The Role of the Main Noncollagenous Domain (NC1) in Type IV Collagen Self-Assembly

Effie C. Tsilibary and Aristidis S. Charonis

Department of Pathology, Yale University School of Medicine, New Haven, Connecticut 06510.
The authors' present address is Department of Laboratory Medicine and Pathology, School of Medicine, University of Minnesota at Minneapolis, P. O. Box 609, Mayo Memorial Building, Minneapolis, Minnesota 55455.

Abstract. Type IV collagen incubated at elevated temperatures in physiologic buffers self-associates (*a*) via its carboxy-terminal (NC1) domain, (*b*) via its amino-terminal (7S) domain, and (*c*) laterally; and it forms a network. When examined with the technique of rotary shadowing, isolated domain NC1 was found to bind along the length of type IV collagen to four distinct sites located at intervals of ∼100 nm each. The same 100-nm distance was observed in domain NC1 of intact type IV collagen bound along the length of the collagen molecules during initial steps of network formation and in complete networks. The presence of anti-NC1 Fab fragments in type IV collagen solutions inhibited lateral association and network formation in rotary shadow images. During the process of self-association type IV collagen develops turbidity; addition of isolated domain NC1 inhibited the development of turbidity in a concentration-dependent manner. These findings indicate that domain NC1 of type IV collagen plays an important role in the process of self-association and suggest that alterations in the structure of NC1 may be partially responsible for impaired functions of basement membranes in certain pathological conditions.

Type IV collagen is one of the exclusive macromolecular components of basement membranes (11). Structurally, it consists of three α-chains [α1(IV)$_2$–α2(IV)] and has in its monomeric form a molecular weight of 500,000 (1, 5). When compared to the α-chains of the other collagen types, the α-chains of type IV collagen are of higher molecular weight and contain along their length many short interruptions of the Gly-X-Y repeating unit (9, 18). Therefore, type IV collagen has multiple short non–triple-helical domains along its length and also has a large globular domain (NC1)[1] at its carboxy-terminal end (22).

Type IV collagen molecules have the ability to self-assemble by interacting via their carboxy termini (NC1) (22), via their amino termini (7S) (22), and laterally (26). The end product of these interactions is a closed network, as visualized with the rotary shadowing technique (26). Pepsin-treated type IV collagen molecules which lack their globular NC1 domain are not able to form networks (26). This observation suggested that the carboxy-terminal globule might play an important role in type IV collagen self-assembly. In this study, we present evidence that domain NC1 is critically involved in lateral association and network formation of type IV collagen.

1. *Abbreviation used in this paper*: NC1, noncollagenous domain 1 (carboxy terminal) of type IV collagen.

Materials and Methods

Preparation of Type IV Collagen

The source of type IV collagen was the Engelbreth-Holm-Swarn (EHS) tumor (16) grown subcutaneously in mice which were rendered lathyritic by the addition of 0.25% β-aminopropionitrile fumarate (Sigma Chemical Co., St. Louis, MO) in their drinking water. Type IV collagen was isolated by a modification of previously described methods (4, 12). Briefly, the tissue was extracted first with 3.4 M NaCl in 0.05 M Tris-HCl, pH 7.4, containing 1 mM EDTA, 50 μg/ml phenylmethylsulfonyl fluoride (PMSF), and 50 μg/ml chloromercurobenzoate, then with 2 M guanidine-HCl (Sigma Chemical Co.) in 0.05 M Tris, pH 7.4, containing the same protease inhibitors and, finally, with 2 M guanidine in 0.05 M Tris-HCl, pH 7.4, containing 2 mM dithiothreitol (DTT) and protease inhibitors. The protein was precipitated with 10% NaCl and the pellet was dissolved in 4 M urea (Schwarz/Mann Div., Cleveland, OH), 0.05 M Tris-HCl, pH 8.6, with protease inhibitors; the solution was then dialyzed against the same buffer overnight at 4°C, and it was incubated with DEAE-cellulose for 12 h at 4°C. The unbound fraction was dialyzed against 2 M guanidine in 0.05 M Tris-HCl, pH 7.4, containing 2 mM DTT and protease inhibitors. Isolated type IV collagen was centrifuged at 60,000 rpm for 90 min to clear aggregates >50S and it was stored on ice. It was found to contain <0.1% type I collagen by the technique of rotary shadowing, a finding which was corroborated previously by ELISA inhibition assays (26). The concentration of type IV collagen was determined by amino acid analysis.

Preparation of Domain NC1 of Type IV Collagen

The main noncollagenous domain (NC1) was isolated from type IV collagen by the use of bacterial collagenase (CLSPA, CooperBiomedical Inc., Malvern, PA) according to published procedures (22, 24). Type IV collagen was centrifuged at 60,000 rpm for 20 min at 3°C to remove large aggregates, it was dialyzed exhaustively against 0.05 M Tris-HCl, pH 7.4, containing

0.2 M NaCl and 2 mM $CaCl_2$, and it was incubated with collagenase (substrate/enzyme ratio, 50:1) at 37°C for 24 h. The solution was then centrifuged at 13,000 rpm for 30 min to remove aggregated material, and the supernatant was concentrated with Aquacide IIA (Calbiochem-Behring Corp., La Jolla, CA), dialyzed against 0.2 M NH_4HCO_3, pH 8.5, and chromatographed on a Sephacryl S-300 (2.5 × 95 cm) column equilibrated in the same buffer. Dimeric NC1 eluted as a distinct peak with a K_{av} 0.430, and its purity was tested by electrophoresis on a 10% SDS polyacrylamide gel (13), and by rotary shadowing, as published previously (22, 24).

