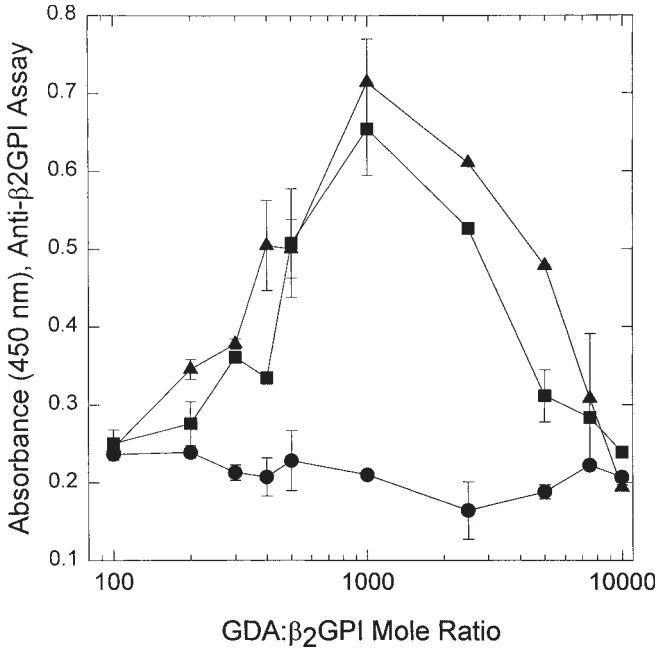

Fig. 3. Anti-β_2GPI binding to GDA-modified β_2GPI adsorbed on polystyrene: effect of GDA:β_2GPI mol ratio. The solid squares (■) and triangles (▲) are two separate trials using plasma with high levels of human IgG anti-β_2GPI (HRM-01); the solid circles (●) are trials using plasma with virtually no anti-β_2GPI (LRM-01). Each point is the mean of duplicate runs, with the error bars indicating the standard deviations about the means. β_2GPI was preincubated with GDA according to the incubation protocol, and then added to the wells of polystyrene microtiter plates. After allowing the modified β_2GPI to adsorb, the wells were rinsed thoroughly, exposed to the plasma with or without anti-β_2GPI, rinsed, and then assayed for human IgG using the horseradish peroxidase-conjugated goat anti-human IgG, as described in Binding Studies. In the absence of GDA, β_2GPI adsorbed onto polystyrene does not bind anti-β_2GPI. Binding does increase well above the control as the GDA:β_2GPI mol ratio is increased up to 1000:1 (■, ▲). Beyond that level, anti-β_2GPI binding drops off logarithmically (■, ▲). Virtually no change in anti-β_2GPI binding is observed with GDA, using the control plasma (●).

formaldehyde at mol ratios ranging from 1:1 up to 10,000:1 aldehyde: β_2GPI. No statistically significant change in anti-β_2GPI binding was observed, suggesting that crosslinking by GDA plays a role in revealing the epitope.

GDA is prone to polymerization, and may readily form intermolecular as well as intramolecular crosslinks *(25)*. In an effort to distinguish these two possibilities, β_2GPI was derivatized with a second crosslinking agent that does not readily polymerize: DMS. DMS crosslinking has no measurable effect on anti-β_2GPI binding to β_2GPI adsorbed onto polystyrene, suggesting that intermolecular crosslinks may be essential in artificially inducing epitope exposure.

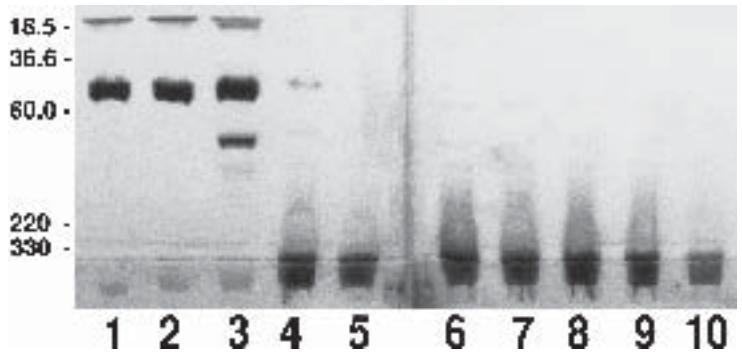

Fig. 4. SDS-PAGE: Detecting protein–protein crosslinks formed by GDA. SDS-PAGE of the native β_2GPI (lane 1) used in these experiments shows a broad band at about 50 kDa and a low mol wt component near the leading edge (top) of the gel. The numbers on the right of the gel label the position of the mol wt standards used to calibrate the gel. Notice that the multiple bands from the isoforms observed in the IEF have collapsed into the single broad band. The low mol wt species at the top is an impurity, and is probably responsible for some of the bands observed in IEF gels. Lanes 2–10 represent the incubation products from GDA:β_2GPI mol ratios of 1, 10, 100, 200, 300, 500, 1000, 2500, and 5000, respectively. Notice that, at 10:1 GDA:β_2GPI (lane 3), there is clear evidence of a dimer crosslink and possibly a trimer species. At 100:1 GDA:β_2GPI, there is only a trace of monomer protein left, and virtually all of the protein is near the origin, suggesting a very high degree of interprotein crosslinking. Some smearing of the protein occurs in lanes 6–9.

