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Purification and Characterization of Type IV Collagen from Mouse Kidney

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Type IV collagen was purified from the pepsin digest of mouse kidneys by heat-gelation and column chromatography on dimethylaminoethyl (DEAE)- and carboxymethyl-cellulose under nondenaturing conditions. The mouse type IV collagen was separated into two fractions on DEAE-cellulose, one (type IV collagen-1) non-adsorbed and the other (type IV collagen-2) adsorbed on the column, although both gave similar results in amino acid analysis.

Indirect immunofluorescence using rabbit antiserum against the purified collagen revealed specific localization of the collagenous protein in the basement membrane of both mouse and human kidneys. This finding strongly suggests that the basement membrane collagens of both species share certain antigenic determinants. In sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) without 2-mercaptoethanol (2-ME), both type IV collagen-1 and -2 migrated poorly into the 5% separating gel, suggesting high molecular weight. After reduction with 2-ME, the proteins penetrated better into the gel and were resolved into three major components with apparent molecular weights of 150000, 135000 and 90000, though slight differences were found in the ratios of these polypeptides and minor peptides. In addition, the purified collagen preparations were sensitive to bacterial collagenase, indicating that the preparations did not contain any other constituents of the basement membrane (proteoglycan, laminin and entactin). Mouse type IV collagen-1 and -2 gave amino acid compositions characteristic of the type IV collagen reported earlier, although they had 20 to 30% higher glutamic acid content than the pepsin-extracted collagens from other sources.

These results suggest that the present collagenous protein and the antiserum against it might be useful tools for elucidating the roles of type IV collagen *in vivo*, since the mouse is utilized in various models of diseases.

Keywords——type IV collagen; heat-gelation; indirect immunofluorescence; interspecies cross-reaction; basement membrane; amino acid analysis; mouse kidney

It is generally accepted that five or more distinct types of collagens occur in animal tissues.[1] Type I, II and III collagens are widely distributed in the interstitium. Type V collagen seems to exist in the cell surface (but not in the basement membrane) in the placenta and muscle.[1a] More recently, a new type of collagen, designated as type VI, was isolated from the human placenta.[2] This collagen is found in the connective tissues of the large vessel, kidney, skin, liver and muscle. On the other hand, type IV collagen has been isolated from various tissues such as human[3] and bovine[4] kidneys, human placenta[5] and bovine lens capsule.[6] The collagen is characterized by its specific localization in the basement membrane, by its higher hydroxyproline and hydroxylysine and lower alanine and arginine contents as compared with the interstitial collagens and by its peculiar behavior on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).[1] However, contradictory results

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have been presented with regard to the immunological species-specificity of type IV collagen. Trüeb *et al.*[4b] and Konomi *et al.*[7] reported no cross-reactivity between human and bovine type IV collagens using passive hemagglutination and/or indirect immunofluorescence. On the contrary, Odermatt *et al.*[8] demonstrated by radioimmunoassay that one of the monoclonal antibodies raised against human kidney type IV collagen reacted well with type IV collagenous proteins from both human and bovine kidneys.

Type IV collagen has many biological functions including tissue morphogenesis.[1a,c] The collagen has been suggested to be associated with various disorders. In glomerulonephrosis and diabetes mellitis, alterations in the chemical structure of glomerular basement membrane containing type IV collagen as a main constituent have been observed. The Type IV collagen, together with other constituents of the basement membrane, also appears to serve as an important barrier against invasion and metastasis of tumors.[1c,9] Type IV collagen and laminin were immunologically undetectable in the majority of invasive breast, pancreas and prostate cancers, but were detected in the benign counterparts.[10] Matsuzawa *et al.* have suggested a progressive decline in the collagen content in tumor tissue along with progression toward greater autonomy or malignancy of hormone-dependent mouse mammary tumors.[11]