Preparation of Anti-NC1 Antibodies and Their FAB Fragments

Isolated NC1 in complete Freund's adjuvant was injected subcutaneously in female New Zealand rabbits. The animals received a total of three injections (100 μg per injection) at 2-wk intervals. 2 wk after the last injection, the animals were bled and the antiserum was tested by ELISA and was found to be equally reactive with domain NC1 and intact type IV collagen. With the same type of assay, this antiserum reacted minimally with pepsin-treated type IV collagen which lacks domain NC1. Monovalent Fab fragments were prepared in order to avoid the formation of large aggregates of type IV collagen by intact, divalent anti-NC1 IgG. Fab fragments were produced by papain digestion of the IgG fraction of the antiserum (8). The IgG fraction was precipitated from the antiserum by 50% ammonium sulfate, the pellet was dissolved in 0.1 M sodium phosphate buffer, pH 7.0, and was dialyzed extensively against the same buffer. Subsequently, cysteine (at a final concentration of 10 mM), EDTA (at a final concentration of 2 mM), and papain (Sigma Chemical Co.) at a substrate/enzyme ratio of 50:1 were added to the solution. The mixture was incubated at 37°C for 16 h with stirring and the digestion was terminated by adding chloromercurobenzoate at a final concentration of 1 mM. The solution was dialyzed against distilled H_2O containing 1 mM chloromercurobenzoate and it was centrifuged at 12,000 *g* for 30 min. The supernatant was passed over a Sepharose 4B-NC1 affinity column, the column was washed with PBS, and the bound fraction was

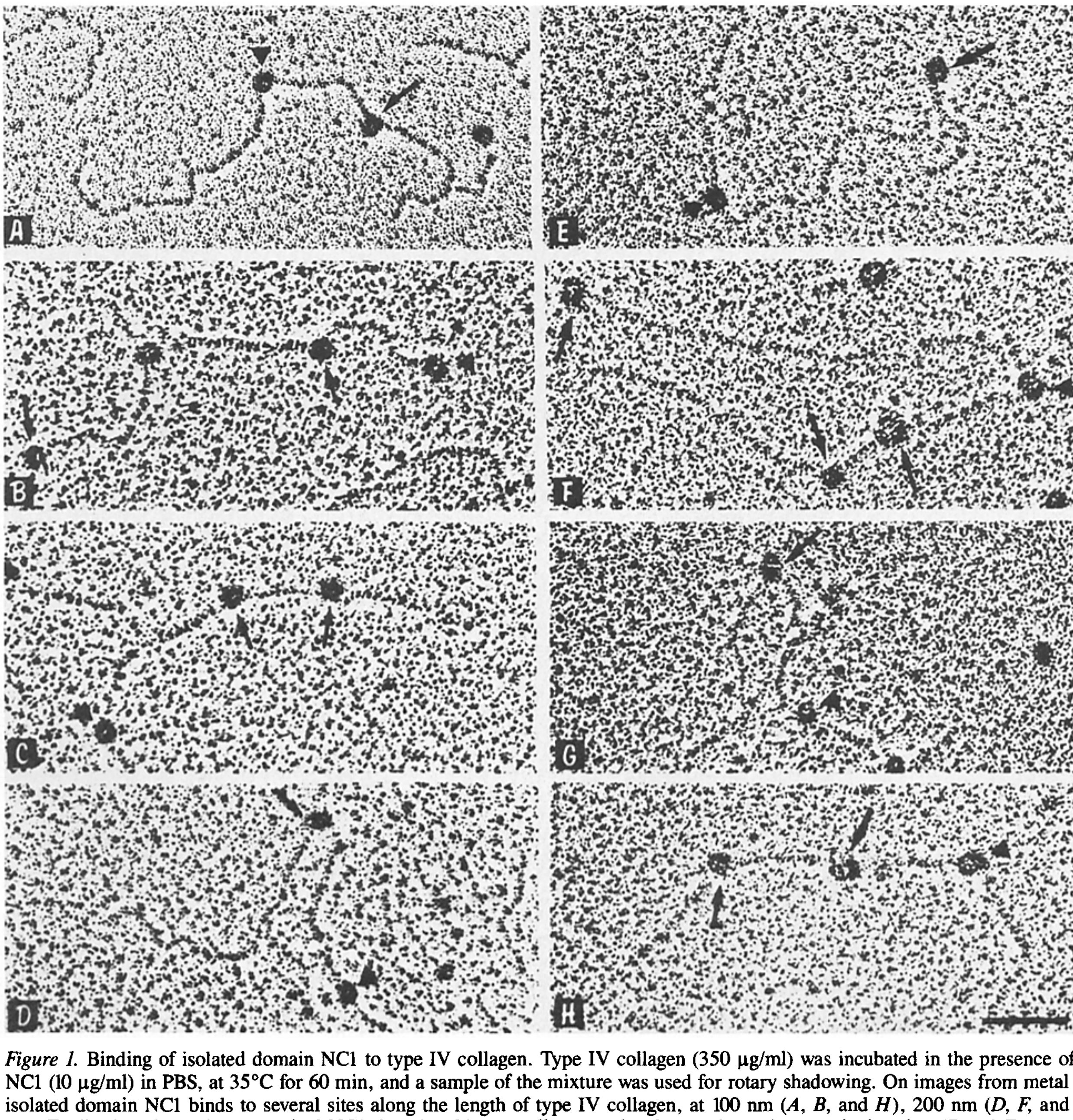

Figure 1. Binding of isolated domain NC1 to type IV collagen. Type IV collagen (350 μg/ml) was incubated in the presence of isolated NC1 (10 μg/ml) in PBS, at 35°C for 60 min, and a sample of the mixture was used for rotary shadowing. On images from metal replicas, isolated domain NC1 binds to several sites along the length of type IV collagen, at 100 nm (*A*, *B*, and *H*), 200 nm (*D*, *F*, and *G*), 300 nm (*E*) distal to the carboxy-terminal NC1 domain of intact collagen molecules, at the amino-terminal region (*B* and *F*), or at various other sites (*C* and *F*). Often more than one NC1 globule is found per collagen molecule (*B*, *C*, *F*, and *H*). Closed triangles point to domain NC1 of intact type IV collagen molecules; arrows point to isolated NC1 bound to collagen. Bar, 100 nm.

eluted with 1 M acetic acid containing 0.15 M NaCl. The eluted peak which contained the anti-NCl Fab fragments was tested by electrophoresis on a 12% SDS polyacrylamide gel (13). Anti-NCl Fab fragments were dialyzed against PBS and stored at −20°C until further use.

