C1q Component of Complement Binds to Fibrinogen and Fibrin†

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ABSTRACT: The interaction of complement component C1q with fibrinogen and fibrin was studied by using a solid-phase direct binding assay. Scatchard analysis of radioiodinated fibrinogen binding to C1q indicated at least two high-affinity binding constants (K_d) calculated as 8.5 and 120 nM. In contrast, binding of radioiodinated fibrin to C1q showed only a single class of binding sites with a calculated K_d of 600 nM. Fibrinogen–C1q binding was shown to decrease as a function of increasing salt concentrations, indicating either the presence of charged amino acids in the binding sites or an ionic strength induced conformational dependency of the binding. In direct binding studies using isolated fragments of C1q, both the collagen-like domain of C1q and the globular domains of C1q were shown to bind fibrinogen, indicating at least one binding site for fibrinogen is located in each of the major domains of C1q. Addition of the thrombin-generated peptides of fibrinogen, fibrinopeptides A and B, enhanced C1q–fibrinogen binding, again indicating a complex binding interaction. These results indicate that C1q and fibrinogen are capable of high-affinity interactions that may serve to sequester these complexes in areas of tumors, immune complex deposition, or wounds.

The C1q component of complement is the recognition protein for the classical complement cascade [reviewed in Reid (1983)]. In vivo, C1q circulates in the plasma either freely or as the calcium-dependent zymogen complex C1, composed of one C1q molecule and two molecules each of complement proteins C1r and C1s (Reid, 1983; Sjoholm et al., 1985). A wide variety of substances have been shown to bind C1q including IgG, IgM, heparin and other glycosaminoglycans, certain types of collagen, fibronectin, laminin, and certain types of polysaccharides (Reid, 1983; Almeda et al., 1983; Menzel et al., 1981; Sorvillo et al., 1983, 1985; Bing et al., 1982; Reid & Edmondson, 1984; Entwistle et al., 1985; Bohnsack et al., 1985). Binding of some of these substances to C1q, for example, IgG and IgM, results in the activation of C1, apparently by inducing a conformational change in the C1q molecule. In contrast, C1q binding to other substances such as fibronectin or laminin does not result in C1 activation but instead occurs only on isolated C1q and not to the $C1qC1r_2C1s_2$ complex. Ligand binding to C1q which does not activate the complement cascade, such as laminin–C1q and fibronectin–C1q binding, has been postulated to be involved in a variety of processes, including the deposition of C1q containing immune complexes along basement membranes, and as a phagocytosis signal for macrophages and monocytes (Sorvillo et al., 1983; Bohnsack et al., 1985).

C1q has a highly unusual protein structure. Approximately half of the amino acid sequence of C1q is collagen-like; the other half is composed of six identical globular regions. The binding of C1q to fibronectin, heparin, laminin, and collagen occurs through the collagen-like region of C1q, while immunoglobulins G and M bind at the globular regions. Fibroblasts, endothelial cells, platelets, macrophages, monocytes, and polymorphonuclear cells have been shown to bind C1q through the collagen-like domain (Wautier et al., 1977; Bordin et al., 1983; Andrews et al., 1981; Tenner & Cooper, 1980). Thus, C1q may be playing a role not only in immune system activation but also in postinflammatory or would healing processes.

Activation of the coagulation system and production of fibrin clots are a common feature of most, if not all, inflammatory processes. Fibrinogen is cleaved by the serine protease thrombin to form fibrin in the final steps of the coagulation cascade [reviewed in Doolittle (1981)]. The protease attacks four peptide bonds near the amino termini of fibrinogen, releasing two molecules each of fibrinopeptide A and fibrinopeptide B. Once these small, charged peptides are removed, the fibrin molecules rapidly laterally associate to form the backbone of the clot, which can then be further stabilized by the action of coagulation factor XIIIa. In light of the many implications of C1q in inflammation and repair, we decided to study the direct interaction between C1q and fibrin(ogen). A direct high-affinity binding between C1q and fibrin(ogen) was observed using a solid-phase binding assay. Furthermore, the binding of C1q to fibrin(ogen) was complex, having multiple binding constants and involving distinct domains on the globular and collagen-like regions of the molecule. These results suggest that C1q may become incorporated into clots as a result of a direct interaction between fibrino(ogen) and C1q.

