Vol. 147, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1987 Pages 275-281

HIGH PRODUCTION OF TYPE VI COLLAGEN IN MULTIPLE FIBROMATOSIS WITH MULTIPLE ARTICULAR DYSPLASIA

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Received July 8, 1987

A patient with multiple fibromatosis occurring at the sites of multiple cartilagenous dysplasia was described. Collagen types solubilized with pepsin from the fibromatous tissue were fractionated by a different salt concentration and analyzed by SDS-polyacrylamide gel electrophoresis, which indicated that the tissue produces predominantly "short-chain" collagen. Western blotting of the subunits indicated a cross reaction with antisera of the type VI collagen. The results of rotatory shadowing electron microscopy confirmed the characteristic short-chain structure. © 1987 Academic Press, Inc.

A certain pathogenesis of inherited diseases has been studied as an aspect of the metabolic disturbance of specific collagen types (1,2). To date, 11 distinctly different collagen gene products and their molecular structures have been reported (3,4). Among them, we found for the first time a tumor which produces a large amount of type VI collagen. Type VI collagen is widely distributed in extracellular matrices mainly produced by fibroblasts (5). Since type VI collagen is characterized by the small size of its collagenous fragments, it has been called "short-chain" collagen or "intima" collagen (6-11). The pepsin extractable subunits of the short-chain collagen have been prepared from human organs and tissues such as placenta (9,12), aorta (13) and cornea (14).

Although the type VI collagenous components can be easily extracted from cultured tissues with pepsin and have been characterized extensively (6,7), their function in intercellular matrix and their role in physiological state

0006-291X/87 $1.50
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have not been elucidated (15). A specific antibody for the collagen has been used as an important tool for confirmation of its heterotrimer subunits (8,9, 13,16). Electron-microscopic studies have been shown the structural assembly (5,7,8,10,15,16), that type VI collagen has a hybrid structure of one collagenous filament with two globular domains (9,10).

This is the first report that a large amount of type VI "short-chain" collagen is found to be present in extracts from multiple fibromatosis with multiple articular dysplasia. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis showed that after pepsin extract macromolecular aggregates appear with partially dissociated forms without reduction, and after reduction they give rise to the unique feature of type VI "short-chain" collagen which was examined by rotatory shadowing electron microscopy (8,10,14-17). Antisera raised against the type VI collagen identified that the majority of collagen formed such tumorous protuberances is identical to the type VI collagen.

MATERIALS AND METHODS

We examined multiple fibromatosis obtained from a patient, who has a hereditary disease, with regions of multiple articular dysplasia surrounded by numerous protuberant tumors. Elastic globe-shaped tumors, weighed about 100 g, were removed from his cervical regions at operation. The tissues were homogenized and suspended in a 0.4 M NaCl solution, pH 7.4, containing protein inhibitors.

Collagen was extracted with limited pepsin digestion, 20 mg of pepsin (Cooper Biochem.) per 1 g dry tissue weight, in 0.5 M acetic acid, with stirring at 4°C for 24 h, and then centrifuged at 15,000 rpm at 4°C for 1 h as reported previously (12,13). The extracted collagen was then precipitated by 0.7 M NaCl in 0.5 M acetic acid. The supernatants consisted mainly of type VI collagen whereas the precipitates contained type I collagen in addition to a small amount of type V collagen.

The supernatants were adjusted to a concentration of 1.2 M NaCl followed by centrifugation, which resulted in precipitation of type VI collagen together with small amounts of type I and V collagens. To the supernatants, NaCl was added to 2.0 M concentration. The precipitates consisted exclusively of type VI collagen. The collagen content of each fraction was determined by hydroxyproline measurement (18). The averaged yield of total collagen extracted from the tumor was 21.9% of dry tissue weight.

Characterization of short-chain collagen

Poly-acrylamide gel electrophoresis was performed in the presence of SDS by a modification (13) of the method originally described by Laemmli (19). Amino acid analysis was performed with a Hitachi Model 835 automatic analyzer after hydrolysis in HCl at 110°C for 24 h (20). Rotatory shadowing electron-

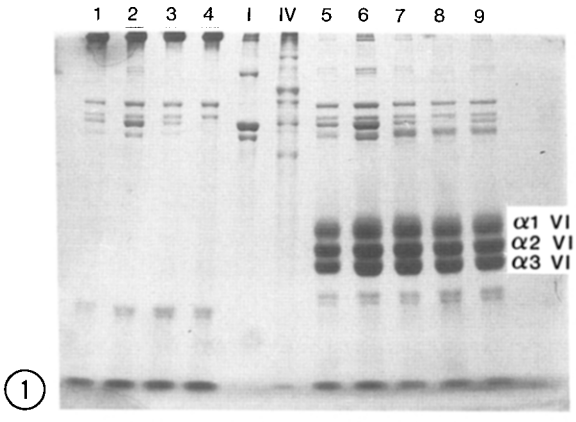
microscopy was carried out as reported previously (10). Rabbit antisera were raised against the non-reduced type VI collagen and the pepsinized α2[VI] subunits: they were prepared on SDS poly-acrylamide gel electrophoresis. The antigen doses injected were 0.5 mg each, emusified with an equal amount of Freund's complete adjuvant followed by booster injections 3-times at 14 day intervals. The antisera were subjected to absorption with human type I - V collagens in order to remove contaminating or cross-reacting antibodies. Immunoblotting of the antigens was carried out after separation of the type VI collagen subunits by SDS-polyacrylamide gel electrophoresis. The separated fragments were blotted to a nitrocellulose membrane and then immunoblotting was carried out (21).