Taking into account the reports described above, studies on type IV collagen isolated from normal tissue rich in authentic basement membrane in the mouse should help to elucidate the roles of type IV collagen in normal and pathological states, especially in nephrosis and neoplasm. However, type IV collagen has been isolated from pathological tissues but not from normal tissues in the mouse, which is widely used in laboratory research. The investigations on mouse type IV collagen reported so far have been performed using EHS sarcoma, a poorly differentiated chondrosarcoma, in C57BL/6 mice.[12] There is no definite evidence to indicate that the type IV collagen from morbid tissue or malignant tumor is identical with that from normal tissue, although the tumor collagen has been used a model for analyses of the molecular structure of type IV collagen and its associated structure in the extracellular matrix.[12d] The present study was conducted to purify the collagenous protein, in native form and without loss of antigenicity, as far as possible, from mouse kidneys and to prepare antibody against it.

Materials and Methods

Chemicals——Pepsin (2500 units/mg) was purchased from Boehringer. Dimethylaminoethyl (DEAE)-cellulose (DE 52) and carboxymethyl (CM)-cellulose were obtained from Whatman. Type I collagen from calf skin was a product of Sigma. Ethylenediaminetetraacetic acid disodium salt (EDTA · 2Na) and 6-amino-*n*-caproic acid from Wako, phenylmethylsulfonyl fluoride from Sigma, *N*-methylmaleimide from Aldrich, α,α'-dipyridyl from Nakarai and bacterial collagenase (Form III) from Advanced Biofactures were used.

Purification of Type IV Collagen from Mouse Kidneys——DDD, C3H, C57BL/6 and other strains of mice weighing 20—25 g were obtained from the Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo, and used. Kidneys were collected from sacrificed mice, freed of the surrounding fat tissue and capsule, and stored at $-80\,°C$ until use. All procedures in the extraction and purification of type IV collagen were carried out at 4 °C, unless otherwise noted.

After thawing, 100 g (wet weight) of the kidneys was minced, and washed five times in 1 l of 0.4 M sodium acetate containing EDTA, phenylmethylsulfonyl fluoride, *N*-methylmaleimide, 6-amino-*n*-caproic acid and α,α'-dipyridyl each added at 1 mM by repeated suspension and centrifugation. The final washing was performed in 1 l of 0.5 M acetic acid containing the above inhibitors and then the minced tissue was homogenized in 2 l of the same solution using a Polytron homogenizer set at 6 for 4×15 s, followed by adjustment to pH 2.5 with 6 M HCl. After addition of pepsin (250 mg) to the homogenate, protease digestion was carried out for 83 h to solubilize collagens. The solubilized collagen fraction was obtained from the incubation mixture by centrifuging it at $30000 \times g$ for 30 min, then crystalline NaCl was added to 10%, and the solution was allowed to stand overnight. Collagens were recovered in the precipitate after centrifugation at $30000 \times g$ for 30 min. The precipitate was suspended in 300 ml of 0.144 M potassium phosphate buffer (0.016 M KH_2PO_4–0.128 M K_2HPO_4, pH 7.6), stirred overnight and subsequently dialyzed against 30 l of the same buffer for at least 24 h with 6 changes of the buffer. The resulting dialysate was ultracentrifuged at $105000 \times g$ for 1 h to eliminate insoluble materials and the supernatant was subjected to 16-h heat-gelation at 37 °C according to the