Materials and Methods

The following reagents were purchased: plasmin(ogen)-free human fibrinogen (Sigma); synthetic human fibrinopeptides A and B (Sigma); porcine stomach mucosa pepsin (Sigma); immobilized pepstatin (Pierce); collagenase form III (Advance Biofactures); and human immune serum globulin (Cutter Biological).

Isolation of C1q and C1q Fragments. C1q was isolated from outdated human plasma from The University of Minnesota hospital blood bank by a modification of the method of Kolb et al. (1978). Briefly, 250 mL of plasma was clotted at 37 °C by the addition of $CaCl_2$ to a final concentration of 20 mM. After removal of the clot by squeezing it through cotton gauze, ethylenediaminetetraacetate (EDTA)[1] was added to the plasma–serum to give a final concentration of 10 mM. A 2.5 × 23 cm human IgG–Sepharose column was prepared

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[1] Abbreviations: EDTA, ethylenediaminetetraacetate; PBS, Dulbecco's phosphate-buffered saline; PBS-E, Dulbecco's phosphate-buffered saline containing 10 mM EDTA and 0.02% NaN_3; LSB, 0.01 M phosphate buffer, pH 7.4, containing 0.015 M NaCl and 0.02% NaN_3; nE_t, total number of ligand binding sites; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)-aminomethane; BSA, bovine serum albumin.

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as described using human immune serum globulin (Cutter Biological). The IgG–Sepharose column was equilibrated with Dulbecco's phosphate-buffered saline (PBS), pH 7.4, containing 0.02% NaN_3 and 10 mM EDTA (PBS-E). All procedures were done at 22 °C, except dialysis which was done at 4 °C. The plasma–serum containing EDTA was applied to the IgG–Sepharose column and washed with 10 column volumes of PBS-E. C1q, as well as some minimal impurities of IgG and IgM, was eluted with 0.65 M NaCl, 0.01 M sodium phosphate, pH 7.4, containing 10 mM EDTA, and 0.02% NaN_3. The peak protein containing fractions were determined by absorbance readings at 280 nm, pooled, and concentrated at 4 °C by addition of saturated ammonium sulfate to a final concentration of 50%. The precipitated protein was collected by centrifugation at 30000*g* for 40 min at 4 °C, then resuspended in one-tenth the original volume in elution buffer, and dialyzed extensively against this buffer. The C1q was then further purified by passing it through a 10-mL protein A–Sepharose column (Pharmacia) and frozen in aliquots at −70 °C. C1q purity was assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using a 5% stacking gel, a 12.5% running gel, and the discontinuous buffer system described by Laemmli (1970). All samples were reduced with 5% (v/v) 2-mercaptoethanol and heated for 5 min at 90 °C. Gels were stained with Coomassie Blue R-250. Ouchterlony analysis was also done to assess the purity of the C1q using commercial antisera to human C1q, IgG, IgM, and albumin (Calbiochem-Behring).

The collagen-like fragment and globular domains of C1q were isolated by pepsin and collagenase digestion, respectively, according to the methods of Reid (1976) and Paques et al. (1979). Briefly, to obtain the C1q globular domains, 5 mg of C1q was dialyzed overnight at 4 °C against 0.2 M NaCl/0.1 M Tris, pH 7.4, containing 20 mM $CaCl_2$. The C1q solution was cleared of aggregates by centrifugation for 20 min, 150000*g*, at 4 °C in a Beckman L2-65B ultracentrifuge using a Beckman type 40 rotor. The C1q was then placed in a 54 °C water bath for 10 min. Collagenase form III (Advance Biofactures) (0.3 mg) was added to the protein and the reaction mixture removed from the waterbath. Aggregates were again cleared by centrifugation, and the protein was dialyzed overnight at 4 °C against 1 M sodium citrate, pH 7.4, containing 0.2 M NaCl. The precipitated protein was collected by centrifugation, washed once, and resuspended in 0.1 M Tris, pH 7.4, containing 0.2 M NaCl. After dialysis against this same buffer, the fragment preparation was chromatographed on a 2.5 × 32 cm Sephacryl S-300 column. Fractions containing protein were monitored by absorbance readings at 280 nm and analyzed by SDS–PAGE as described above.

The collagen-like fragment of C1q was obtained by using the method of Reid (1976). Briefly, 5 mg of C1q was dialyzed against 0.5 M acetic acid overnight at 4 °C. After the solution was cleared of possible aggregates by centrifugation as described above, the C1q was digested for 20 h at 37 °C using porcine mucosa pepsin (Sigma) at a ratio of 10 μg of enzyme:1 mg of C1q. The digestion reaction was stopped by the addition of immobilized pepstatin (Pierce) followed by dialysis against 0.5 M NaCl/10 mM sodium phosphate, pH 7.4, containing 0.02% NaN_3 at 4 °C. The collagen-like fragment was then analyzed by SDS–PAGE as described above.