RESULTS

The components thus prepared seemed to be the helical form of type VI short-chain collagen and the subunits. On SDS-polyacrylamide gel electrophoresis of type VI collagen, the non-reduced forms were found to be remained at macromolecular weights over 300 Kd, whereas the reduced subunits gave monomeric chains corresponding to approximate MW 140 Kd with three main bands, pepsinized α1[VI]-, α2[VI]- and α3[VI]-chains (Fig. 1).

Materials extracted with limited pepsin digestion, either reduced subunits or non-reduced forms, were immunogenic in rabbits, causing the production of antisera which reacted with the constituent pepsinized α1[VI]-, α2[VI]- and α3[VI]-chains obtained on the reduction of the type VI collagen (Fig. 2). The type VI collagen was predominantly found in the acidic 0.7 M NaCl supernatant. The type VI collagen was detected not only in the acidic 1.2 M and 2.0 M NaCl precipitates but also in the acidic 2.0 M NaCl supernatant. Conclusively, the proportions of the pepsin extractable-collagen types I, III, V and VI separated from the tumorous materials were 42%, 5%, 4% and 49% to the total collagen, respectively. However, presence of a very small amount of collagen type II could not be ruled out.

The antibodies raised against pepsin-extractable type VI collagen were then used to identify and characterize the forms of the type VI collagen produced in the tumor. The antibody against the pepsinized α2[VI] subunit recognized the antigens not only for the α2[VI]-chain but also for the pepsinized α1[VI]- and α3[VI]-chains, and moderately for the monomeric domains and lower MW of approximately 45 Kd. Since the type VI collagen had been extracted by means of pepsin digestion, it was likely that some structures might have been lost during the course of solubilization with pepsin and further purification. The molecular sizes estimated for the pepsinized α1[VI]-, α2[VI]- and α3[VI]-chains obtained from the present fibromatosis case were respectively 62 Kd, 57 Kd and 52 Kd on comparison with globular standards. Rotatory shad-



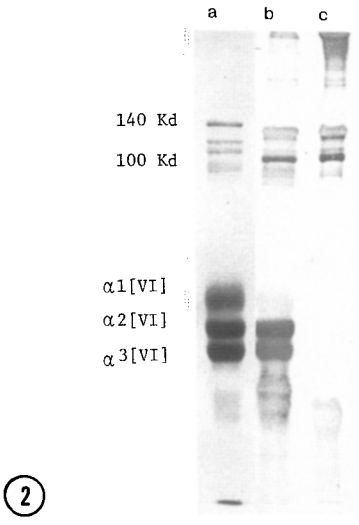


Figure 1.
SDS-polyacrylamide gel electrophresis of the non-reduced (lanes 1-4) and reduced froms (lanes 5-9) of type VI collagen extracted with limited pepsin digestion from different parts of multiple fibromatosis. The collagens presented here are those in the acetic 0.7 M NaCl supernatants separated by different salt fractionation.
Collagens were separated on 8% acrylamide slab gels (2.0 x 174 x 136 mm) using 0.025 M Tris, 0.192 M glycine and 0.1% SDS as the electrode buffer. The disulfide bonds were reduced with 10% 2-mercaptoethanol. The separated collagens were calibrated with reference globular proteins, which were run in parallel slots. Pepsin-extracted type VI collagen was also prepared from human placenta for comparison. The gels were stained with 0.25% Coomassie Brilliant Blue R-250 and the bands automatically scanned with a Video-densitometer (Bio-Rad, Model 620). I and V indicate standard types I and V.

Figure 2.
SDS-polyacrylamide gel electrophoresis of the reduced forms of type VI collagen and immunoblotting staining with treatment with the antibodies for the pepsinized α2[VI] antigen prepared from a multiple fibromatosis with articular dysplasia.
Lane a: reduced, pepsin-extractable type VI collagen prepared from the tumor, showing main bands of the pepsinized α1[VI]-, α2[VI]- and α3[VI]-chains, monomer domains from 100 Kd to 140 Kd, and a chain band of the pepsinized lower MW chain of approximately 45 Kd. Lanes b and c: reduced (b) and non-reduced collagen subunits (c) were transferred to nitrocellulose and then stained with purified antibodies against pepsinized α2[VI] antigen.

owing electron microscopy of type VI collagen revealed abundant amounts of unique structures (Fig. 3), as previously found in pepsin-extracted type VI collagen of human placenta (6-8,10).