Production of Anti-BSA Fab Fragments

Rabbit anti-BSA serum was generously provided by H. Furthmayr (Yale University) and anti-BSA Fab fragments were produced by papain digestion of the IgG fraction of the antiserum, as mentioned above. After the end of the digestion, the mixture was passed over a Sepharose 4B-BSA affinity column, and the bound fraction containing anti-BSA Fab fragments was eluted, dialyzed against PBS, and stored as mentioned above.

Turbidity Measurements

Type IV collagen and isolated noncollagenous NCl domain were dialyzed against PBS overnight at 4°C and were centrifuged at 40,000 rpm for 20 min at 3°C to remove aggregated material. Mixtures of the above components were incubated for various time intervals in pre-warmed quartz cuvettes at 35°C (maintained with a water jacket) and the change in absorbance at 360 nm was followed over time with a Beckman spectrophotometer.

Rotary Shadowing

Type IV collagen and domain NCl were dialyzed in PBS overnight at 4°C. Each protein was centrifuged at 40,000 rpm for 20 min to remove large aggregates. Solutions of type IV collagen alone, or mixtures containing type IV collagen and isolated domain NCl or anti-NCl Fab fragments or anti-BSA Fab fragments were incubated at 35°C for 1 h; then they were added to a solution containing 50% glycerol in 0.15 M NH_4HCO_3, pH 7.8, at a final concentration of 5–10 μg/ml, and the samples were sprayed on freshly cleaved mica sheets. The mica sheets were then placed on a rotary stage and shadowed under vacuum with a mixture of 95% platinum–5% carbon in a Balzers apparatus (Balzers Union, Hudson, NH) (4, 19). The replicas were floated in distilled H_2O, placed on 300-mesh uncoated copper grids, and examined with a Philips 300 transmission electron microscope operating at 60 kV.

To evaluate in a quantitative way the binding of isolated domain NCl to type IV collagen, association events were photographed at a magnification of 26,930 and prints were made at a final magnification of 403, 950. Measurements were then performed on the prints using a Zeiss videoplan computer with a digitizer measuring tablet attachment and a (Y)l videoplan program. We measured the distance from domain NCl of the type IV collagen molecule to the site of binding of the isolated NCl globule and expressed this value as a ratio, by dividing it by the total length of type IV collagen. The following criteria were applied in order to include association events in the measurements: (*a*) the whole type IV collagen molecule should be clearly traced from its carboxyl to its amino end; (*b*) its length should be 410 ± 40 nm; and (*c*) the binding of added NCl should be clearly visualized. The results from these measurements were used to construct a histogram in which the absolute frequency of binding events was plotted against the length of the type IV collagen molecule, divided into 20 equal segments (∼20 nm per segment). When intermediate or complete forms of assembly of type IV collagen were examined, the distance between NCl globules was measured directly, and histograms were made in which the absolute frequency of binding events was plotted against length, divided into 20-nm segments.

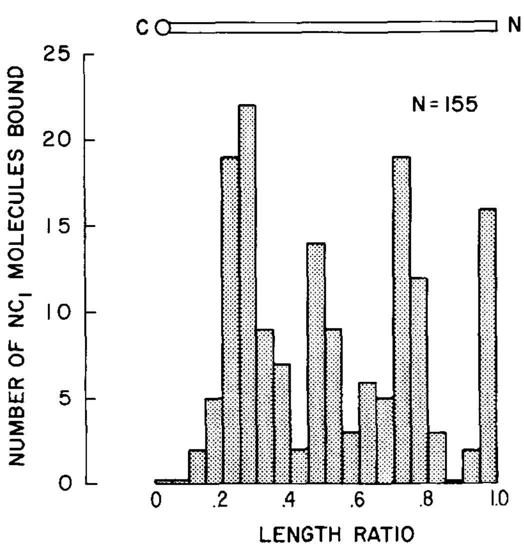

Figure 2. Histogram of the distribution of binding domain NCl to segments of the type IV collagen molecule shown diagrammatically on top (COOH terminal [*C*], *left*; NH_2 terminal [*N*], *right*). The collagen molecule was divided in 20 segments. Four different peaks equally spaced are observed along the length of type IV collagen. Statistical analysis demonstrates that the distribution is nonrandom at a statistically significant level ($P < 0.001$) and ordered.

Results

Isolated, dimeric domain NCl was visualized by rotary shadowing as a globule 10–12 nm in diameter, as described previously (22, 24). Intact type IV collagen, EHS-derived, was 80–90% dimeric, and appeared as two flexible rods connected by a single globule corresponding to domain NCl. The average length of the monomeric form was 410 nm.