SDS-PAGE: Evidence of GDA-Induced Aggregation of β_2GPI

In order to determine whether GDA and DMS yield intermolecular crosslinks, SDS-PAGE experiments were run, with β_2GPI exposed to crosslinking agent at levels ranging from 1:1 up to 5000:1. Native β_2GPI appears as a single broad band with an apparent mol wt of about 50 kDa (lane 1 in Fig. 4A). The SDS has blurred the resolution of the various isoforms.

There is no change in the β_2GPI band at 1:1 (lane 2) GDA:β_2GPI; however, at 10:1 (lane 3), a dark band appears at about 100 kDa, along with a fainter band at higher mol wt. This strongly suggests dimer and trimer formation caused by GDA intermolecular links. At 100:1 (lane 4), almost no monomer β_2GPI remains, the dimer band is also very faint, and nearly all of the protein is close to the origin in the gel. All of the protein does appear to remain at the origin at 200:1 (lane 5). This strongly suggests clusters of crosslinked protein involving five or more β_2GPI molecules. Beyond 200:1 GDA:β_2GPI, faint bands reappear between the origin and 60 kDa, which do not coincide with the bands observed at lower mol ratios. These may be monomers, dimers, and so on, with enough GDA attached to significantly alter the apparent mol wt.

DMS incubated with β_2GPI under similar conditions shows no evidence of intermolecular crosslinking by SDS-PAGE. This supports the idea that clustering of the β_2GPI may be essential for anti-β_2GPI to bind.

CD Evidence for Conformational Changes in β_2GPI

The SDS-PAGE experiments suggest that, at 200:1 GDA:β_2GPI, virtually all of the β_2GPI is present as crosslinked aggregates of more than five protein molecules per cluster (Fig. 4A). However, the level of anti-β_2GPI binding at 200:1 is just slightly above the control (Fig. 3). The maximum in anti-β_2GPI binding to GDA-modified β_2GPI occurs near 1000:1 GDA:β_2GPI (Fig. 3). These observations indicate that extensive intermolecular crosslinking is not sufficient for anti-β_2GPI binding. There may be some specific conformational change required that is not necessarily induced by cluster formation.

CD experiments are used here to detect GDA-induced conformational changes. The ultraviolet CD spectrum of native β_2GPI has three main features: a broad, negative aromatic band near 290 nm; a positive shoulder near 235 nm, with both amide and aromatic contributions; and a strong negative amide band near 210 nm (Fig. 5A and B, solid line in each figure). The negative amide band at 210 nm is indicative of a disordered peptide *(30)*. In fact, analysis of the CD spectrum suggests that there is virtually no α-helix content in native β_2GPI *(31)*, although overlap of chromophores in this region complicates the analysis *(32)*.

GDA (Fig. 5A) and formaldehyde (Fig. 5B) both cause the 210 nm band to become more positive, suggesting an increase in α-helix content. However, the two aldehydes differ in effect on the 235 nm band. Formaldehyde induces only a small decrease in the amplitude of this band, even though the IEF shows that it is modifying the protein extensively. The effect of GDA on the 235 nm band appears to be triphasic, with an immediate drop in intensity at 1:1 GDA:β_2GPI, which remains relatively constant until about 100:1. The band then increases in amplitude to that of native β_2GPI in approaching 500:1 GDA:β_2GPI, after which the amplitude gradually decreases to zero (Fig. 6). These observations suggest that, after the GDA has induced aggregation of β_2GPI through intermolecular crosslinks, further reaction with GDA induces a conformational change that correlates with anti-β_2GPI binding. The eventual decrease in the 235 nm band to zero amplitude is also observed with guanidinium chloride at concentrations in excess of 1 *M*, presumably as a result of denaturation *(32)*.

