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cDNA cloning and characterization of Type V/XI procollagen α1 chain in the skate, *Raja kenojei*

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Abstract

The full-length cDNA of the Type V/XI procollagen α1 [pro-α1(V/XI)] chain was determined by the RACE technique, using a cDNA library constructed from the 4-week embryo of the skate, *Raja kenojei*. The expression property and phylogenetic analysis confirmed that the skate pro-α1(V/XI) chain was close to other vertebrate pro-α1(V) chains rather than pro-α1(XI) chains. The present study is the first evidence for the primary structure of full-length cDNA of the pro-α1(V/XI) chain in an elasmobranch.

Keywords: cDNA; Cloning; Collagen; Embryo; RT-PCR; Fish

1. Introduction

The fibrillar collagens are the most abundant proteins of extracellular matrices. Among them, Type V and XI collagens are quantitatively minor components, which participate in the formation of the fibrillar collagen network. The current view on the tissue localization of fibrillar collagen is that Type V collagen is associated with Type I and III collagens in non-cartilaginous tissues (Fitch, Gross, Mayne, Johnson-Wint & Linsenmayer, 1984; Linsenmayer et al., 1983). On the other hand, Type XI collagen, associated with Type II collagen, has been discovered in cartilaginous matrices (Mendler, Eich-Bender, Vaughan, Winterhalter & Bruckner, 1989). The tissue localizations of Type V and XI collagens are different, but their structural and biological properties seem to be closely related. Fichard, Kleman, and Ruggiero (1994) demonstrated that several molecules are heterotypic associates of Type V and XI collagen chains, suggesting that these two collagens are not distinct types but a single type, which can be called Type V/XI collagen. Moreover, minor collagens in the fishes have been recognized as important factors relating to *post-mortem* tenderization under chilled storage (Sato et al., 1997).

We have isolated and characterized molecular species of collagens from the muscle, cartilage and skin of skate (Hwang, Mizuta, Yokoyama & Yoshinaka, 2007; Mizuta, Hwang & Yoshinaka, 2002, 2003). In the course of the work, we found different biochemical characteristics among the minor collagens, in contrast to the fact that the distribution of molecular identity of collagen is fundamentally the same between skin and muscle of minor collagens from some teleosts (Yata, Yoshida, Fujisawa, Mizuta & Yoshinaka, 2001). As for fish minor collagen, there is only one report on the full-length cDNA of Type V/XI procollagen α1 [pro-α1(V/XI)] chain from the red seabream *Pagrus major* (Touhata, Tanaka, Yokoyama, Sakaguchi & Toyohara, 2001). Its sole examination is still insufficient for understanding characteristics of fish Type V/XI collagen. The present study describes a full-length cDNA of pro-α1(V/XI) chain from the skate, *Raja kenojei*.

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2. Materials and methods

2.1. Materials

Skate, *Raja kenojei* (body weight, 450–500 g), was obtained alive from local fishermen in Obama, Fukui, Japan, and reared in the running water tank of the Research Center for Marine Bioresources, Fukui Prefectural University in Obama, Fukui, Japan. Newborn embryos in the egg capsules were independently reared in the plastic box, with supply of enough aeration.

2.2. RNA isolation

Various tissues (muscle, skin, cartilage, gill, orbit, brain, liver, intestine, heart, stomach, spleen, pancreas, egg, ovary, uterine tube, shell gland, spiral valve and whole blood) were dissected out from the adult skate and immediately placed in liquid nitrogen. Frozen tissues were pulverized in a mortar with a pestle and then homogenized with Sepasol (Nacalai tesque, Japan), and total RNAs were isolated according to the manufacturer's instructions. Total RNAs of 4- and 8-week embryos were prepared, as described above, and used for cDNA library construction and rapid amplification of cDNA ends (RACE), respectively.

2.3. cDNA cloning of skate pro-α1(V/XI) chain

Total RNA (2 μg) from adult liver was reverse-transcribed by using an oligo$(dT)_{20}$ primer and ThermoScript reverse transcription (RT)-PCR system (Invitrogen, USA) according to the manufacturer's instructions. A pair of oligonucleotide mixed primers, 5′-TT(TC)CC-(TCAG)GA (TC)GG(TCAG)GA(AG)TA(TC)TGG-3′ and 5′-TC(AG)AA(TCAG)CC(AG)AA(TC)TT(TC)TG-3′, was designed, based on highly conserved amino acid sequences in the C-terminal propeptides of vertebrate pro-α1(V) and pro-α1(XI) chains, which are conserved in the highest similarity to vertebrate pro-α1(V) and pro-α1(XI) chains (Dion & Myers, 1987).