When isolated domain NCl (final concentration, 10 μg/ml) was co-incubated with type IV collagen (final concentration, 350 μg/ml) at 35°C for 60 min in PBS, it was found by rotary shadowing to bind along the length of the collagen molecule (Fig. 1). Often more than one NCl globule was observed to be bound (Fig. 1, *B*, *C*, *F*, and *H*). When a histogram of the binding events was constructed, as mentioned in Materials and Methods, four peaks were observed, approximately one evey 100 nm, at intervals of about one fourth the length of the type IV collagen molecule (Fig. 2). When examined by statistical analysis, with the goodness of fit of the Poisson distribution, the binding was found to be nonrandom at a statistically significant level ($P < 0.001$) and to have an ordered distribution (27). Bound NCl was usually easily discernible, because the NCl domain of native intact collagen was located in the middle of well-resolved dimers and the rodlike portion of each monomer emanated from the center of this globular domain. In contrast, added NCl was bound to the rodlike portion of type IV collagen, usually via the edge of the globule (Fig. 1). Because isolated domain NCl was generated by enzymatic digestion of type IV collagen, the possibility existed that sticky ends were created and that the observed binding, although repeated at regular intervals, could be due to nonspecific sticking. To eliminate this possibility, we determined whether this binding occurred in intact type IV collagen. Type IV collagen at 300 μg/ml was incubated in PBS at 35°C for 1 h, and was then examined by rotary shadowing. Under these conditions, it self-associated end-to-end and laterally as well, to form intermediate complexes and an irregular polygonal network (26). In this instance, we observed that the NCl globules of intact type IV collagen dimers which were not treated enzymatically also appeared to bind to the rodlike part of collagen. This binding was apparent both in intermediate forms of self-association and in completely formed networks. When the distance between NCl globules was measured in intermediate forms of assembly (Fig. 3), a prominent peak was observed at 100 nm, followed by another peak at 200 nm and a minor peak at 300 nm. No peak was observed at 400 nm (Fig. 4). The reduction and disappearance of peaks at 300 and 400 nm, respectively, can be explained by the difficulty of tracing the total length of individual type IV collagen

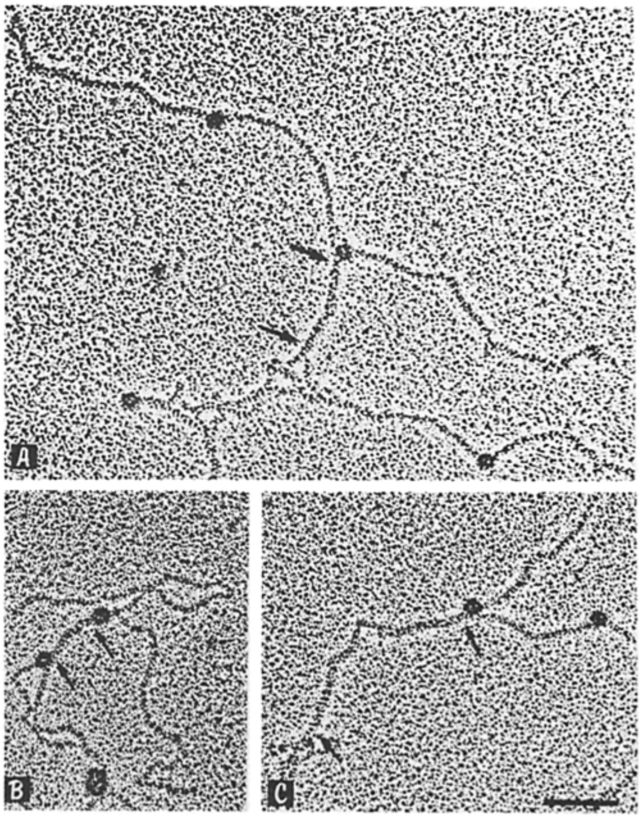

Figure 3. Lateral association of type IV collagen in initial stages of self-assembly. Rotary shadow images obtained from type IV collagen (300 μg/ml) incubated at 35°C for 60 min in PBS. Lateral association can be observed between adjacent type IV collagen molecules (*A–C*), next to areas where the NCl globule of one molecule binds to another collagen molecule, as indicated by arrows. The thickness of collagen in areas contained within the arrows is approximately double due to lateral association. Bar, 100 nm.

molecules all the way from the carboxy- to the amino-terminal end when assembly occurs (Fig. 3).

In completely formed networks, it is impossible to trace the whole length of individual type IV collagen molecules, but the distance between neighboring NCl globules can be traced easily (see Fig. 6 *B*). When this distance was measured, a single peak was observed at 100 nm (Fig. 5).

It appears therefore that domain NCl binds to four sites along the length of type IV collagen molecules with an apparent periodicity of ∼100 nm.

Furthermore, we observed that in intermediate forms of assembly, binding events between domain NCl and the rod-like part of collagen were accompanied by lateral association between adjacent molecules. Lateral assembly was evident due to the increased thickness of associated collagen molecules (Fig. 3, *A–C*). The question then arises, whether lateral association is a consequence of the binding of NCl to collagen or whether it is initiated by other binding events.

To answer this question, we used anti-NCl Fab fragments to block this domain and examined the effects on the assembly of type IV collagen by rotary shadowing. Under control conditions, type IV collagen (350 μg/ml) was incubated at 35°C in PBS for 1 h in the presence of ∼15 M excess anti-BSA Fab fragments (60 μg/ml) and was examined by rotary shadowing. Intermediate forms of assembly (Fig. 6 *A*)

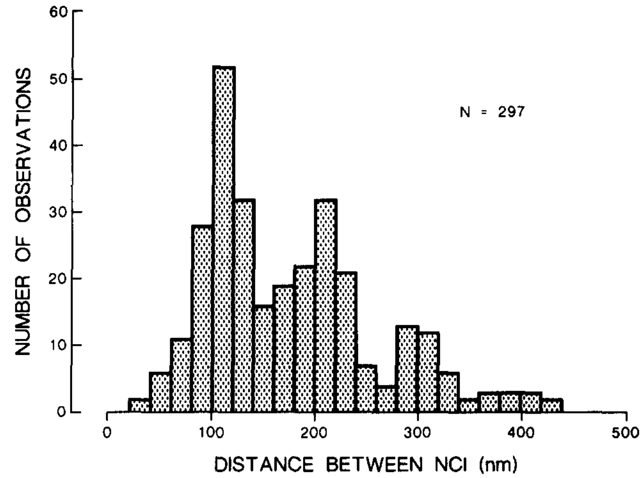

Figure 4. Histogram of the distribution of domain NCl of intact type IV collagen bound along the length of collagen, in initial forms of assembly, after incubation of type IV collagen (300 μg/ml) at 35°C for 60 min in PBS. Three different peaks, one major and two minor ones, are observed 100 nm apart.