Discussion

Anti-β_2GPI antibodies from APS patients bind to β_2GPI when it is adsorbed onto anionic phospholipid surfaces *(7,8)*, platelets *(7)*, and oxidized polystyrene surfaces *(9,10)*, but have very low affinity for β_2GPI adsorbed on untreated polystyrene assay wells *(11)*. It may be that specific interactions with the anionic groups of the lipid and the oxidized sites on the polystyrene trigger a conformational change required to expose the epitope; adsorption alone is not effective. Because the lipid-binding region of the protein is believed to be the lysine-rich fifth domain, it is reasonable to assume that the lysines are involved in the interactions with the surface

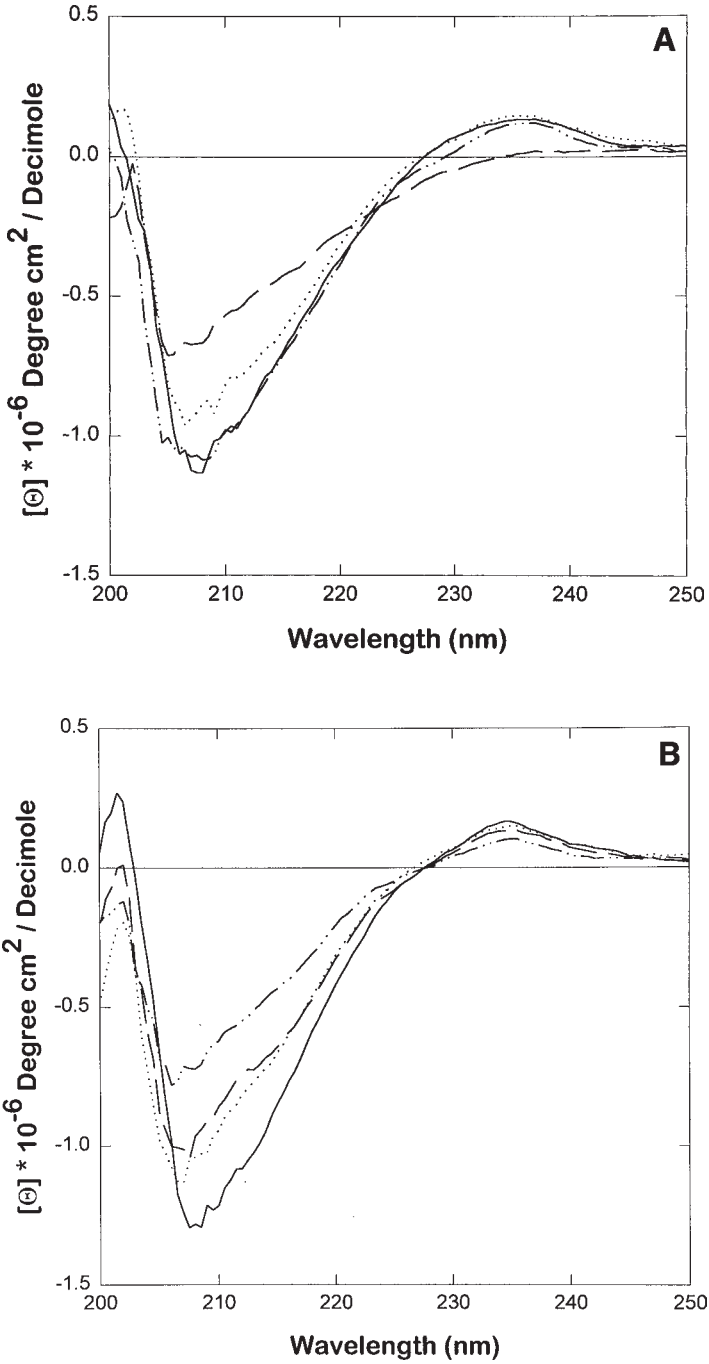

Fig. 5. Changes in CD spectrum of β_2GPI induced by reaction with aldehydes. β_2GPI was incubated with GDA **(A)** and formaldehyde **(B)** at mol ratios ranging from 1:1 up to 10,000:1. Spectra are shown for aldehyde:β_2GPI ratios of 0:1 (solid line; β_2GPI without aldehyde), 100:1 (dotted line), 1000:1 (dash-dot-dot), and 10,000:1 (long dash). GDA (A) induces changes in both bands, but formaldehyde (B) has little effect on the 235 nm band.

