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Gel Formation from the Type IV Collagen Isolated from Bovine Lens Capsule in Guanidine-HCl and Dithiothreitol[1]

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Type IV collagen was prepared from bovine lens capsule by acetic acid extraction, followed by purification including DEAE-Sephacel chromatography and dialysis. The type IV collagen solution became viscous and eventually gelated upon dialysis against 2 M guanidine-HCl and 10 mM dithiothreitol. Gelation was not observed for heat-denatured type IV collagen, suggesting that collagenous conformation may be required for the gelation. A reducing agent, dithiothreitol, was essential for the gelation of type IV collagen in 2 M guanidine-HCl. An optimal concentration of guanidine-HCl for the gelation lays between 1.5 and 2.5 M: gelation did not occur at 1 M or lower and at 3 M or higher, although the circular dichroism spectrum characteristic of the collagenous triple-helix was not changed in 3 M guanidine-HCl. This suggests that an appropriate change in conformation of the type IV collagen at a region other than the triple-helical region or/and partial dissociation of complexed type IV collagen aggregates may drive intermolecular interactions of the type IV collagen leading to polymerization and eventually to gelation. To our knowledge, this is the first report that the type IV collagen alone has the ability to form a rigid gel. The assembled structure of the type IV collagen in gel form might be related to the skeletal architecture of basement membrane(s).

Key words: basement membrane, gel, guanidine-HCl, self-assembly, type IV collagen.

Skeletal structures of extracellular matrices consist of supramolecular assemblies of matrix components, particularly of the collagen protein family. Isolated type I collagen molecules in solution under appropriate conditions, such as physiological pH, ionic strength, and temperature, assembled into fibrils eventually forming a gel. Basement membranes comprise a thin layer of extracellular matrix, which separates epithelia from underlying connective tissues. Type IV collagen is a major constituent of basement membranes (*1*). Hence the possibility that type IV collagen has the ability to aggregate into a gel form has been pursued. Kleinman *et al.* reported that the extract from EHS tumor tissues containing type IV collagen formed a gel under physiological conditions. The major components required for the gelation included laminin, heparan sulfate proteoglycan, and nidogen other than type IV collagen. These components polymerized in a constant proportion, implying that all these components interact in a defined way to form a basement membrane-like structure in a neutral phosphate buffer (*2*). Yurchenco and Furthmayr demonstrated that the purified type IV collagen from EHS tumor in a neutral phosphate buffer increased its viscosity upon incubation at 28°C, presumably due to self-assembly of the type IV collagen. They did not say whether the aggregates eventually gelated or not (*3*). Instead, they discussed the mechanism of association of the type IV collagen and proposed a model of the assembled structure of type IV collagen. We report here that the type IV collagen purified from bovine lens capsule formed a rigid gel in a buffer containing 2 M guanidine-HCl (Gn-HCl) and DTT. The phenomenon is peculiar in that this condition in general has been believed to be dissociative for macromolecular complexes. Therefore, the present finding implies that the type IV collagen protein has a unique intermolecular interaction which has not been reported previously

MATERIALS AND METHODS

Materials—Bovine lens capsules isolated from fresh bovine eyes and stored after freezing were provided by Nitta Gelatin, Osaka. Urea and Gn-HCl were purchased from Nacalai Tesque. DEAE-Sephacel was obtained from Pharmacia LKB Biotechnology. Spectra/Por Cellulose Ester Membrane (molecular weight cut-off: 300,000) was obtained from Spectrum Medical Industries. Goat polyclonal antibody against type IV collagen was purchased from Southern Biotechnology Associates. Horseradish peroxidase-conjugated rabbit anti-goat IgG was purchased

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Abbreviations: CD, circular dichroism: DTT, dithiothreitol; EHS, Engelbreth-Holm-Swarm; ELISA, enzyme-linked immunosorbent assay; Gn-HCl, guanidine-HCl; NC, non-collagenous; PBS, phosphate-buffered saline.

from Zymed Laboratories. Glass micropipets were obtained from Drummond Scientific.