and areas containing the polygonal network (Fig. 6 *B*) were present in 80% of the fields examined. Lateral associations were evident in both stages of assembly. The remaining 20% contained randomly oriented collagen molecules. However, when type IV collagen at 350 μg/ml was incubated under the same conditions in the presence of 15 M excess anti-NCl Fab fragments (60 μg/ml), the NCl globule was not observed to bind along the length of the collagen molecule. In this instance, no lateral association or network was observed (Fig. 6, *C–D*). Instead, randomly oriented molecules (Fig. 6 *C*) and various aggregates (Fig. 6 *D*) were seen in 90% of the fields examined. Only 10% of the fields contained initial stages of assembly. Thus, it appears that when the binding

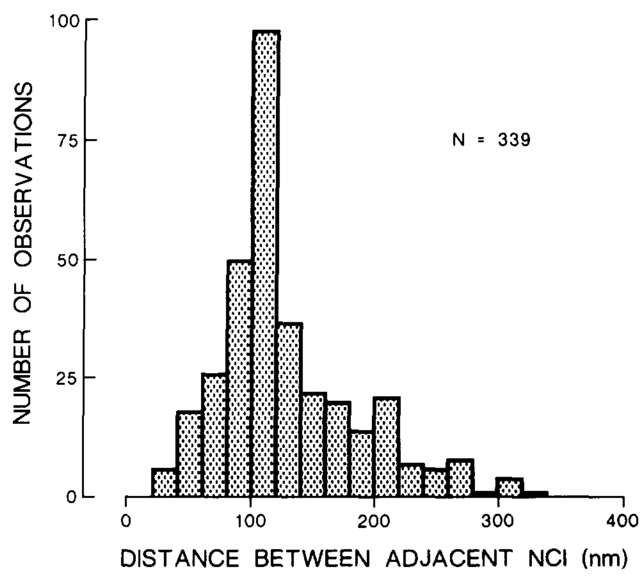

Figure 5. Histogram of the distribution of domain NCl of intact type IV collagen bound along the length of collagen, in complete networks formed by incubation of type IV collagen (300 μg/ml) at 35°C for 60 min in PBS. The nearest neighbor distance between NCl globules was measured. A single peak is observed at 100 nm along the length of type IV collagen.