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method of Trelstad and Lawley.[13] The heat-treated sample was centrifuged at $30000 \times g$ for 30 min at 22—25 °C to obtain type IV collagen in a non-gelled form in the supernatant, and dialyzed against 30 l of 5% NaCl in 50 mM Tris–HCl buffer, pH 7.5 with 6 changes of the buffer to eliminate the insoluble materials including aggregates of collagens. The dialysate was ultracentrifuged at $105000 \times g$ for 1 h, crystalline NaCl was added to the supernatant to 30%, and the solution was allowed to stand overnight. Collagens were precipitated again by ultracentrifugation at $105000 \times g$ for 1 h and dissolved in 50 ml of 0.5 M acetic acid. The resulting solution was dialyzed against 6 l of 2 M urea in 50 mM Tris–HCl buffer, pH 8.6 with 3 changes of the buffer, and then the insoluble materials formed were removed by ultracentrifugation at $105000 \times g$ for 1 h. The supernatant was applied to a column (1.5 × 12 cm) of DEAE-cellulose previously equilibrated with the dialysis buffer. The column was eluted at a flow rate of 22 ml/h with the starting buffer until all unretained materials were eluted as judged by return to the baseline of the absorbance at 230 nm in the effluent. Then, gradient elution was initiated using a two-chambered constant-level device containing 100 ml of the starting buffer in the mixing chamber and 100 ml of the starting buffer containing 0.1 M NaCl in the reservoir chamber. Type IV collagen was resolved into two fractions; one (designated as type IV collagen-1) appeared in the flow-through fraction, and the other (designated as type IV collagen-2) was eluted at 25 mM NaCl. Each fraction was dialyzed against 20 l of 2 M urea in 40 mM sodium acetate buffer, pH 4.8, with 4 changes of the buffer and applied to a CM-cellulose column (1.5 × 10 cm) previously equilibrated with the dialysis buffer. The column was eluted with 200 ml of a linear gradient from 0 to 0.2 M NaCl in the same buffer at a flow rate of 50 ml. Type IV collagen-1 was further rechromatographed on the CM-cellulose column under the same conditions. Both type IV collagen-1 and -2 fractions thus obtained were dialyzed against 5 × 2 l of 0.1 M acetic acid and lyophilized.

Preparation of Rabbit Antiserum against Type IV Collagen-2——Antiserum was raised against the mouse type IV collagen-2. Japanese white male rabbits weighing about 3 kg were immunized by subcutaneous injection of 1 mg of the lyophilized protein dissolved in 1 ml of 0.1% (v/v) acetic acid mixed with an equal volume of complete Freund's adjuvant. After two weeks, the second injection of 0.8 mg of the protein with incomplete Freund's adjuvant was given and antisera were collected 2 weeks later.

Indirect Immunofluorescence——Frozen mouse and human kidney cortices were cut at 5 μm at −20 °C. The sections obtained were fixed with acetone–methanol (1 : 1, v/v) for 20 min and dried in air. Indirect immunofluorescence was carried out on the fixed sections using the rabbit antiserum prepared as mentioned above at 1/30 dilution and fluorescein-conjugated goat anti-rabbit immunoglobulin (Miles–Yeda, at 1/40 dilution). Control staining was performed using the serum from non-immunized normal rabbits in place of the antiserum.

SDS–PAGE——Collagenous proteins from mouse kidneys were dissolved at 1 mg/ml in the sample buffer with or without 5% 2-mercaptoethanol (2-ME) and denatured at 56 °C for 30 min by the method of Miller and Rhodes[1b] with minor modifications. Aliquots (30 μg) of the denatured samples were analyzed by SDS–PAGE in 5% separating gel with 3% stacking gel by the method of Laemmli.[14] The electrophoresed gels were stained with 0.25% Coomassie Brilliant Blue-250 in 10% acetic acid and 50% methanol, and destained with 7.5% acetic acid and 5% methanol.

Amino Acid Analysis——Samples (1 μg) were hydrolyzed in 0.2 ml of 6 M HCl in the presence of 5% (v/v) thioglycollic acid for 24 h at 110 °C under reduced pressure according to the method of Matsubara and Sasaki,[15] and analyzed for amino acids with a Hitachi amino acid analyzer, type 835-50.

Collagenase Digestion——Both purified type IV collagen-1 and -2 as well as type I collagen (included for comparison) were dissolved in 0.5 M acetic acid at 1 mg/ml and dialyzed against 50 mM Tris–HCl buffer, pH 7.4 containing 150 mM NaCl, 5 mM $CaCl_2$ and 10 mM *N*-methylmaleimide. Insoluble materials were removed by ultracentrifugation at $105000 \times g$ for 1 h at 4 °C. Aliquots of each collagen solution were incubated with bacterial collagenase in a final volume of 0.24 ml at an enzyme–substrate concentration of 1 : 50 (w/w) for 16 h at 37 °C. Control digestion was carried out with either the substrate or the enzyme alone. The reaction was terminated by adding a half volume of twice concentrated sample buffer containing 10% 2-ME prior to analysis by SDS–PAGE.