Degenerate PCR was conducted to obtain cDNA fragment by using the above primer set (0.1 μM each), the skate liver RT product as a template and HotStarTaq master mix kit (Qiagen, The Netherlands) in a PC-808 (Astec, Japan) with one cycle of 95 °C for 15 min, 30 cycles at 94 °C for 0.5 min, at 50 °C for 0.5 min and at 72 °C for 1 min, and one cycle of 72 °C for 10 min. The resultant products, showing expected size, were subcloned and sequenced. The amplified fragment was subcloned and its nucleotide sequence was determined. Analysis of the nucleotide and deduced amino acid sequences revealed a significant similarity to the C-terminal domains of other vertebrate pro-α1(V) and pro-α1(XI) chains (data not shown). From this result, it was speculated that the clone encodes a part of the skate pro-α1(V/XI) chain. Gene specific primers (GSPs) were designed from the sequence of the degenerate PCR fragment for extending the skate pro-α1(V/XI) chain cDNA.

The mRNA was purified from 4-week skate embryo by Oligotex™-dT30⟨Super⟩ kit (Roche, Switzerland). Approximately 3 μg of the mRNA was applied for constructing the cDNA library with a Marathon cDNA amplification kit (BD Biosciences Clontech, USA). Using the cDNA library, rapid amplification of cDNA ends (RACE) was conducted by GSPs, based on the sequence obtained from the degenerate PCR, and Marathon adapter primers. The PCR reaction was conducted by using an Adavantage-GC 2 PCR kit (BD Biosciences) with one cycle of 94 °C for 3 min, 30 cycles at 94 °C for 0.5 min, at 68 °C for 3 min, and one cycle of 68 °C for 3 min. The resultant 5′ and 3′ RACE products were subcloned and sequenced. Moreover, the 5′ RACE was further utilized to obtain 5′ end of the skate pro-α1(V/XI) chain cDNA according to the method of Zhi (1996) with some modifications.

2.4. Expression analysis by RT-PCR

Total RNA was determined by measuring the absorbance of 260 nm in a spectrophotometer, and the purity of the RNA was confirmed by readings of the 260 nm/280 nm ratio in 10 mM Tris–HCl, pH 7.5. Total RNA (5 μg) was treated with deoxyribonuclease (Nippon Gene, Japan) to remove contaminant genomic DNA traces, and reverse-transcribed into first strand cDNA with the oligo$(dT)_{20}$ primer. PCR amplification was performed by using a pair of the gene specific primer (0.1 μM each), and various RT products from skate adult origin as template, and Adavantage-GC 2 PCR kit (BD Biosciences, USA) in a PC-808 (Astec, Japan) with one cycle of 94 °C for 3 min, 30 cycles at 94 °C for 0.5 min, at 60 °C for 0.5 min, and at 72 °C for 1 min, and one cycle of 72 °C for 1 min.

2.5. Sequence analysis

The nucleotide and amino acid sequence analyses were performed by using FASTA (http:www.ddbj.nig.ac.jp/search/fasta-j.html) and CLUSTAL W (http:www.ddbj.nig.ac.jp/search/clustalw-j.html).

3. Results and discussion

Full-length cDNA of the skate pro-α1(V/XI) chain (accession no. AB201248) was derived from sequences of six overlapping cDNA clones, and contained a 5580bp open reading frame encoding a protein of 1860 amino acids with a calculated molecular mass of 187,813.2 Da.

Alignment with amino acid sequences of other vertebrate pro-α1(V), pro-α1(XI) and pro-α1(V/XI) chains demonstrates, more clearly, both the similarity and dissimilarity among them (Chernousov, Rothblum, Tyler, Stahl & Carey, 2000; Gordon et al., 1999; Greenspan, Cheng & Hoffman, 1991; Takahara et al., 1991; Touhata,