Iodination of Fibrinogen, C1q, and C1q Fragments. Proteins and protein fragments were radiolabeled with carrier-free $Na^{125}I$ (100 mCi/mL) (Amersham) using Iodogen (Pierce) following the protocol recommended by the manufacturer. Radiolabeled proteins were separated from free $Na^{125}I$ by chromatography through 7-mL Sephadex G-25 (Pharmacia) columns equilibrated with 0.5 M NaCl/0.01 M phosphate, pH 7.4. Specific activities were calculated to be 7×10^6 dpm/μg for fibrinogen, 5×10^6 dpm/μg for C1q, and 3×10^6 and 3×10^5 dpm/μg, respectively, for the collagen-like and globular domains of C1q. The functional activity of C1q has been shown to be sensitive both to the radiolabeling method and also to the extent of iodination (Tenner et al., 1981). The Iodogen method of radiolabeling the proteins was chosen as this procedure has been shown to be simple, reproducible, and nondenaturing using a number of different proteins (Knight et al., 1981; Low & Cunningham, 1982; Parker & Strominger, 1983). Aliquots of the radioiodinated proteins were precipitated by the addition of 50% (w/v) trichloroacetic acid to give a final concentration of 10% acid and shown to contain greater than 90% precipitable counts.

Solid-Phase Binding Assays. Polystyrene 12 × 75 mm tubes (Falcon) were coated with 0.5 mL of C1q at 10 μg/mL in PBS containing 0.02% NaN_3 for 16 h at 4 °C. Unbound C1q was removed by aspirating and washing 3 times with PBS. To determine the amount of C1q bound to the tubes, some tubes were coated with C1q solutions containing trace amounts of ^{125}I-C1q (50000 dpm per tube) (Table I). Residual protein binding sites on the polystyrene were then blocked by incubation with 1% (w/v) bovine serum albumin (BSA, Sigma, fraction V) in PBS for 2 h at 22 °C. Control tubes coated with BSA only were prepared in the same manner. To determine the binding affinity of fibrinogen for solid-phase C1q, tubes were washed 3 times with a low-salt buffer (LSB) containing 0.015 M NaCl, 0.01 M sodium phosphate, pH 7.4, and 0.02% NaN_3. Radiolabeled fibrinogen (40000 cpm/tube) and increasing amounts of unlabeled fibrinogen (1–800 nM) were diluted in LSB containing 10 μg/mL BSA in a total volume of 0.5 mL per tube. All assay points were measured in triplicate, and experiments were performed a minimum of 3 times. Background binding to tubes coated only with BSA was determined for all fibrinogen concentrations used. Binding was measured after various incubation times at 37 °C, but in general, data are presented for binding after 18 h. Radiolabeled protein bound to the tubes was quantitated in a TM Analytic γ counter after aspirating and washing the assay tubes 3 times with LSB.

Direct binding of labeled fibrinogen to purified fragments of C1q was measured by using polystyrene tubes coated with various concentrations of C1q globular fragments or C1q collagen-like fragments. Trace amounts of radiolabeled fragments [0.05 μg, (5–10) × 10^4 dpm] were added to some tubes to determine the amount of protein actually bound to the solid phase (Table I).

Binding of fibrin to solid-phase C1q was measured by adding human thrombin (0.1 unit) obtained from Dr. John Fenton, New York State Department of Health, Albany, NY, to the diluted fibrinogen immediately before adding it to the protein-coated tubes. As performed in the fibrinogen–C1q studies, fibrin–C1q binding was done by using a wide range of fibrin concentrations (1–800 nM) and a trace amount (0.1 nM, 30000 cpm) of ^{125}I-fibrin diluted in LSB containing 10 μg/mL BSA.