Results

Purification of Type IV Collagens from Mouse Kidneys

Type IV collagens were precipitated from the pepsin digest of mouse kidneys with 10% NaCl in 0.5 M acetic acid (adjusted to pH 2.5 with 6 M HCl). The precipitate was dissolved in 0.144 M potassium phosphate buffer, pH 7.6, and incubated at 37 °C for 16 h. Heat-treatment caused gelation of interstitial collagens. The non-gelled supernatant obtained by centrifugation was chromatographed on a column of DEAE-cellulose with the results shown in Fig. 1. Type IV collagen was resolved into two fractions: one non-adsorbed, named type IV collagen-1, and the other adsorbed, named type IV collagen-2. Each collagen was subjected to CM-cellulose column chromatography to eliminate other collagens such as type V collagen present in small quantities (Fig. 2), as previously described.[1c,5a,b] The type IV collagen-1 was

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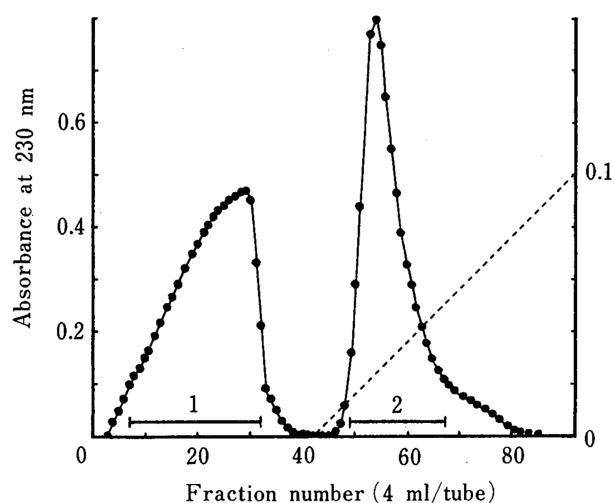

Fig. 1. DEAE-Cellulose Column Chromatography of Type IV Collagen from Mouse Kidney

Fractions under the lines were collected. ●, absorbance at 230 nm; ----, NaCl concentration.

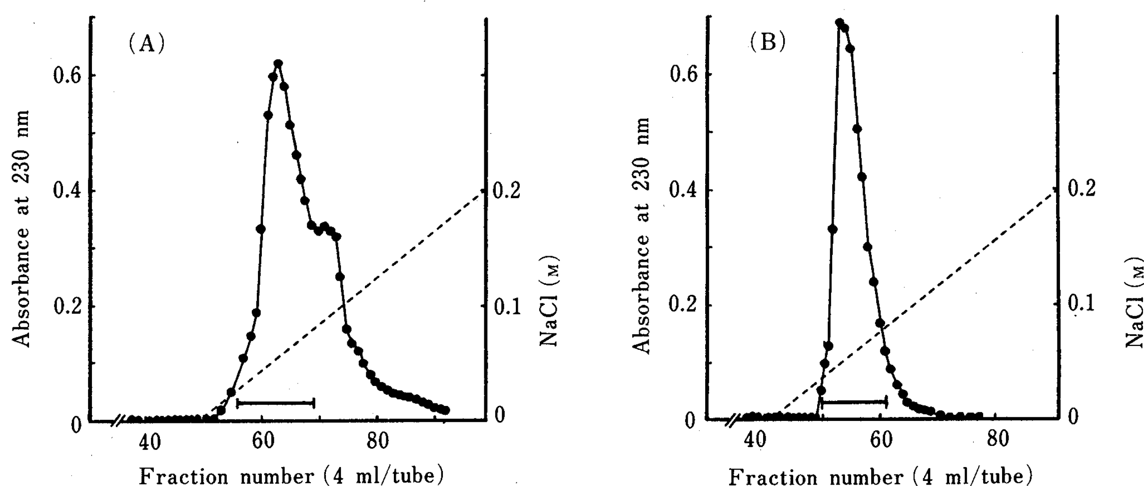

Fig. 2. CM-Cellulose Column Chromatography of Type IV Collagen-1 (A) and -2 (B) from Mouse Kidney