Tanaka, Yokoyama, Sakaguchi & Toyohara, 2001; Wu, Sumiyoshi, Khaleduzzaman, Ninomiya & Yoshioka, 1998; Yoshioka et al., 1995; Yoshioka & Ramirez, 1990). The difference between pro-α1(V) and pro-α1(XI) chains is the number of amino acid residues in the N-domain. The N-domains of pro-α1(V) chains (554–560 residues) are longer (over 25 amino acid residues) than those of pro-α1(XI) chains (526–528 residues). The N-domain of the skate pro-α1(V/XI) chain was found to consist of 579 amino acid residues. From the result, the skate pro-α1(V/XI) chain seems to be closer to pro-α1(V) chains than pro-α1(XI) chains. Like the N-propeptide of other vertebrate pro-α1(V) and pro-α1(XI) chains, the N-propeptide of the skate pro-α1(V/XI) chain contains a long globular domain, followed by an interrupted collagenous domain. A concentration of mismatches and gaps was seen in the acidic globular subdomains, which lie between the cluster of Cys (234 and 241, Fig. 1) and small collagenous domains (468–559, Fig. 1) in the N-propeptides of the skate pro-α1(V/XI) chain, as with other vertebrate counterparts.

Of note is the presence of two potential Asn-linked carbohydrate attachment sites (Asn-Leu-Ala, 156–158; Asn-Ala-Thr, 393–395, Fig. 1), conserved in the skate pro-α1(V/XI) chain, in the two globular subdomains of the N-propeptide. Similar potential sites are not found in the N-propeptides of mammalian pro-α1(I), pro-α1(II) and pro-α1(III) chains. Moreover, the other two sites (Asn-Glu-Thr, 379–381; Asn-Ser-Glu, 400–402, Fig. 1) were located around the second potential Asn-linked site. In the skate pro-α1(V/XI) chain, at least one of the potential glycosylation sites may be utilized and be of functional significance.