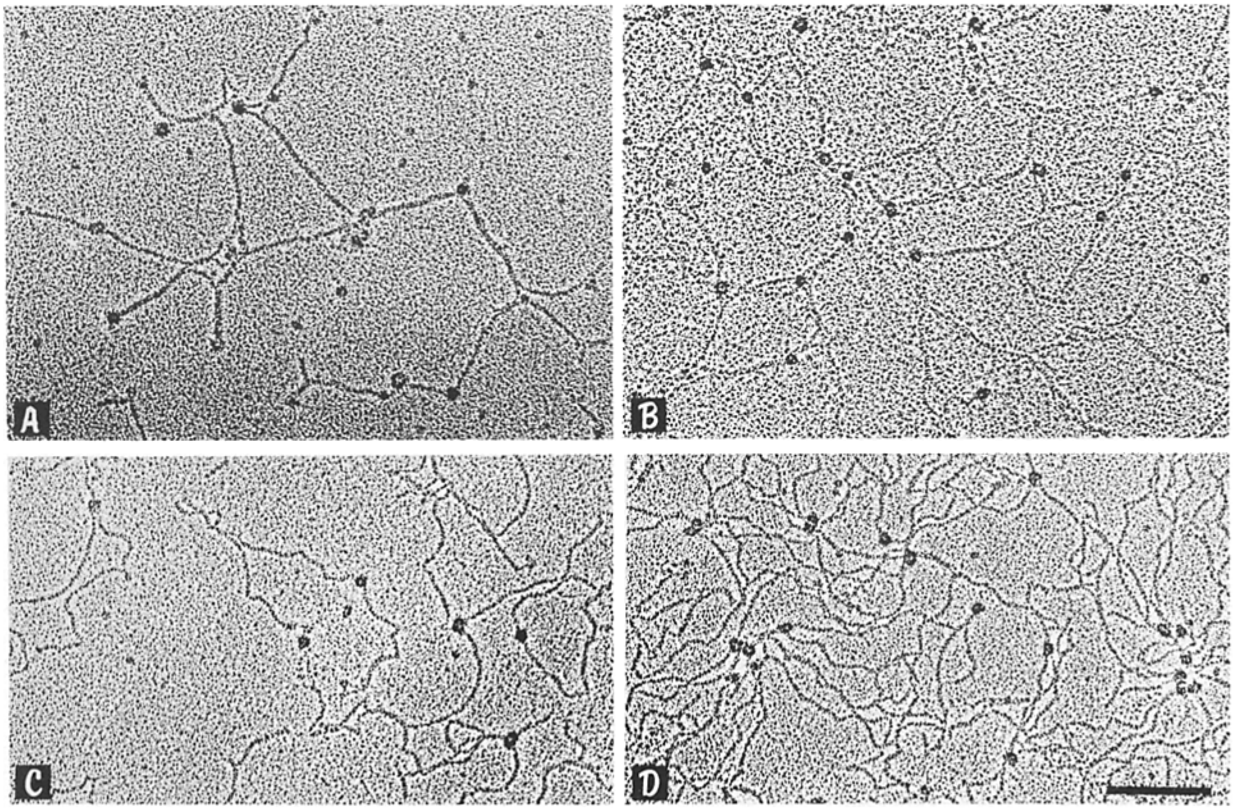

Figure 6. Effect of anti-NC1 Fab fragments on type IV collagen lateral association and network formation. Rotary shadow images obtained from type IV collagen (350 μg/ml) incubated at 35°C for 60 min in PBS, in the presence of anti-BSA Fab (*A* and *B*), or anti-NC1 Fab (*C* and *D*) fragments. The concentration of Fab fragments in both cases was 60 μg/ml. Type IV collagen incubated in the presence of anti-BSA Fab fragments was associated laterally (*A*) and formed an extensive polygonal network (*B*). The presence of anti-NC1 Fab fragments in incubated type IV collagen solutions inhibited both lateral association (*C*) and network formation (*D*) although aggregates of various forms were observed (*D*). Bar, 200 nm.

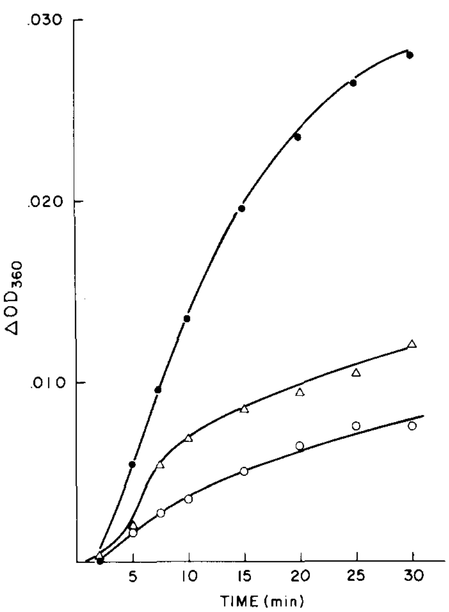

Figure 7. Turbidity of type IV collagen incubated under control conditions and in the presence of isolated domain NC1. Type IV collagen (200 μg/ml) was incubated at 35°C for 30 min, in PBS, either alone (*solid circles*) or in the presence of isolated domain NC1 at 50 μg/ml (*open triangles*), and at 100 μg/ml (*open circles*). The presence of domain NC1 considerably suppressed the development of turbidity, in a concentration-dependent manner.

of domain NC1 along the length of type IV collagen was blocked by specific antibodies, the assembly of collagen to a polygonal network that is mediated by lateral associations was inhibited. Therefore, binding of domain NC1 to type IV collagen appears to be crucial for lateral assembly.

Finally, we examined the effect of isolated domain NC1 on type IV collagen self-assembly by turbidometry. When type IV collagen is incubated at elevated temperatures in physiologic buffers, it develops turbidity without an apparent lag, in a concentration-dependent manner (26). Indeed, we observed that type IV collagen at 200 μg/ml incubated at 35°C in PBS raises turbidity and reaches a plateau within 30 min (Fig. 7). When isolated domain NC1 was added in the incubation mixture, it suppressed turbidity in a concentration-dependent manner; the higher the concentration of added NC1, the lower the observed maximal turbidity. Thus, the presence of NC1 at 50 μg/ml suppressed turbidity by 65%, while the presence of NC1 at 100 μg/ml suppressed turbidity by 75% (Fig. 7). These data indicate that added, isolated domain NC1 competes with the NC1 domain of intact type IV collagen for binding and formation of aggregates. Apparently, then, this domain is very important for the process of self-assembly and lateral association of type IV collagen.

Discussion

In this report we describe a novel type of association between the main noncollagenous NCl domain of type IV collagen and the rodlike part of collagen. This association appears to be important for lateral assembly of collagen and formation of the irregular polygonal network which has been described elsewhere (26). To our knowledge, this is the first study which reveals a specific function of domain NCl other than the binding to itself which results in the formation of dimeric collagen molecules (22, 24).

We present evidence that domain NCl binds at intervals of 100 nm along the length of type IV collagen. This 100-nm periodicity was observed in the binding of both isolated and native domain NCl, during the process of self-assembly. In a previous study, the distance between adjacent NCl globules of the complete network was measured and two peaks were observed, one at 170 nm and a second, smaller peak at 300 nm (26). We have no obvious explanation for this discrepancy although in the latter instance, the conditions used for incubation of type IV collagen were different. The temperature used during the incubation was lower (28°C instead of 35°C) and the buffer (PBS) also contained 100 μM DTT and 100 μM EDTA. Furthermore, the buffer used for the technique of rotary shadowing was ammonium acetate instead of ammonium bicarbonate and most of the experiments were done in the absence of glycerol. The possibility exists that one or more of these different experimental conditions could account for the differences we observe in the periodicity of the binding of domain NCl to type IV collagen. Nevertheless, even under these conditions, a substantial number of measurements were observed at ~100, 200 and 300 nm (Fig. 5 of reference 26). The nature of the binding site which apparently is repeated every 100 nm is unknown. Type IV collagen differs from most interstitial collagens because it contains interruptions of the triplet Gly-X-Y sequence. It has been suggested that these discontinuities make the molecule more flexible (10). Twelve interruptions, 1–11 amino acid residues long, have been found in the α1 chain of human type IV collagen (2). The interruptions of the α2 chain are not totally known and it remains to be substantiated whether they are aligned in the three polypeptide chains which form each collagen molecule. It is possible that the binding site for domain NCl involves some of the interruptions of the triple helix. This would be an exciting possibility, because it could assign important functions to several interruptions, other than flexibility. Alternatively, the binding site could lie in the collagenous, triple-helical part of collagen.