Fractions under the lines were collected. ●, absorbance at 230 nm; ----, NaCl concentration.

further purified by CM-cellulose column rechromatography in a similar manner. Both type IV collagen-1 and -2 were dialyzed against 10 l of 0.1 M acetic acid with 5 changes of the solution, and lyophilized. Finally, 3.3 mg of type IV collagen-1 and 6.8 mg of type IV collagen-2 were recovered (in lyophilized form) from 100 g of wet kidney tissues.

Indirect Immunofluorescence

In indirect immunofluorescence analysis, the rabbit antiserum raised against the purified type IV collagen-2 stained the basement membrane of glomeruli, tubules and capillaries and Bowman's capsule in a specific manner, but not the interstitium containing type I and type III collagens in the mouse kidney cortex (Fig. 3A). In addition, the antiserum produced practically the same pattern of fluorescence in the human kidney cortex (Fig. 3B). However, the normal rabbit serum did not give any fluorescence at all in these sections (data not shown). These results clearly indicate that the basement membrane or type IV collagen of the mouse might share certain antigenic determinants with that of the human.

SDS–PAGE of Mouse Type IV Collagens

Purified type IV collagen-1 and -2 were subjected to SDS–PAGE in the absence or

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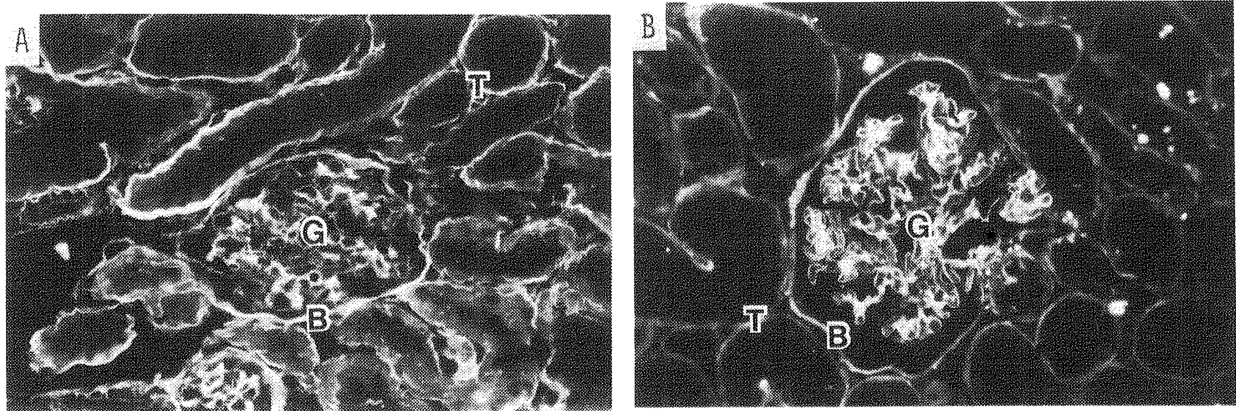

Fig. 3. Indirect Immunofluorescence of Mouse (A) and Human (B) Kidneys

G, glomerulus; T, tubule; B, Bowman's capsule; magnification, × 100.