Finally, experiments were also performed to determine whether the presence of the thrombin-released fibrinopeptides A and B could perturb C1q–fibrinogen binding. Synthetic human fibrinopeptides A and B (Sigma) were added at 50–500 nM concentrations to solid-phase C1q in the presence of ^{125}I-fibrinogen, and the binding assay was performed as usual. The relative binding of ^{125}I-fibrinogen alone to C1q was in-

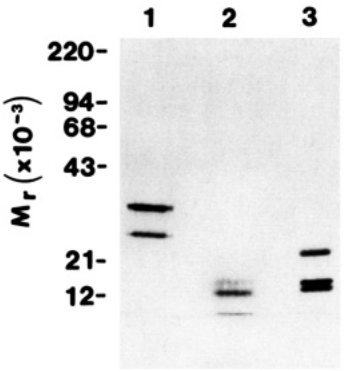

FIGURE 1: SDS–PAGE of C1q and C1q fragments. All proteins were run under reducing conditions as described under Materials and Methods. Lane 1, intact C1q (3 μg); lane 2, pepsin-generated collagen-like fragment of C1q (2 μg); lane 3, collagenase-generated C1q globular domains (3 μg).

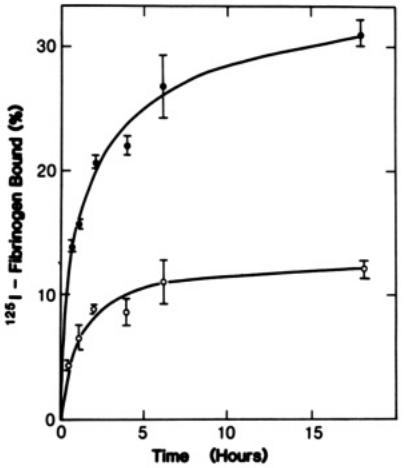

FIGURE 2: ^{125}I-Fibrinogen binding to C1q as a function of time. Binding of ^{125}I-fibrinogen to polystyrene tubes coated with 10 μg/mL C1q was measured in a low-salt buffer at 37 °C for the indicated times as described under Materials and Methods. (●) ^{125}I-Fibrinogen (50000 dpm, 0.3 nM); (○) ^{125}I-fibrinogen (0.3 nM) + 150 nM fibrinogen. Background binding to tubes coated with albumin represented less than 4% and was subtracted from the plotted values. Each data point represents the mean of triplicate determinations that varied by less than 3% from the mean.

cluded for comparison purposes.

RESULTS

The purity of the C1q and its fragments generated for this study was assessed by SDS–PAGE and Ouchterlony analysis. Figure 1 shows a representative 12.5% SDS–PAGE gel of C1q, pepsin-digested C1q, and collagenase-digested C1q run under reducing conditions. Densitometer scans indicated that these proteins were >90% pure. Ouchterlony analysis of the intact C1q showed a positive reaction with antisera to human C1q and no reaction with antisera to human albumin, IgG, or IgM.

The binding of radiolabeled fibrinogen to solid-phase C1q was determined after different incubation times (Figure 2). The equilibrium binding time, i.e., the incubation time after which no further increase of bound fibrinogen occurred, was approximately 6 h. Increasing the fibrinogen concentration to 150 nM using a combination of labeled and unlabeled protein also required approximately 6 h to achieve equilibrium. Increasing the incubation time up to 36 h did not further increase the fibrinogen binding (data not shown). In order to ensure equilibrium conditions for all concentrations of ligand used, subsequent binding and inhibition assays were measured after overnight incubations.

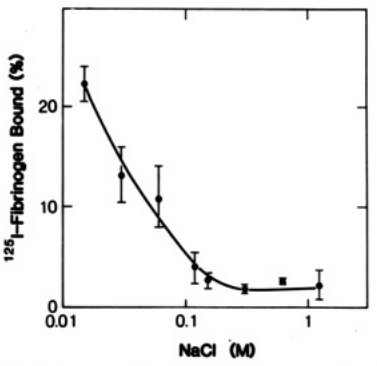

FIGURE 3: ^{125}I-Fibrinogen binding to C1q as a function of NaCl concentration. Binding of 0.3 nM radiolabeled fibrinogen to solid-phase C1q (10 μg/mL coating concentration) was measured after 18 h at 37 °C in 0.01 M phosphate buffer, pH 7.4, in the presence of increasing NaCl concentrations. Background binding to albumin-coated tubes has been subtracted from the plotted values and was never more than 4%. Each data point represents the mean of triplicate determinations that varied by less than 3% from the mean.