An interesting observation was that when binding events between NCl globules of intact type IV collagen and the rodlike part of collagen were seen with the technique of rotary shadowing, lateral associations were found next to the bound globules. Furthermore, when domain NCl was blocked by anti-NCl Fab fragments, lateral association and network formation were inhibited almost completely. In addition, it has been observed that pepsin-treated type IV collagen, which is cleaved to produce a smaller structure deprived of domain NCl, cannot self-assemble laterally. In fact, it can only associate via the amino-terminal domain to form small intermediate forms, up to tetramers, which have a spider-like appearance (22, 26). These data taken together strongly suggest that the binding of domain NCl which is repeated every 100 nm along the length of type IV collagen is required for lateral association and network formation.

The turbidity experiments further indicate a possible role for domain NCl in type IV collagen self-assembly. Type IV collagen has been described as raising turbidity readily when incubated at elevated temperatures in physiologic buffers (26). Co-incubation of type IV collagen with isolated domain NCl suppressed the development of turbidity depending upon the concentration of added NCl (Fig. 7). Interestingly, this competition by added NCl for binding and subsequent formation of turbid aggregates indicates that only one site for binding to the rodlike part of collagen exists per NCl globule. If two or more binding sites were available, this would facilitate the association of neighboring type IV collagen molecules and the formation of aggregates and would thus result in increased turbidity compared to that of control collagen. The suggestion that only one site exists per NCl globule is reinforced by morphological findings which indicate that the maximal observed thickness of laterally associated type IV collagen molecules corresponds to three strands (26). Indeed, if four binding sites exist in the rodlike part of each collagen molecule for domain NCl (Fig. 2) and if all are occupied by only one NCl globule each, the result would be a staggered arrangement with three strands in its thicker portions.

It is exciting to begin to understand the reason why type IV collagen is not enzymatically cleaved like other interstitial collagens. Maintenance of procollagen peptides could serve several important functions, including the assembly of type IV collagen in a polygonal network. This network would provide a scaffolding and could contribute to the sieving properties of basement membranes.

It would be important to determine the binding site in the sequence of domain NCl which is responsible for the initiation of lateral assembly of type IV collagen. In fact, the whole amino acid sequence of the NCl domain of the α1 chain has been deciphered from cDNA clones for the human (17) and murine (15) NCl. There are several striking observations. First, the two sequences, 229 amino acids long each, are nearly identical, indicating a possible conservation of this domain. Second, the sequence shows pronounced homology between the first and second parts of the structure (15, 17). It is exciting to determine if conservation of the amino acid sequence of this domain exists between various species.

The whole amino acid sequence of domain NCl contains five lysines. These residues are important because they are commonly involved in cross-linking and they are known to become chemically modified in diabetes. Under diabetic conditions, when the levels of glucose in the plasma are abnormally high, glucose binds non-enzymatically to the ε-amino group of lysine (14). Non-enzymatic glucosylation occurs in proteins with slow turnover rates such as albumin (6), hemoglobin (3), crystallins (20), fibronectin (21), etc., in diabetic conditions.

In preliminary experiments, non-enzymatically glucosylated domain NCl was tested for binding to type IV collagen by turbidometry and was found to cause minimal or no decrease of turbidity. In contrast, control NCl maintained the ability to suppress turbidity substantially (23) as mentioned previously in this report. This effect could be due to modified lysines. This would indicate that at least one lysine residue could participate in the binding site or be very close to it. In diabetes, the glomerular basement membrane becomes leaky to plasma proteins, an indication that the sieve formed by basement membrane components became defective. It is tempting to speculate that in vivo non-enzymatic glucosylation of domain NCl of collagen is related to aberrant leakage of proteins, since the binding needed for lateral assembly and network formation is defective. Furthermore, domain NCl appears to be the main antigen in patients with Goodpasture syndrome, in which auto-antibodies to this domain of collagen are most commonly observed (25). As a result, immune deposits are found in various basement membranes including the glomerular basement membrane, and cause proteinuria. It remains to be determined how these antibodies which are directed specifically against domain NCl affect the structure of basement membranes in such a way as to cause leakage of plasma proteins in the urine.

In diabetes, Goodpasture syndrome and other nephrotic syndromes (i.e., Alport's syndrome) proteinuria could be related in part to abnormal functions of domain NCl. It is possible that chemical or other modifications of this domain and antibodies directed against it could affect not only network assembly but also binding to other components of basement membranes such as nidogen (entactin), which has been reported to bind to domain NCl and to other basement membrane proteins as well (7).

The authors wish to express their thankfulness to Dr. H. Furthmayr (Yale University) for providing facilities and for helpful discussions, Dr. V. Marchesi (Yale University) for his support, and to Ms. V. Rosenzweig and Ms. Carol El-Ghandour for typing the manuscript.

This study was supported in part by a Research and Development Award from the American Diabetes Association to E. C. Tsilibary and by training grant GM-07562 from the National Institutes of Health to A. S. Charonis.

Received for publication 30 June 1986, and in revised form 3 September 1986.

References

1. Alitalo, K., A. Vaheri, T. Krieg, and R. Timpl. 1980. Biosynthesis of two subunits of type IV procollagen and of other basement membrane proteins by a human tumor cell line. *Eur. J. Biochem.* 109:247–255.

2. Babel, A., and R. W. Glanville. 1984. Structure of human-basement-membrane (type IV) collagen. Complete amino-acid sequence of a 914-residue-long pepsin fragment from the a1 (IV) chain. *Eur. J. Biochem.* 143:545–556.

3. Bunn, H. F., R. Shapiro, M. McManus, L. Garrick, M. J. McDonald, P. M. Gallop, and K. H . Gabbay. 1979. Structural heterogeneity of human hemoglobin A due to nonenzymatic glucosylation. *J. Biol. Chem.* 254:3892–3898.