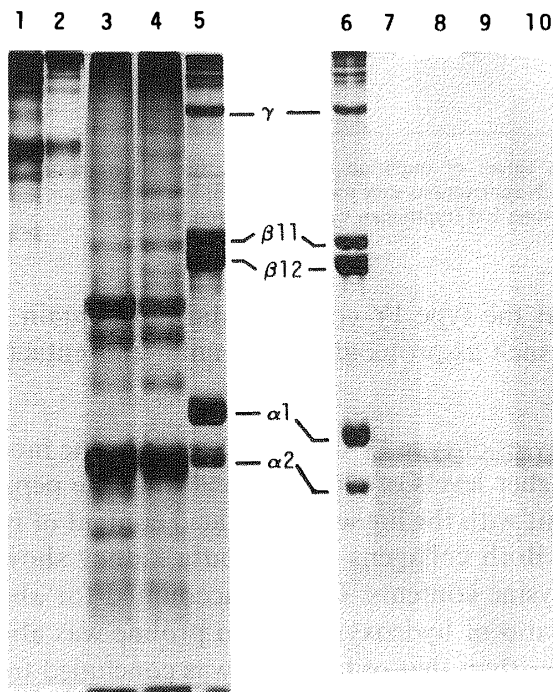

Fig. 4. SDS–PAGE of Type IV Collagen from Mouse Kidney

1, type IV collagen-1 without 2-ME;. 2, type IV collagen-2 without 2-ME; 3, type IV collagen-1 with 2-ME; 4, type IV collagen-2 with 2-ME; 5 and 6, type I collagen from calf skin (α, molecular weight of 95000; β, molecular weight of 190000; γ, molecular weight of 285000) used as a marker without or with 2-ME; 7, collagenase digest of type IV collagen-1; 8, collagenase digest of type IV collagen-2; 9, collagenase digest of type I collagen; 10, collagenase alone.

presence of 5% 2-ME to examine their chain components, and to determine whether or not there are differences between the two collagens. In the absence of 2-ME, type IV collagen-1 and -2 showed no major difference in behavior and hardly penetrated into 5% separating gel. After reduction of disulfide bonds with 5% 2-ME, both collagens were also very similar in behavior although they appeared to have slightly different proportions of the three major polypeptides with apparent molecular weights of 150000, 135000 and 90000, as well as showing some differences in the distribution of minor peptides. In addition, the intensity of the 150000-peptide was rather greater than that of the 135000-peptide in all of four different preparations of type IV collagen-1, while the former was somewhat greater than or equal to the latter in type IV collagen-2 (Fig. 4, lanes 1—4).

When type IV collagen-1 and -2 were digested with bacterial collagenase and analyzed by SDS–PAGE with 2-ME, all protein bands except for a weak one disappeared (Fig. 4, lanes 7 and 8). The weak band was accounted for by the collagenase added, since the same band was also observed after incubation of the enzyme alone (lane 10) or even in the presence of type I

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TABLE I. Amino Acid Composition of Type IV Collagen from Mouse Kidney

Amino acid[a]	Type IV collagen-1	Type IV collagen-2
Hydroxyproline	129	127
Aspartic acid	50	50
Threonine	25	25
Serine	34	31
Glutamic acid	103	107
Proline	72	78
Glycine	326	323
Alanine	30	29
Valine	23	23
Methionine	17	17
Isoleucine	24	23
Leucine	51	50
Tyrosine	4	5
Phenylalanine	28	28
Hydroxylysine	41	41
Lysine	5	6
Histidine	4	5
Arginine	31	33

a) The values of amino acid residues are mean values of duplicate analyses of three different preparations, and are given per 1000 amino acid residues. No corrections were made for incomplete release or destruction of amino acid residue during hydrolysis. Cystine and tryptophan were not determined.

collagen (lane 9). The results demonstrated that the type IV collagens did not contain any other constituents of the basement membrane, such as proteoglycan, laminin and entactin.

Amino Acid Composition

Table I shows the results of amino acid analyses of type IV collagen-1 and -2. The mouse kidney type IV collagens contained 20—30% higher levels of glutamic acid than the pepsin-extracted type IV collagens reported, in agreement with the higher amino acid content of type IV-like procollagen from the mouse tumor.[12a] Both collagens from mouse kidney showed practically the same hydroxylated proline and lysine contents. Glycine accounted for about one-third of the total amino acid residues, the ratio of hydroxyproline to proline was about two, and the level of hydroxylysine was far higher than that of lysine. It was concluded from these results that the isolated proteins in this study were type IV collagens.