Table I: Quantitation of Solid-Phase Protein Coatings

coating protein (μg/mL)		protein bound[a] ng	protein bound[a] pmol	^{125}I-fibrinogen bound (%)
C1q	0.2	50	0.1	2
	1.1	320	0.7	13
	10	1900	4.1	25
	50	3200	6.9	27
	100	4000	8.7	27
C1q globular fragment	0.4	150	2.6	4
	1.3	490	8.5	1
	10	2200	39	11
	50	3100	54	16
	100	4300	75	18
C1q collagen-like fragment	0.1	40	0.2	1
	1	170	0.9	6
	10	330	1.7	12
	30	220	1.1	11

[a] Polystyrene tubes were coated with different concentrations of C1q and C1q fragments containing trace amounts of iodinated proteins [0.05 μg, (5–10) × 10^4 dpm] in order to quantitate the amount of protein bound to the assay tubes as described under Materials and Methods.

The binding of fibrinogen to solid-phase C1q was shown to be dependent upon the NaCl concentration (Figure 3). Fibrinogen–C1q binding dropped rapidly with increasing ionic strength until it leveled off at approximately 3% above background at 0.15 M NaCl and remained at this level up to 1.2 M NaCl. Binding of fibrinogen to C1q at all salt concentrations was always greater than the binding to control tubes in all experiments. In order to measure accurately the binding affinity of fibrinogen and C1q, equilibrium binding studies were performed using 0.01 M sodium phosphate buffer, pH 7.4, containing 0.015 M NaCl and 10 μg/mL BSA.

Binding of radiolabeled fibrinogen to C1q in the presence of increasing amounts of unlabeled fibrinogen is shown in Figure 4A. Scatchard analysis (Scatchard, 1949) of these data (Figure 4B) indicated the existence of two dissociation constants, 8.5 and 120 nM. The total number of binding sites (nE_t) calculated by linear regression analysis for these two equilibria were 4.0 and 35 nM, respectively, for the higher and lower equilibria. Using the number of moles of C1q on the solid phase (Table I) and these nE_t values, there were calculated to be 2 high-affinity sites and 18 moderately high-affinity sites for fibrinogen on C1q. Correlation coefficients for these values were 0.81 and 0.96, respectively, and the corresponding P values were $0.01 \leq p \leq 0.05$ and $p \leq 0.01$. These disso-

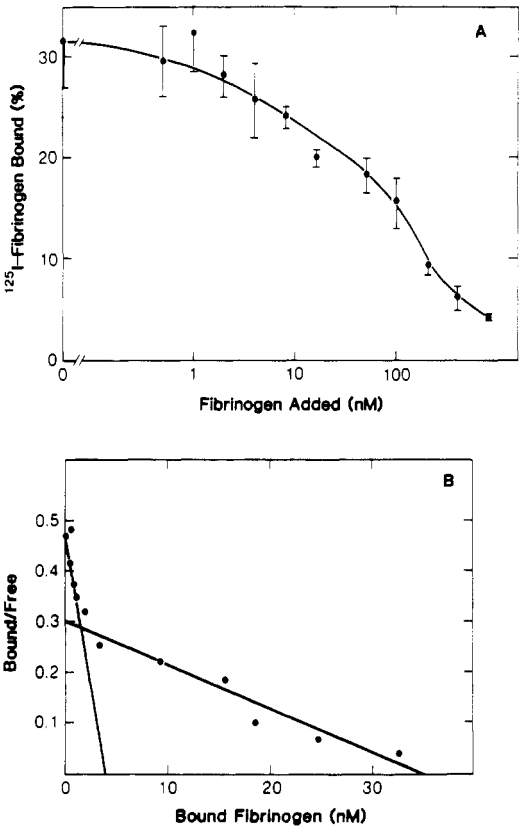

FIGURE 4: Direct binding curve and Scatchard plot of ^{125}I-fibrinogen binding to tubes coated with a 10 μg/mL C1q solution. (A) Binding of ^{125}I-fibrinogen (0.1 nM) to C1q in the presence of increasing amounts of unlabeled fibrinogen. Background binding (average <4%) was subtracted before plotting. The ordinate represents binding of ^{125}I-fibrinogen alone. Each data point represents the mean of triplicate determinations that varied by less than 3%. (B) Scatchard plot of fibrinogen–C1q binding. Affinity constants calculated from these data equaled 8.5 and 120 nM with respective nE$_t$ values of 4 and 35 nM.

ciation constants indicate two high-affinity interactions of C1q and fibrinogen.