Methods—Preparation of lens capsule type IV collagen: All the following procedures were carried out at 4°C. Acid extract of bovine lens capsules was prepared as previously described (*4*). Briefly, lens capsules were washed with 20 mM sodium phosphate, pH 7.2, containing a mixture of protease inhibitors, 5 mM EDTA, 1 mM *N*-ethylmaleimide, 100 μM phenylmethylsulfonyl fluoride, and 1 μM pepstatin A, and 0.02% (w/v) sodium azide. Lens capsules were homogenized with a Polytron homogenizer in 0.5 M acetic acid (5-10 ml per 1 g wet weight of lens capsule) and stirred in suspension for several days. After centrifugation at 800 × *g* for 15 min, the supernatant was collected (designated as acid extract). The type IV collagen in the acid extract was purified as follows. The acid extract was dialyzed to equilibrium against 50 mM Tris-HCl, pH 8.5, containing 6 M urea. The dialysate then was passed through a DEAE-Sephacel column equilibrated with the same buffer. The unbound fraction was extensively dialyzed against 50 mM Tris-HCl, pH 8.5, containing 6 M urea, using Spectra/Por Cellulose Ester Membrane (molecular weight cut-off: 300,000) to remove low-molecular-weight substances. The sample prepared was dialyzed against 50 mM Tris-HCl, pH 8.1, containing 2 M Gn-HCl, and stored at 4°C until used.

The preparation was treated with bacterial collagenase as described previously (*4*).

SDS-polyacrylamide gel electrophoresis: SDS-PAGE was carried out in the buffer system of Laemmli on a vertical slab gel (1 mm thick) at a constant current of 20 mA (*5*).

Electrophoretic transfer for immunoblotting: Protein blotting and immunological identification of the proteins were carried out as previously described (*4*). Briefly, the proteins were transferred to a nitrocellulose sheet, which was then incubated with a goat polyclonal antibody to type IV collagen diluted 1:1,000 in PBS containing 0.25% BSA and 0.1% Tween 20. The sheet was exposed to horseradish peroxidase-conjugated rabbit anti-goat IgG in PBS containing 0.25% BSA and 0.1% Tween 20, followed by exposure to PBS containing H_2O_2 and diaminobenzidine for color development.

Amino acid analysis: Samples were hydrolyzed at 110°C for 24 h or 72 h in 6 M HCl. Amino acid analyses were performed with an auto-amino acid analyzer L-8500 (Hitachi, Tokyo). Amino acid contents were obtained as the average values for 24 and 72 h hydrolysates, except for serine, threonine, and tyrosine, which were quantified by extrapolation to 0 h of hydrolysis, and for valine and isoleucine, which were determined from the 72 h hydrolysates.

Measurement of relative viscosity: Relative viscosity was measured by a method similar to the one previously described (*3, 6*). A sample was sucked up into a 100-μl glass micropipet. Then the micropipet was capped at one end and incubated for a predetermined time at 4°C. After incubation the micropipet was placed at an angle of 45° and the time required for a 1-mm steel ball to fall between two fixed points was measured.

Circular dichroism measurement: Circular dichroism spectra were recorded at 10°C on a Jasco J-600 spectropolarimeter in a water-jacketed cell with a light path length of 2 mm. Sample concentrations (0.086 mg/ml) were determined from the results of amino acid analysis.