Discussion

Type IV collagen has been purified from the pepsin digest of various sources, and proved to be clearly different from other collagens.[1] The procollagen-like protein resembling type IV collagen in the authentic basement membrane was also isolated from poorly differentiated EHS chondrosarcoma in C57BL/6, which is likely to produce exclusively type II collagen, one of the interstitial collagens, but not type IV collagen in a differentiated state.[12a] The tumor collagen was extracted with dilute acetic acid alone without pepsin digestion of EHS sarcomas from mice given β-aminopropionitrile, a lysyl oxidase inhibitor. The collagen appeared to be in a nearly intact form in the tumor matrix, differing from the pepsin-solubilized type IV collagen from the authentic basement membrane in normal tissue. Investigations on the tumor collagen have provided useful information on the molecular structure of type IV collagen.

It is not yet known whether the type IV collagen from the tumor matrix is identical with

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that from the authentic basement membrane in normal tissue. There are few reports on the tissue-specificity of type IV collagen in the same species or on the species-specificity of the collagen. Furthermore, the chemical structure of basement membrane, composed mainly of type IV collagen, has been implied to alter in various disorders, especially glomerulonephrosis and diabetes mellitis, during development and with aging.

However, no work has been done on the type IV collagen from normal tissue; the only reports deal with that from morbid tissue or tumor tissue in the mouse. We consider that the study of type IV collagen from mouse normal tissue should cast light on the roles of the collagen in the body. Thus, we attempted to purify type IV collagen from mouse kidneys, which are rich in basement membrane. We firstly purified type IV collagen from kidneys according to the method of Dixit,[4a)] which was applied to the purification of type IV collagen from bovine kidney cortex. The protein obtained was identified as type IV collagen by an immunofluorescence study using antiserum raised against it in a rabbit. Contrary to our expectation, however, the recovery (mean yield: 0.67 mg of purified collagen per 100 g of wet tissue, $n=3$) of the protein from mouse kidneys was far lower than that (50 mg collagen per 100 g wet tissue) reported by Dixit,[4a)] using heat-gelation, low ionic strength precipitation and DEAE-cellulose chromatography. The low recovery in this study might be due to the different animal species used and/or the use of whole kidney as a starting material instead of the cortex alone. However, the real reasons for this result are not clear. Therefore, we made some modification to the method of Dixit[4a)] to increase the yield of the collagen from the whole mouse kidney. As a result, the combined yield of the type IV collagen-1 and -2 was increased to approximately 10 mg of lyophilized powder per 100 g of wet tissue, which is comparable to that reported by Dixit.[4a)] In the course of the purification, the collagenous protein was separated into two fractions on a DEAE-cellulose column (Fig. 1); one, called type IV collagen-1, appeared in the flow-through fraction and the other, called type IV collagen-2, was adsorbed on the column. However, no significant differences were found between them by amino acid analysis. Similar observations were made by Timpl *et al.*[12a)] in the purification of the type IV collagen-like protein from mouse sarcoma, by Trüeb *et al.*[4b)] in the fractionation of the purified protein from the bovine kidney cortex, and by Nagai and Fujiwara[1a)] in the final step of purification of type IV collagen. The result might be associated with the microheterogeneity of type IV collagen suggested by Timpl.[1c,12a)] However, it could not be attributed to overcharging of type IV collagen on the DEAE-cellulose, since the preliminary studies established that almost all of the type IV collagen-1 was again recovered in the flow-through fraction on rechromatography. The above investigators[4b,12a)] charged a higher amount of type IV collagen sample on the column than we used in the present study, under similar conditions.