The binding of fibrin to solid-phase C1q was also measured. Human thrombin (0.1 unit) was added to each dilution of fibrinogen immediately before addition to the C1q-coated tubes. With these conditions, the conversion of fibrinogen to fibrin is virtually instantaneous as determined by SDS–PAGE followed by Coomassie blue staining or autoradiography of the thrombin–fibrinogen solutions (data not shown). In addition, exposure of C1q to thrombin for up to 18 h at 37 °C did not result in any apparent proteolysis of C1q (data not shown). Total binding of fibrin to C1q was less than that of fibrinogen (Figure 5A). In addition, only a single class of binding sites was apparent for the C1q–fibrin interaction (Figure 5B), with a dissociation constant of 600 nM and an nE$_t$ value of 80 nM, indicating a significantly weaker binding than that obtained for the C1q–fibrinogen interaction. Again, 10 moderate affinity binding sites on C1q for fibrin were calculated using the number of moles of C1q on the solid phase (Table I).

Thrombin cleavage of fibrinogen releases 2 mol each of fibrinopeptides A and B (Doolittle, 1981) of 14 and 16 amino acid residues, respectively. Increasing concentrations of fibrinopeptides resulted in enhanced fibrinogen binding (Figure 6). This effect was most evident with fibrinopeptide A but was also seen with fibrinopeptide B. Increasing the concentration of fibrinopeptide A from 0 to 500 nM resulted in a 20% increase in ^{125}I-fibrinogen binding. Increasing fibrinopeptide

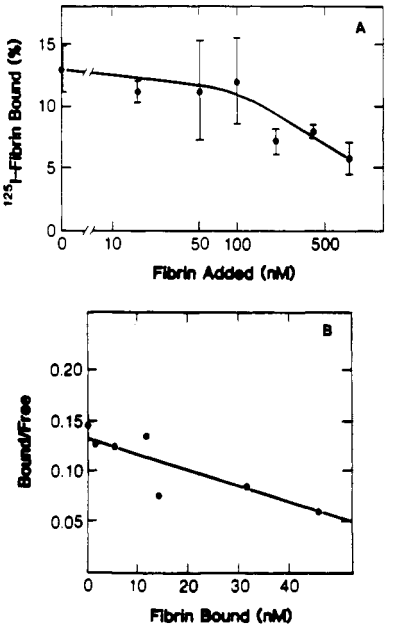

FIGURE 5: Binding curve and Scatchard plot of fibrin–C1q binding. ^{125}I-Fibrin binding to tubes coated with a 10 μg/mL solution of C1q was performed as described under Materials and Methods. (A) Binding of ^{125}I-fibrin to C1q as a function of increasing concentrations of unlabeled fibrin. The ordinate represents binding of ^{125}I-fibrin alone. Background values have been subtracted from the plot and were always less than 4%. Each data point represents the mean of triplicate determinations, which varied by less than 4%. (B) Scatchard plot of fibrin–C1q binding. The calculated K_d is 600 nM, and the nE$_t$ value is 80 nM.

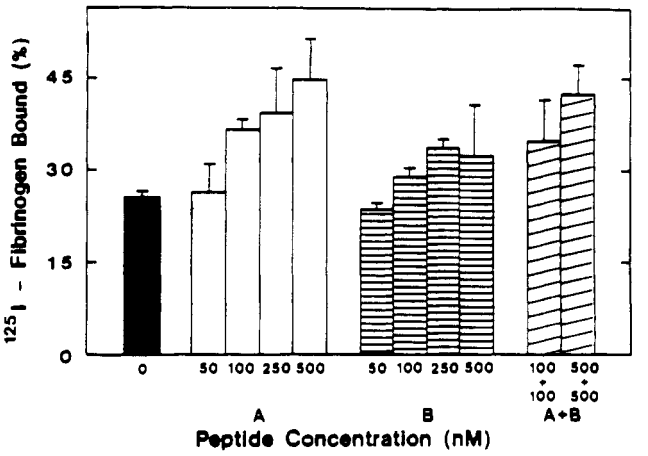

FIGURE 6: Effect of fibrinopeptides A and B on fibrinogen–C1q binding. Binding of fibrinogen to solid-phase C1q was measured in the presence of 50–500 nM fibrinopeptide A (white bars), fibrinopeptide B (striped bars), or equimolar concentrations of A + B (diagonally striped bars). Binding of ^{125}I-fibrinogen alone (black bar) is given for comparison. Background binding has been subtracted from all data points and was never more than 4%.

B concentrations from 0 to 500 nM resulted in a 10% increase in fibrinogen binding. The effect of the two fibrinopeptides was not additive, as the addition of 100 nM each of fibrinopeptides A and B enhanced the binding only as much as that seen with the single peptides. Background binding of labeled fibrinogen to tubes coated with BSA only was unaffected by changing fibrinopeptide concentrations, remaining constant at approximately 4%.