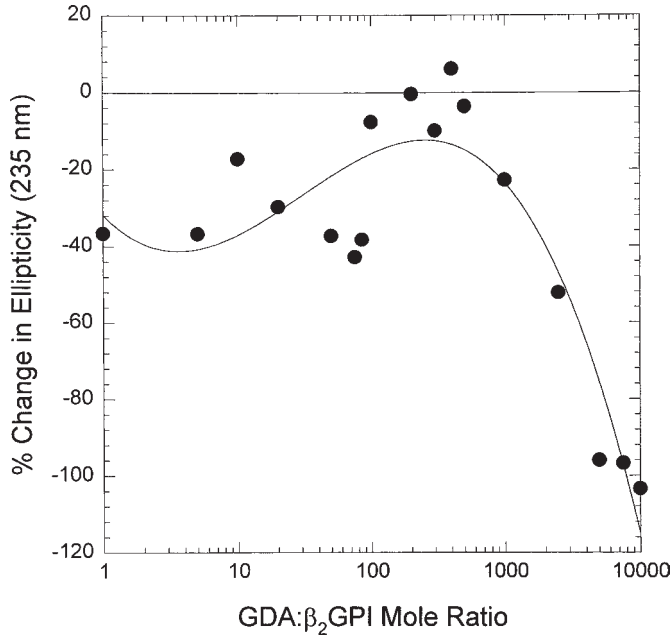

Fig. 6. GDA-induced biphasic change in 235 nm CD band of β_2GPI parallels the changes in anti-β_2GPI binding. The percent change in ellipticity of the 235 nm band relative to native β_2GPI is shown for GDA:β_2GPI ratios ranging from 1:1 up to 10,000:1. There is an initial drop in amplitude of 40% at 1:1 GDA:β_2GPI, followed by a gradual increase to the original amplitude by 500:1. Beyond that level the amplitude decreases by 100%.

sites. This was recently demonstrated through site-directed mutagenesis of recombinant β_2GPI by Sheng et al. *(24)*, i.e., Lys^{284}, Lys^{286}, and Lys^{287} appear to be involved in lipid binding.

When β_2GPI binds to oxidized polystyrene, it is likely that aldehyde groups on the surface react covalently with the lysines to form Schiff bases, which in some way leads to exposure of the epitope. IEF experiments show that formaldehyde does react with the lysine residues, but this alone is not enough to expose the epitope when the modified β_2GPI is deposited on an untreated polystyrene surface. The bifunctional crosslinking reagent, GDA, also reacts with the lysines in β_2GPI, as suggested by IEF experiments, and it does induce changes in the protein leading to anti-β_2GPI binding. Thus crosslinking of lysines appears to be necessary in order to expose the epitope on untreated polystyrene. However, another lysine crosslinking agent, DMS, does not trigger exposure of the epitope, indicating that GDA is crosslinking in a different manner.

SDS-PAGE analysis of GDA-modified β_2GPI demonstrates that, at 10:1 GDA:β_2GPI, a significant fraction of the β_2GPI is present as crosslinked dimers and trimers, and, at 100:1, there is almost no monomer β_2GPI present. Similar analysis of DMS-modified β_2GPI shows no evidence of intermolecular crosslinking. Roubey *(2)* has suggested that the surfaces that are

effective in triggering epitope exposure may in fact be responsible for generating clusters of β_2GPI, which are the actual target for the anti-β_2GPI antibodies. The results presented here are consistent with this view, because anti-β_2GPI binding to β_2GPI on untreated polystyrene only occurs under conditions of extensive intermolecular crosslinking by GDA.

Comparing the electrophoresis results with the anti-β_2GPI binding studies suggests that cluster formation on the surface does not, by itself, explain these observations. Both the IEF and SDS-PAGE data indicate that, by 200:1 GDA:β_2GPI, cluster formation is virtually complete; however, antibody binding does not reach a maximum until about 1000:1 GDA:β_2GPI. CD studies show that there is a significant conformational change that occurs between 100:1 and 1000:1 GDA:β_2GPI, which correlates with the trends observed in the antibody binding studies. At GDA levels higher than 1000:1, the CD spectrum shows a trend toward complete denaturation of the protein by 10,000:1 GDA:β_2GPI, which may explain the decrease in anti-β_2GPI binding at these higher GDA levels.

Events leading to the autoimmune response against β_2GPI in APS are not understood. Observations presented here confirm that clustering of β_2GPI on a surface may be important in anti-β_2GPI binding; however, the results also suggest that clustering alone may not be sufficient. It may be that antibody binding requires a specific conformational change that can be brought about by further modification of the lysines following formation of the intermolecular crosslinks. These conformational changes appear to be distinct from those that occur when the most accessible lysines are modified; that is, epitope exposure may involve events that are distinct from initial association of β_2GPI with anionic lipids or oxidized polystyrene. On an anionic lipid surface, the clustering and conformational changes are probably induced by electrostatic interactions with the lysines.

In conclusion, the authors have clearly demonstrated that GDA adducts of β_2GPI present the epitope recognized by human autoantibodies. In addition to potential therapeutic applications in apheresis systems and their likely utility in diagnostic devices for anti-β_2GPI antibody assays, GDA-modified β_2GPI has allowed us to define conditions necessary for epitope presentation. These conditions provide insight into the biochemical processes leading to the immunopathology of APS, and could lead to the development of site-specific pharmacologic agents useful in therapeutic intervention for APS.

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