RESULTS

Preparation and Characterization of the Type IV Collagen from Bovine Lens Capsule—The acid extract of bovine lens capsules was subjected to SDS-5% acrylamide gel electrophoresis (lane 1, Fig. 1A) and immunoblotting with polyclonal antibody to type IV collagen (lane 3, Fig. 1A). All the amidoblack-stained bands reacted with the anti-type IV collagen antibody except for the band with an apparent M_r of 100K and the bands at the gel front, which were resolved into several bands, including a polypeptide with an apparent M_r of 25K, when 12.5% gel was used (lane 1, Fig. 1B). The 100K polypeptide reacted with anti-α2(IV) monoclonal antibody (Iwata *et al.*, unpublished observation). Small polypeptides in the acid extract without immunoreaction were removed by DEAE-Sephacel chromatography as a bound fraction, followed by dialysis with cellulose ester membrane tubing (molecular weight cut-off: 300,000). The SDS-PAGE band pattern of the purified material showed only the type IV collagen-derived

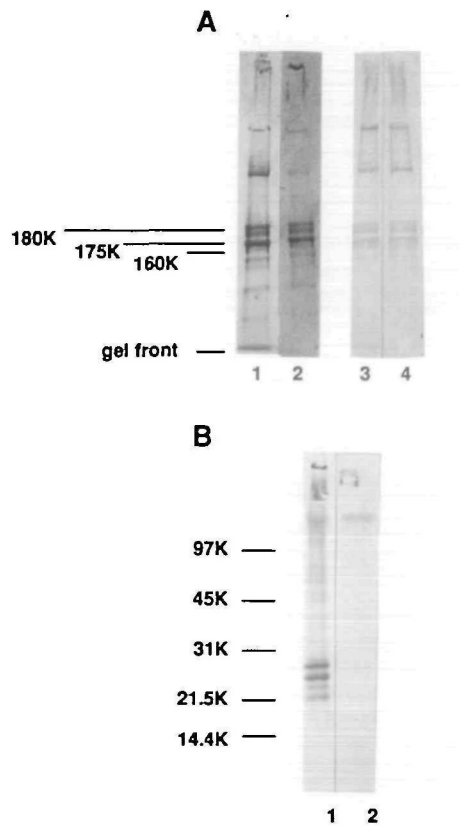

Fig. 1. **Analysis by SDS-PAGE and immunoblotting of the type IV collagen preparation from bovine lens capsule.** Acid extract of bovine lens capsule (A· lanes 1, 3; B: lane 1) and the type IV collagen purified from the acid extract (A: lanes 2, 4, B: lane 2) were subjected to 5% (A) and 12.5% (B) SDS-polyacrylamide gel electrophoresis under reducing conditions After the electrophoresis, proteins were transferred to a nitrocellulose membrane. The membranes were stained with amidoblack (A, B. lanes 1, 2) or immunoblotted with the polyclonal antibody to type IV collagen (A: lanes 3, 4).

four bands as major polypeptides, with no bands at the gel front (lane 2, Fig. 1A). All the bands seen in 5% acrylamide gel disappeared and a new 25K band appeared upon treatment with a purified bacterial collagenase. The 25K polypeptides were presumably derived from NC1 domains of the type IV collagen chains (Muraoka M., unpublished observation). The results confirmed that the preparation contained little noncollagenous protein. Three polypeptides with M_r of 180K, 175K, and 160K may represent the α chains of type IV collagen and one with M_r of 320K may represent the β chain (*4*). The amino acid composition of the purified material is shown in Table I, along with those of human type IV collagen (*7*) and mouse type IV collagen (*8*) deduced from the nucleotide sequences, as well as those obtained from the isolated type IV collagen (*9*) and laminin (*10*) from mouse EHS tumor tissues. The amino acid composition of the purified material is similar to those of type IV collagen and far from that of laminin, suggesting that laminin or other non-collagenous protein can not be the major component in the preparation.