C1q was digested with collagenase and pepsin in order to obtain the globular domains and collagen-like fragment, respectively, of C1q. Their purity and electrophoretic mobility are shown in Figure 1. Fibrinogen bound to both the collagen-like and the globular fragments of C1q (Figure 7).

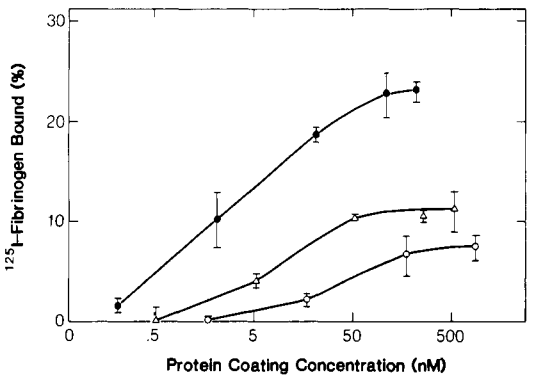

FIGURE 7: Binding of ^{125}I-fibrinogen to solid-phase C1q and C1q fragments. The direct binding of ^{125}I-fibrinogen to tubes coated with the indicated nanomolar amounts of C1q, the collagen-like fragment of C1q, or the globular domains of C1q was measured as described under Materials and Methods. Background binding (~4%) to tubes coated with albumin has been subtracted from the plotted values. Each point represents the mean of three separate determinations that agreed within 3%. Duplicate experiments gave similar results. C1q (●); C1q collagen-like fragment (△), and C1q globular domains (○).

However, fibrinogen did not bind as well to either fragment, even at high coating concentrations, as it bound to intact C1q. For the collagen-like fragment of C1q, decreased fibrinogen binding can in part be explained by the lower coating efficiency of this fragment to the tubes (Table I). However, a comparison of equivalent moles of solid-phase C1q and the collagen-like fragment of C1q show that the fragment binds less fibrinogen than does intact C1q. In addition, the globular fragment of C1q on a weight basis bound to the polystyrene almost as well as C1q, but bound significantly less fibrinogen (Table I, Figure 7). When ^{125}I-fibrin binding to tubes coated with different concentrations of C1q or C1q fragments was measured, similar results were obtained, although the maximum amounts of ^{125}I-fibrin bound was less than that of ^{125}I-fibrinogen (data not shown). These results further support the existence of two discrete binding sites for fibrinogen on C1q, one located in the globular domain and the other in the collagen-like region of C1q.

Discussion

This study shows that binding of complement component C1q to fibrinogen involves at least two kinds of binding interactions. The two calculated binding constants (K_d) for this association were 8.5 and 120 nM derived from the Scatchard plot shown in Figure 4. These values indicate that both classes of binding sites are of relatively high affinity. One binding site is apparently in C1q's globular domain, while the second is found in C1q's collagen-like domain (Figure 7). Further studies would be required to establish if these are cooperative sites or independent, heterogeneous sites.

Multiple, heterogeneous binding of C1q has been reported for both heparin and fibronectin. Heparin binds C1q with two dissociation constants of 76.6 nM and 1.01 μM. Both of the heparin binding sites were localized to the collagen-like region of C1q (Almeda et al., 1983). Fibronectin has been shown to bind C1q via two high-affinity interactions with K_{d1} = 0.16 nM and K_{d2} = 26 nM (Entwistle et al., 1985). Interestingly, fibronectin also contains two distinct binding domains for fibrin(ogen) and three distinct binding domains for heparin (Hayashi & Yamada, 1982; Smith & Furcht, 1982; Richter et al., 1981; Yamada et al., 1980; Mosher, 1980; Stemberger & Hormann, 1976). Thus, a complex interactive network model can be postulated for the binding of fibrinogen, C1q, fibronectin, and heparin, with the association of any two of these components being strengthened by the addition of a third component and by the formation of a ternary complex of high functional affinity.