This tumor-derived collagen showed, on rotary shadowing electron microscopy, the typical collagen structures which, however, were contaminated with rod-like helical structures lacking globular domains, presumably due to the pepsin treatment (6). In the present study, the glycine contents of the non-reduced type VI collagen and pepsinized α2[VI]-chain were found to account for less than one-third of the collagenous protein, suggesting that the collagen contains other than helical regions (Table 1).

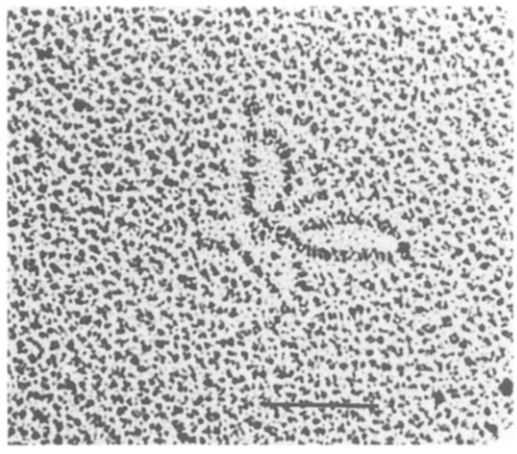


Figure 3.
Rotatory shadowing electron microscopic features of type VI short chain collagen extracted with pepsin from the multiple fibromatosis case. The type VI short chain collagen shows various rod-like structure connected with globular domains, forming looped forms. The bar indicates 100 nm length. Collagen samples (50 μg/ml) dissolved in 0.1 M acetic acid or in 0.2 M ammonium bicarbonate, pH 7.9, were mixed with 50% glycerol and then subjected to rotatory shadowing electron microscopy (10).

DISCUSSION

There has previously been no report on multiple fibromatosis occurring in the site of articular joints of a patient with multiple articular dysplasia, and the tumor produced predominantly type VI short-chain collagen.

Table 1. Amino acid composition of pepsin-extracted type VI collagen

	Pepsinized α2[VI] subunit	Non-reduced VI collagen
Asparatic acid	65.5	69.5
Threonine	22.9	32.5
Serine	65.3	91.2
Glutamic acid	104.4	106.1
Glycine	285.9	248.4
Alanine	55.8	69.7
Valine	25.5	26.3
Cysteine	8.1	4.2
Methionine	4.8	2.9
Isoleucine	11.9	22.6
Leucine	29.8	39.5
Tyrosine	6.8	15.6
Phenylalanine	15.5	19.5
Hydroxylysine	51.7	21.3
Lysine	30.8	46.2
Histidine	16.1	24.6
Arginine	63.3	58.5
Hydroxyproline	53.0	32.1
Proline	83.0	69.1

Residues are given per 1000 amino acid residues. No corrections were made for incomplete hydrolsis or hydrolytic loss. The results are the avarages of two determinations.

The formation of type VI collagen subunits for end-to-end assembly and the the globular domains suggested that type VI collagen was composed of the characteristic short-chain structure (7,10,15,16). The chemical properties have been studied, as to the three different polypeptide chains in the pepsinized α1[VI]-, α2[VI]- and α3[VI]-chains. Accumulating studies have indicated MW from 110 Kd to 150 Kd for the type VI chains of non-reduced collagen VI obtained from cell culture and tissue (8). In spite of the fragility of the collagen VI chains, the present study showed that the structure still retains essential features of the monomeric assembly, i.e., a strand structure of an averaged 105 nm long triple helix connected by globular domains.

Previous ultra-structural work on a strand form of type VI collagen, as an end-to-end assembly, suggested that the ability of contact constitution of microfibril structures in tissues (15). Present studies on the 105 nm periodicity of such microfibrils occasionally concomitant with looped and petal-shaped assemblies suggested that even larger aggregates of collagen VI may exist in intercellular matrices.

The filamentous fibrils are abundant in the various states of tumorous substances, which may be composed of a variety of different microfibriles (7). Recent electron microscopic study indicated that the formation of such stranded filaments might result in significant clustering of the chain leading to banding patterns with a periodicity of approximately 100 nm along to type I collagen fibrils (8), which have been, in fact, observed in many pathological and some normal tissues (7). Such fibrils solubilized with pepsin were found to contain type VI collagen, but they may contain some additional structural components of intercellular matrices with type I collagen (8). The biological and pathological roles of type VI collagen with these fibrils in the present tumorous tissue remain to be clarified. But it may be presumed that the highly increased type VI collagen replaces the region of interstitial collagens in the articular joints. Further study on the specificity of tumor types for high production of such short-chain collagen should be necessary to elucidate a certain role of the specific collagen species in excessive tumorous production possibly due to intercellular interaction with either a growth stimulating factor or other matrix components.

ACKNOWLEDGMENTS

We thank Drs Timpl, R. and Miller, P. Max Planck Institute in Martinsried for valuable discussions and Drs von der Mark, H. and Aumailley, M. for supplying antisera for placental type VI. The present study was supported, in part, by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan and a grant from the Adult Disease Memorial Foundation.

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