Gelation of the Lens Capsule Type IV Collagen—The purified type IV collagen remained in solution upon dialysis against 50 mM Tris-HCl, pH 8.1, containing 2 M Gn-HCl. In contrast, dialysis against the same solution but containing 10 mM DTT resulted in gel formation. Since an increase in solution viscosity was observed prior to gelation, the conditions required for gelation were examined by the measurement of viscosity increase. Gelation of purified type IV collagen solution that had been dialyzed against 50 mM Tris-HCl, pH 8.1, containing 2 M Gn-HCl, was initiated by adding DTT. As incubation time was prolonged at 4°C, the falling speed of the ball decreased until the ball would no longer begin to fall owing to the high viscosity that the solution reached (Fig. 2). In contrast, there was no change in viscosity in the absence of DTT. The protein solution after heat treatment at 80°C for 15 min did not show any increase in viscosity upon incubation with DTT, suggesting that heat-denatured type IV collagen had lost the ability to form gel. The solution treated at 80°C showed even lower viscosity than the solution without heat treatment, presumably because of the low intrinsic viscosity of the heat-denatured type IV collagen due to the destruction of

TABLE I. **The amino acid compositions of the purified material and other basement membrane components.**

	Purified material from bovine lens capsule	Type IV collagen			Laminin
		Human[a]	Mouse[b]	EHS tumor[c]	EHS tumor[d]
		(residues/1000)			
Asn	N.D.	10	11	N.D.	N.D.
Asp	39	40	37	62	109
Thr	31	27	30	61	58
Ser	45	41	46	38	77
Gln	N.D.	41	46	N.D.	N.D.
Glu	102	40	41	102	122
Gly	308	283	282	234	93
Ala	39	40	35	54	76
Val	32	30	35	46	48
Cys	8	12	12	N.D.	30
Met	15	17	19	16	14
Ile	31	36	28	33	42
Leu	58	57	54	63	92
Tyr	7	13	11	15	27
Phe	32	30	32	34	31
His	9	10	11	17	24
Arg	31	33	33	37	50
Trp	N.D.	4	4	N.D.	N.D.
Pro	72	186	182	64	53
3-Hyp	N.D.	–	–	3	N.D.
4-Hyp	108	–	–	70	N.D.
Lys	11	53	51	26	52
Hyl	22	–	–	31	2

[a]From Ref. 7. [b]From Ref. 8. [c]From Ref. 9. [d]From Ref. 10, N.D. not determined. [a,b]The results are deduced from nucleotide sequences. Calculations are based on the assumption that the type IV collagen molecule consists of two α1(IV) and one α2(IV) chains.

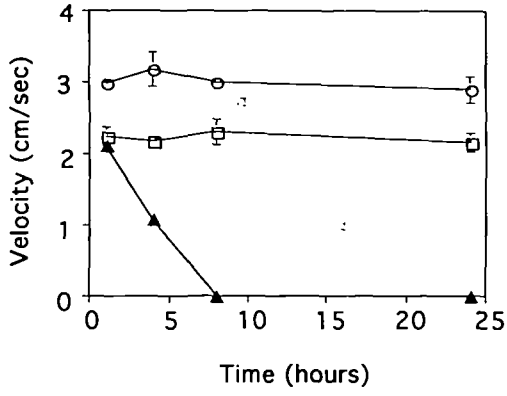

Fig. 2. **Increase in viscosity of type IV collagen in Gn-HCl solution with dithiothreitol.** After addition of DTT to a final concentration of 10 mM, the type IV collagen solutions in 50 mM Tris-HCl, pH 8.1, containing 2 M Gn-HCl were incubated at 4°C for 1 h (▲) or incubated at 80°C for 15 min followed by incubation at 4°C for 45 min (○). The samples were sucked up into micropipets and incubated at 4°C for the times indicated (0 time indicates the time when DTT was added), then the falling velocity of a steel ball was measured as described under "MATERIALS AND METHODS." The ordinate, falling ball velocity, represents the inverse of relative viscosity. As a control, samples to which DTT was not added were incubated at 4°C and the velocity was measured in a similar manner as above (□). Final concentration of type IV collagen was adjusted to 0.27 mg/ml.