Multiple, homogeneous binding sites on C1q have been described for immunoglobulins G and M in the six globular regions (Reid, 1983; Hughes-Jones & Gardner, 1979; Tschopp et al., 1980). In addition, homogeneous interactions of C1q have also been described for laminin, collagen, and fibronectin binding to the collagen-like domain of C1q (Menzel et al., 1981; Sorvillo et al., 1983; Bing et al., 1982; Reid & Edmondson, 1984; Bohnsack et al., 1985). In each of these studies determining the C1q–ligand affinity, buffers of lower than physiologic ionic strength were used in order to optimize the assay measurements. In agreement with these previous studies, C1q–fibrinogen binding was demonstrable at physiologic salt concentrations but was 5-fold greater in the reduced ionic strength buffer. In a C1q–immune complex binding assay, Faaber et al. (1981) showed that C1q and fibrinogen in PBS would form significant complexes in the presence of poly(ethylene glycol) 6000. They found this binding to be inhibited by protamine sulfate, indicating the fibrinogen–C1q interaction might be electrostatic in nature. Using affinity columns of Affigel 15–C1q (Bio-Rad) (prepared according to the manufacturer's instructions), we measured ^{125}I-fibrinogen–C1q binding in both PBS and LSB buffers. As seen with the polystyrene assay system, fibrinogen binding was detectable in PBS but significantly increased in LSB (data not shown). This sensitivity indicates either that charged amino acids are involved in the protein binding sites or that an ionic strength supported conformation of the proteins is critical to the binding reaction. In addition, as the time study in Figure 2 shows, C1q–fibrinogen binding reached equilibrium only after 6 h. The rate-limiting step could perhaps be due to a conformational constraint on either or both of the proteins. It is possible that the necessary conformation favored by these experimental conditions could be attained in vivo by the influence of other ligands such as cell surface receptors or heparin.

Binding of fibrin to C1q showed a much lower affinity constant than fibrinogen–C1q binding. In addition, only a single class of fibrin–C1q binding sites was demonstrated. Because the four fibrinopeptides (two each of fibrinopeptides A and B) that are removed from fibrinogen to form fibrin represent only a small fraction (2% by weight) of the total fibrinogen molecule, it was expected that fibrin contained at least one of the C1q binding sites. However, once thrombin cleaves the fibrinopeptides off of fibrinogen, fibrin monomers rapidly polymerize both laterally and in an end to end manner, so a large proportion of potential C1q binding sites would thus be blocked (Doolittle, 1981). Monomeric fibrin binding to solid-phase C1q could not be measured by the assay system described here, because the conditions necessary to prevent fibrin polymerization, such as 1 M NaBr, would also prevent fibrin–C1q binding. Nevertheless, fibrin binding to solid-phase C1q was still demonstrable in this assay system, indicating that although fibrin–fibin binding is of higher affinity than fibrin–C1q binding, the fibrin–C1q affinity is still significant.

It is noteworthy that measurable fibrin–C1q binding occurred only at very high fibrin concentrations. With these high fibrin concentrations, fibrinopeptide concentrations would also be very high. The effect of chemically synthesized fibrinopeptides A and B on C1q–fibrinogen binding was measured to further define the fibrinogen fragment that binds C1q. If these small (14 and 16 amino acid) peptides contained a C1q binding site, it was expected that they would compete for C1q–fibrinogen binding. Paradoxically, increasing fibrino-

peptide concentrations did not inhibit C1q–fibrinogen binding but actually increased the binding of fibrinogen to C1q. The enhanced binding of fibrinogen to C1q in the presence of the fibrinopeptides was specific, as no increase in radiolabeled fibrinogen binding was found in control tubes coated with albumin only. This enhancement indicates an effect of the fibrinopeptides either on the C1q or on the fibrinogen molecule. Fibrinopeptides binding to fibrinogen do not seem probable as the postulated role of these highly charged peptides is to prevent fibrinogen polymerization (Doolittle, 1981). Once thrombin cleaves the fibrinopeptides off of fibrinogen, fibrin monomers rapidly polymerize. It therefore seems most likely that the fibrinopeptides represent either one of the two binding sites for C1q or part of a cooperative binding mechanism.

The binding of C1q to fibrinogen and fibrin could be another way to localize C1q at a wound site. Since the binding of C1q to fibrinogen occurs through both the collagen-like and globular domains of C1q, the globular domains of C1q would be available for opsonic functions. Macrophages, fibroblasts, and endothelial cells which all have C1q receptors (Reid, 1983; Wautier et al., 1977; Bordin et al., 1983; Andrews et al., 1981; Tenner & Cooper, 1980) would then have an additional signal to localize them at the wound site. It is therefore possible that the C1q–fibrin(ogen) interaction provides one of a number of positive influences in inflammation, wound healing, fibrosis, and angiogenesis.

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