4. Charonis, A. S., E. C. Tsilibary, P. D. Yurchenco, and H. Furthmayr. 1985. Binding of type IV collagen to laminin. A morphologic study. *J. Cell Biol.* 100:1848–1852.

5. Crouch, E., H. Sage, and P. Bornstein. 1980. Structural basis for apparent heterogeneity of collagens in human basement membranes: type IV collagen contains two distinct chains. *Proc. Natl. Acad. Sci. USA.* 77:745–749.

6. Day, J. F., R. W. Thornburg, S. R. Thorpe, and J. W. Baynes. 1979. Nonenzymatic glucosylation of rat albumin. *J. Biol. Chem.* 254:9394–9400.

7. Dziadek, M., M. Paulsson, and R. Timpl. 1985. Identification and interaction repertoire of large forms of the basement membrane protein midogen. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:2513–2518.

8. Edelman, G. M., and J. J. Marchalonis. 1967. Methods used in the studies of the structure of immunoglobulins. *In* Methods in Immunology and Immunochemistry. C. A. Williams and M. W. Chase, editors. Academic Press, Inc., New York. 405–424.

9. Glanville, R. W., and A. Rauter. 1981. Pepsin fragments of human placental basement membrane collagens showing interrupted triple-helical amino acid sequences. *Hoppe-Seyler's Z. Physiol. Chem.* 362:943–951.

10. Glanville, R. W., T. Voss, and K. Kuehn. 1982. A comparison of the flexibility of molecules of basement membrane and interstitial collagens. *In* New Trends in Basement Membrane Research. K. Kuehn, H. Schoene, and R. Timpl, editors. Raven Press, New York. 69–77.

11. Kleinman, H. K., R. J. Klebe, and G. R. Martin. 1981. Role of collagenous matrices in the adhesion and growth of cells. *J. Cell Biol.* 88:473–485.

12. Kleinman, H. K., M. L. McGarvey, L. A. Liotta, P. Gehron-Robey, K. Tryggvason, and G. R. Martin. 1982. Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma. *Biochemistry.* 21:6188–6193.

13. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

14. Monnier, V. M., and A. Cerami. 1981. Nonenzymatic browning *in vivo*: possible process for aging of long-lived proteins. *Science (Wash. DC).* 211: 491–493.

15. Oberbäumer, I., M. Laurent, U. Schwarz, Y. Sakurai, Y. Yamada, G. Vogeli, T. Voss, B. Siebold, R. W. Glanville, and K. Kühn. 1985. Amino acid sequence of the non-collagenous globular domain (NC1) of the a1(IV) chain of basement membrane collagen as derived from complementary DNA. *Eur. J. Biochem.* 147:217–224.

16. Orkin, R. W., P. Gehron, E. B. McGoodwin, G. R. Martin, T. Valentine, and R. Swarm. 1977. A murine tumor producing a matrix of basement membrane. *J. Exp. Med.* 145:204–220.

17. Pihlajaniemi, T., K. Tryggvason, J. C. Myers, M. Kurkinen, R. Lebo, M.-C. Cheung, D. J. Prockop, and C. D. Boyd. 1985. cDNA clones coding for the pro-a1 (IV) chain of human type IV procollagen reveal an unusual homology of amino acid sequences in two halves of the carboxy-terminal domain. *J. Biol. Chem.* 260:7681–7687.

18. Schuppan, D., R. W. Glanville, and R. Timpl. 1982. Covalent structure of mouse type IV collagen. *Eur. J. Biochem.* 123:505–512.

19. Shotten, D. M., B. E. Burke, and D. Branton. 1979. The molecular structure of human erythrocyte spectrin. Biophysical and electron microscopic studies. *J. Mol. Biol.* 131:303–329.

20. Stevens, V. J., C. A. Rouzer, V. M. Monnier, and A. Cerami. 1978. Diabetic cataract formation: potential role of glycosylation of lens crystallins. *Proc. Natl. Acad. Sci. USA.* 75:2918–2922.

21. Tarsio, J. F., B. Wigness, T. D. Rhode, W. M. Rupp, H. Buchwald, and L. T. Furcht. 1985. Nonenzymatic glycation of fibronectin and alterations in the molecular association of cell matrix and basement membrane components in diabetes mellitus. *Diabetes.* 34:477–484.

22. Timpl, R., H. Wiedemann, V. Van Delden, H. Furthmayr, and K, Kühn. 1981. A network model for the organization of type IV collagen molecules in basement membranes. *Eur. J. Biochem.* 120:203–211.

23. Tsilibary, E. C., and A S. Charonis. 1986. The effect of nonenzymatic glucosylation on the binding of the main non-collagenous NC1 domain to type IV collagen. *J. Cell Biol.* 103(4, Pt. 2):388*a*. (Abstr.)

24. Weber, S., J. Engel, H. Wiedemann, R. W. Glanville, and R. Timpl. 1984. Subunit structure and assembly of the globular domain of basement membrane collagen type IV. *Eur. J. Biochem.* 139:401–410.

25. Wieslander, J., J. F. Barr, R. J. Butkowski, S. J. Edwards, P. Bygren, D. Heinegard, and B. G. Hudson. 1984. Goodpasture antigen of the glomerular basement membrane: localization to noncollagenous regions of type IV collagen. *Proc. Natl. Acad. Sci. USA.* 81:3838–3842.

26. Yurchenco, P. D., and H. Furthmayr. 1984. Self-assembly of basement membrane collagen. *Biochemistry.* 23:1839–1850.

27. Zar, J. H., 1974. Biostatistical Analysis. Prentice-Hall Inc., Englewood Cliffs, New Jersey. 301–306.