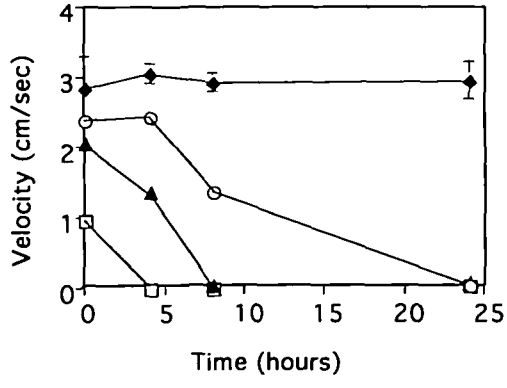

Fig. 3. **Effect of type IV collagen concentration on viscosity.** After addition of DTT to a final concentration of 10 mM, the type IV collagen solutions in 50 mM Tris-HCl, pH 8.1, containing 2 M Gn-HCl, were sucked up into micropipets and incubated at 4°C for the times indicated. Final concentrations of type IV collagen were adjusted to 0.80 (□), at 0.40 (▲), at 0.20 (○) and at 0.08 (◆) mg/ml. Then the falling velocity of a ball was measured as described under "MATERIALS AND METHODS." The ordinate, falling ball velocity, represents the inverse of relative viscosity.

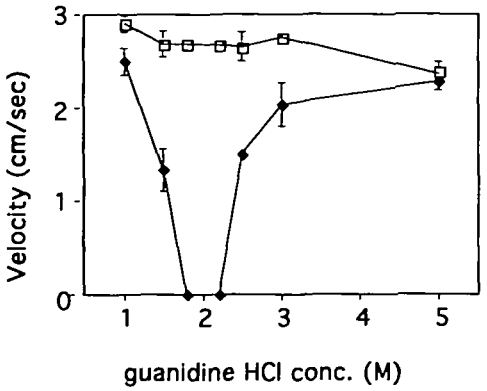

Fig. 4. **Effect of Gn-HCl concentration on increase in viscosity.** To the purified type IV collagen in 50 mM Tris-HCl, pH 8.1, containing 2 M Gn-HCl, 50 mM Tris-HCl, pH 8.1, containing 0, 2, or 6.3 M Gn-HCl was added to give a concentration of 1-5 M. After addition of DTT to a final concentration of 10 mM, the samples were incubated at 4°C for 24 h. Final concentration of type IV collagen was 0.27 mg/ml. The falling velocity of a ball was measured as described under "MATERIALS AND METHODS" (◆). The ordinate, falling ball velocity, represents the inverse of relative viscosity. Open squares (□) show the falling ball velocity in the buffer under each experimental condition without the type IV collagen.

the long collagenous triple-helix structure with its rod-like shape.

Effect of Concentrations of Type IV Collagen and Gn-HCl on the Gelation—Figure 3 illustrates the effect of type IV collagen concentration on the viscosity increase. The type IV collagen solution was dialyzed against 50 mM Tris-HCl, pH 8.1, containing 2 M Gn-HCl and diluted to give a final type IV collagen concentration of 0.08-0.80 mg/ml. At concentrations above 0.20 mg/ml, solution viscosity increased upon addition of DTT. The time required to attain the maximum viscosity (a velocity of 0 cm/s) depended on the protein concentration. At a concentration of 0.20 mg/ml, the viscosity increased after a lag period of incubation. At a concentration of 0.08 mg/ml or less, the viscosity did not increase.

The dependence of the viscosity on Gn-HCl concentration is shown in Fig. 4. When the type IV collagen solution at a concentration of 0.27 mg/ml was incubated at 4°C for 24 h with DTT, increased viscosity was observed at a concentration of Gn-HCl between 1.5-2.5 M, but not at 1, 3, or 5 M. An optimal concentration of Gn-HCl for the gelation was between 1.5 and 2.5 M, suggesting that a conformational change of the type IV collagen or/and partial dissociation of complexed type IV collagen aggregates was induced by 2 M Gn-HCl appropriately for the initiation of self-assembly. Conformational change or/and partial dissociation may be insufficient at Gn-HCl concentrations below 1.5 M, while conformational change or/and partial dissociation was excessive above 2.5 M.