In the present purification procedure, heat-gelation at 37 °C was utilized instead of salt precipitation for the fractionation of different types of collagen from the collagen extract, since little or no type IV collagen solubilized from mouse kidneys was recovered in the precipitate at 1.8 M NaCl in 50 mM Tris–HCl buffer, pH 7.5 (data not shown), in conflict with the results reported previously.[1b,c,5b)] The type IV collagen thus obtained was practically precipitated at about 3.5 M NaCl in the Tris–HCl buffer after mild digestion with pepsin in the present study. This finding agrees well with the observation by Nagai *et al.*[1a,16)] that type IV collagen was precipitated at 1.7 to 4.0 M NaCl in the same buffer. Addition of 2 M urea to the basic buffer for the DEAE-cellulose column or the acidic buffer for the CM-cellulose column made type IV collagens more soluble, as observed for the collagen from other sources.[1,5b,12)]

Type IV collagen has been reported to be more antigenic than other collagens such as type I, II and III. In accordance with this, antiserum against the mouse type IV collagen was prepared in rabbits with ease. Immunofluorescence studies using it demonstrated specific localization of the protein in the basement membrane not only in mouse but also in human

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kidneys (Fig. 3). In addition, the basement membrane in the kidney cortex from the immunized rabbit was stained by an indirect immunofluorescence method (data not shown), suggesting that the rabbit basement membrane collagen may also share some antigenic determinants with the mouse type IV collagen and that the injection of the mouse kidney type IV collagen may cause autoimmune disease in the mouse, as reported by von der Mark *et al.*[17] Opposing reports have been published on the species–specificity of type IV collagen. Trüeb *et al.* found no cross-reactivity between bovine and human type IV collagens by either passive hemagglutination or indirect immunofluorescence analyses.[4b] A similar result was presented by Konomi *et al.*[7] In contrast, Odermatt *et al.*[8] demonstrated by radioimmunoassay that the monoclonal antibody against type IV collagen from the human kidney reacted about equally well with both human and bovine type IV collagens in native conformation, and von der Mark *et al.*[17] confirmed by immunochemical analysis that some antigenic determinants in the globular domain NCl of type IV collagen from the human placenta and mouse tumor were shared among several animal species. Further investigations utilizing various antibodies, monoclonal and polyclonal, against type IV collagens from many species of animals and various assay methods will be required to reach a final conclusion concerning this issue. The mouse kidney type IV collagen and the antiserum against it obtained in the current study should be useful for this purpose.

Recent *in vitro* biosynthetic studies using several systems including the EHS tumor,[18] parietal yolk sac tissue,[18] amniotic fluid cells[19] and tetracarcinoma cell line PYS-2,[20] have demonstrated secretion of type IV collagen-like protein composed of two genetically distinct polypeptides, which are designated pro-α1(IV) and pro-α2(IV) and have similar molecular weights of about 180000, though the former has a slightly slower migration rate on SDS–PAGE in the presence of 2-ME. However, it remains controversial whether or not the type IV procollagen is processed to the type IV collagen.

The two components with molecular weights of 150000 and 135000 in the present study may correspond to a doublet of polypeptides from the tumor matrix,[12] which migrated between β components and α chains of type I collagen, and the similar polypeptides from human amniotic fluid cell matrix, placenta and kidney[19] rather than to a doublet of forms from bovine kidney[4] and the two smaller chain components of the EHS tumor type IV procollagen chains.[18] In addition, the distribution of bands on SDS–PAGE following reduction of the mouse kidney type IV collagens appeared to be comparable to those of the human preparations obtained from three different sources by Crouch *et al.*[19] They proposed that the different pepsin-degraded fragments of type IV collagen can be identified as derivatives of either the pro-α1(IV) or the pro-α2(IV) in each case on the basis of their analyses of placental type IV collagen and amniotic fluid cell type IV procollagen. Considering their hypothesis together with the report of Tryggvason *et al.*,[18] indicating rapid degradation of the 170000-peptide or pro-α2(IV) on pepsin digestion of the EHS type IV procollagen, it is likely that the 150000- and 135000-polypeptides in this study could originate from pro-α1(IV). The α-chain sized 90000-peptide band could be composed of two distinct fragments derived from pro-α1(IV) and pro-α2(IV).

A study on the components of each chain of the present collagens and type IV procollagen chains from mouse kidneys is in progress.

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