Circular Dichroism Examination—Circular dichroism spectra (CD) of the type IV collagen in the solution and in the gel were obtained. The CD spectrum of type IV collagen in 2 M Gn-HCl showed a positive peak near 220 nm, characteristic of the collagen triple-helix (Fig. 5a). The type IV collagen in 2 M Gn-HCl incubated at 4°C for 24 h with DTT showed a CD spectrum similar to that in 2 M Gn-HCl without DTT (Fig. 5b). Furthermore, type IV

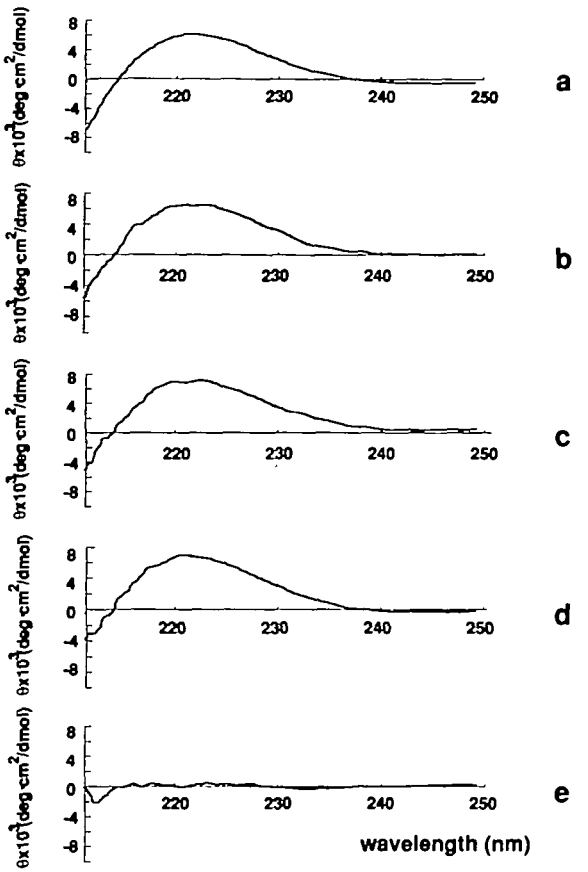

Fig. 5. **Circular dichroism spectra of the type IV collagen in Gn-HCl.** (a) The type IV collagen incubated at 4°C in 50 mM Tris-HCl, pH 8.1, containing 2 M Gn-HCl. The type IV collagen incubated at 4°C in 50 mM Tris-HCl, pH 8.1, with 10 mM DTT, containing (b) 2 M, (c) 1 M, and (d) 3 M Gn-HCl. (e) The type IV collagen incubated in 50 mM Tris-HCl, pH 8.1, containing 2 M Gn-HCl, at 80°C for 15 min after addition of DTT, followed by further incubation at 4°C for 45 min. All spectra were recorded at 10°C.

collagen in 1 or 3 M Gn-HCl incubated for 24 h at 4°C with DTT also showed the same CD spectrum as that in 2 M Gn-HCl with or without DTT (Fig. 5, c and d). On the other hand, the type IV collagen incubated at 80°C in 2 M Gn-HCl and DTT showed CD a spectrum without a peak near 220 nm (Fig. 5e). These results indicated that the addition of DTT caused no detectable change in the triple-helical conformation, in terms of the CD spectra, and that the type IV collagen maintained the triple-helical conformation in 1-3 M Gn-HCl.

DISCUSSION

The present study demonstrated that the type IV collagen, prepared from bovine lens capsule by acetic acid extraction followed by purification procedures including DEAE-Sephacel chromatography and dialysis, self-assembled into gel in 2 M Gn-HCl and DTT. The preparation reported previously by Brinker *et al.* (*11*), obtained essentially in the same way, contained only type IV collagen and little noncollagenous protein, such as laminin or heparansulfate proteoglycan. The preparation used in the present study contained type IV collagen or peptides derived from type

IV collagen as the major constituent. The amino acid composition is similar to that expected for pure type IV collagen (Table I). Treatment with collagenase, that only cleaves collagenous proteins, digested all the protein bands and no band was detectable on 5% SDS gel except at the gel front. Immunoblotting with polyclonal antibody to type IV collagen revealed all the bands detected by amidoblack staining except one (M_r 100K), which was found to react with H21 monoclonal antibody recognizing the NC1 domain of α2(IV) chain (Iwata *et al.*, unpublished observation). The polyclonal antibody was initially raised against pepsin-treated type IV collagen, which presumably lacks the non-collagenous region. The above results imply that the polypeptide with the M_r of 100K was derived from α2(IV) chain, and it lost a part of the collagenous region of the chain located at the side opposite to NC1 domain.

Recently, α3-α6 chains in addition to α1 and α2 chains of type IV collagen were discovered (*12, 13*). Analysis of NC1 domains of type IV collagen obtained by bacterial collagenase digestion of bovine lens capsule demonstrated that the major constituents of type IV collagen in bovine lens capsule were α1(IV) and α2(IV) chains, and that small amounts of α3(IV) and α4(IV) were also present (*13, 14*). Western blotting analysis showed that 180K and 160K polypeptides in the acid extract from bovine lens capsule reacted with anti-α1(IV) monoclonal antibody, and that 175K polypeptide reacted with anti-α2(IV) monoclonal antibody. No positive staining with anti-α3(IV), α4(IV), α5(IV), or α6(IV) was obtained, suggesting that native α3(IV), α4(IV), α5(IV), or α6(IV) was not enriched in the acid extract. In a preliminary ELISA study using anti-α1(IV), α2(IV), α3(IV), α4(IV), α5(IV), and α6(IV) monoclonal antibodies, only α1(IV) and α2(IV) chains were detected in the acid extract from bovine lens capsule (M. Iwata *et al.*, manuscripts in preparation). From these results, it was concluded that major α chains of type IV collagen in the acid extract of lens capsule were α1(IV) and α2(IV), and that little α3(IV), α4(IV), α5(IV), or α6(IV) existed in the acid extract.

Taking into account the minimum polymer concentration required for gelation in general, it is likely that the gelation was driven by self polymerization of the type IV collagen. It is unlikely that other components, present in undetectable amounts, if at all, could be responsible for the gelation. Heat treatment of the preparation inactivated gel formation in the same buffer, suggesting that the aggregation of type IV collagen depended on the conformation of a heat-labile domain, as found for the gelation of type I collagen. The CD studies implied that type IV collagen maintained the collagenous triple-helical conformation in 2 M Gn-HCl and DTT, but that the heat treatment destroyed the conformation. Thus, it is most likely that the triple-helical conformation of type IV collagen is involved in aggregation of the protein with subsequent gelation.

Four types of interdomain associations between the molecules of type IV collagen have been reported: (i) between carboxy terminal (NC1) domains, (ii) among four amino terminal (7S) domains, (iii) between NC1 domain and the collagenous domain, and (iv) between the collagenous domains in lateral interaction (*3, 15, 16*). Aggregation of the EHS type IV collagen molecules was observed *in vitro* at a physiological pH and ionic strength. It was repressed in 2 M urea and DTT, where only the first type of association, the NC1-NC1 interactions which may lead to dimer formation, could be retained (*3*). We observed that the lens capsule type IV collagen formed a gel in a buffer containing 6 M urea and DTT (Muraoka M., unpublished observation). Even though the association between NC1 domains might be retained in 6 M urea and DTT, NC1-NC1 interactions would yield only dimeric collagen or the combination of two molecules, being inadequate for the formation of a network assembly of the molecules. Hence, we speculate that the gelation of lens capsule type IV collagen may involve another interdomain interaction not previously reported. The optimal Gn-HCl concentration of between 1.5 and 2.5 M implies that moderate, but not major, conformational change of the type IV collagen was required for the association. For example, the addition of DTT to 2 M Gn-HCl may change the conformation of type IV collagen molecules in a specific way, which may contribute to the putative fifth association mode by uncovering hidden association sites. The CD spectrum of the type IV collagen in 2 M Gn-HCl and DTT showed a positive peak near 220 nm and was similar to that in 1 M Gn-HCl and DTT or that in 3 M Gn-HCl and DTT. This suggests that the overall collagenous conformation of type IV collagen was little changed under these conditions. Since the type IV collagen did not form a gel in 1 M Gn-HCl and DTT or in 3 M Gn-HCl and DTT, a conformational change of a domain(s) other than the triple-helical domain may be important. Alternatively, since the affinity of pepsin-treated type I collagen for heparin was altered in urea without change in the overall triple-helical conformation, at least in terms of the CD spectra (*17*), Gn-HCl with DTT may have caused a subtle change in the conformation of type IV collagen, not affecting the CD spectra, but sufficient to modify the intermolecular interactions, generating the putative fifth association mode.

Gn-HCl and DTT in general have been used as a dissociative reagent for macromolecular complexes, including extracellular matrix. In fact, the condition used in the present report for the lens capsule type IV collagen gelation was employed for the extraction of EHS tumor type IV collagen (*3, 9*). Since the lens capsule type IV collagen did not gelate at all in the absence of DTT, the reduction of disulfide-bonds in the type IV collagen molecules must have played a key role in the molecular associations which eventually resulted in gelation. We have observed that DTT accelerated the multimerization of plasma fibronectin

TABLE II. **Comparison of Gn-HCl-DTT gel and Matrigel.**

	Gn-HCl-DTT gel	Matrigel[a]
Starting material	Bovine lens capsule	EHS tumor
Extraction with	0.5 M acetic acid	2 M urea-50 mM Tris-HCl (pH 7.4)
Purification	DEAE-Sephacel and dialysis (MWCO:300,000[b])	–
Gelation conditions	2 M Gn-HCl, 10 mM DTT, 50 mM Tris-HCl, pH 8.1 at 4°C	0.15 M NaCl-50 mM Tris-HCl (pH 7.4) at 35°C
Components	Type IV collagen	Laminin 60%, type IV collagen 30%, heparan sulfate proteoglycan 3%, nidogen 5%

[a]From Ref. 2. [b]Molecular weight cut-off.

at 37°C, and appeared to induce a conformational change of fibronectin (*18*). It is known that type IV collagen possesses two disulfide-rich domains. One is the NC1 domain (*15*) and the other is the 7S domain (*19*). Thus, the reduction of the disulfide-bonds in terminal portions of the type IV collagen might induce conformational change of these regions and/or dissociation of the type IV collagen complex. Newly formed and/or unmasked association sites in the presence of DTT may well have driven type IV collagen association into gelation. Alternatively, disulfide-bonds in the collagenous domain could be responsible for the association with extended conformations of the NC1-NC1 or 7S regions.

The gelation from basement membrane components of EHS tumor under physiological conditions was reported by Kleinman *et al.* (*2*). They claimed that laminin, heparan sulfate proteoglycan and nidogen other than type IV collagen were required. The gel of this study and that of Kleinman *et al.* (Matrigel) are compared in Table II. Matrigel comprised laminin, type IV collagen, heparan sulfate proteoglycan and nidogen and all these components were suggested to be required for the gelation. Laminin accounts for almost 60% of the material in the gel, type IV collagen for 30%, heparan sulfate proteoglycan for less than 3% and nidogen for less than 6% (*2*). Thus, cooperative interaction between the components was needed to form Matrigel. In contrast, the major component responsible for the gelation in Gn-HCl and DTT was the type IV collagen, suggesting that type IV collagen extracted from bovine lens capsule has the ability to form a gel by itself.

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