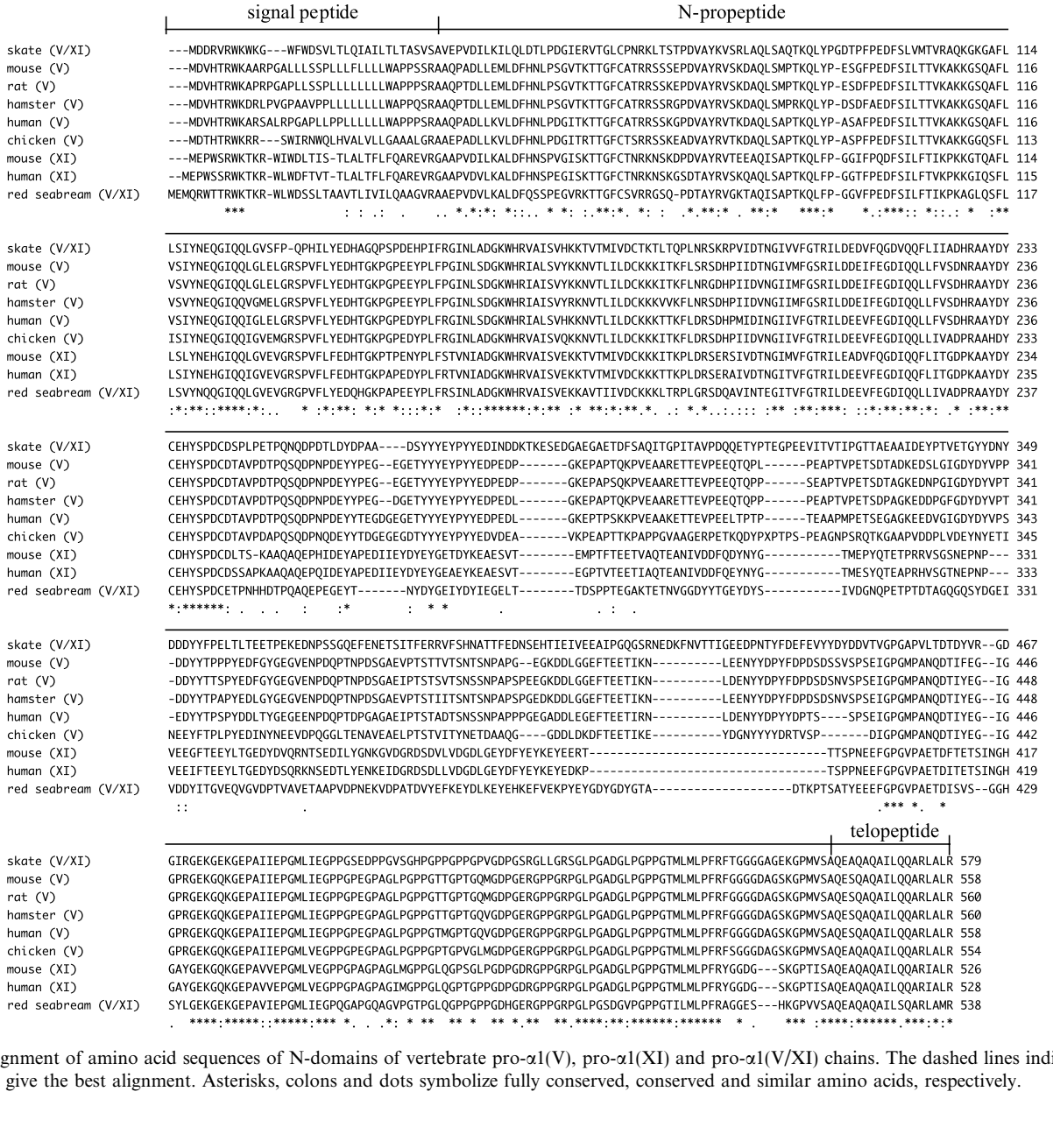

Fig. 1. Alignment of amino acid sequences of N-domains of vertebrate pro-α1(V), pro-α1(XI) and pro-α1(V/XI) chains. The dashed lines indicate gaps inserted to give the best alignment. Asterisks, colons and dots symbolize fully conserved, conserved and similar amino acids, respectively.

Lys residues are important in the formation of intermolecular covalent cross-links, responsible for much of the mechanical stability of collagen fibrils, and have previously been found in either one or both telopeptides of the major fibrillar collagen chains (Bernard et al., 1983, 1988; de Wet et al., 1987; Greenspan, Cheng & Hoffman, 1991; Kimura et al., 1989; Loidl et al., 1984; Myers, Loidl, Seyer & Dion, 1985; Sandell, Prentice, Kravis & Upholt, 1984). The skate pro-α1(V/XI) chain lacks a Lys residue in the N-telopeptide, as like other vertebrate pro-α1(V) and pro-α1(XI) chains (Fig. 1). On the other hand, one Lys residue (1606, Fig. 2) in the C-telopeptide was observed in the non-identical position with other counterparts (Fig. 2). It was, therefore, suggested that a relative paucity of cross-links in fibrils containing the Type V and XI collagen may utilize forms of cross-linking different from those employed by other fibrillar collagens.

As shown in Fig. 2, amino acid sequences, including those sequences in the C-telopeptide and amino-terminal side of the C-propeptide, are relatively divergent when Type I, II and III procollagen α chains are compared (Dion & Myers, 1987). This localized variability is highlighted in the present comparison between pro-α1(V) and pro-α1(XI) chains and suggests that this region may be important in providing the specificity for combining with other procollagen chains of the appropriate type (Dion & Myers, 1987). Most parts of C-propeptides, which are highly conserved between pro-α1(V) and pro-α1(XI) chains, may reflect the ability of the two chains, under certain conditions, to substitute for each other in the formation of cross-type heterotrimers (Mayne & Burgeson, 1987a; Niyibizi & Eyre, 1989). A similar function could be retained in the skate pro-α1(V/XI) chain, because of high similarity to other vertebrates (Fig. 2). A putative C-proteinase cleavage site (Glu-Glu, 1616–1617) of the skate pro-α1(V/XI) chain is assigned in Fig. 2, based on comparisons of the demonstrated and postulated cleavage sites of the other vertebrate procollagen chains (Bernard et al., 1988; Greenspan et al., 1991). In contrast to the C-proteinase cleavage site of pro-α1(XI) chain, shown to be in a different position, the skate pro-α1(V/XI) chain seems to be close to the pro-α1(V) chain group, however, the Asp residue of the cleavage site in others was substituted for the same acidic amino acid, Glu (Fig. 2).

The locations of the eight Cys residues (1661, 1667, 1684, 1693, 1702, 1768, 1811, 1857) found within the C-propeptide of the skate pro-α1(V/XI) chain were identical to the locations of the Cys residues found within the C-propeptides of other vertebrate pro-α1(V) chains (Fig. 2). The presence of eight conserved Cys residues in the skate pro-α1(V/XI) chain assures that it could be close to the pro-α1(V) chains, in contrast to seven Cys residues in the pro-α1(XI) chains, including the red seabream counterpart (Fichard et al., 1994; Touhata et al., 2001; Vuorio & de Crombrugghe, 1990).

In the skate pro-α1(V/XI) chain, Lys residues of two sites (661–666, 1519–1524) were conserved toward the N- and C-ends of the main triple helical domain. These two Lys residues, as well as those of other vertebrate pro-α1(V/XI) chains, are thought to be important as an attach-

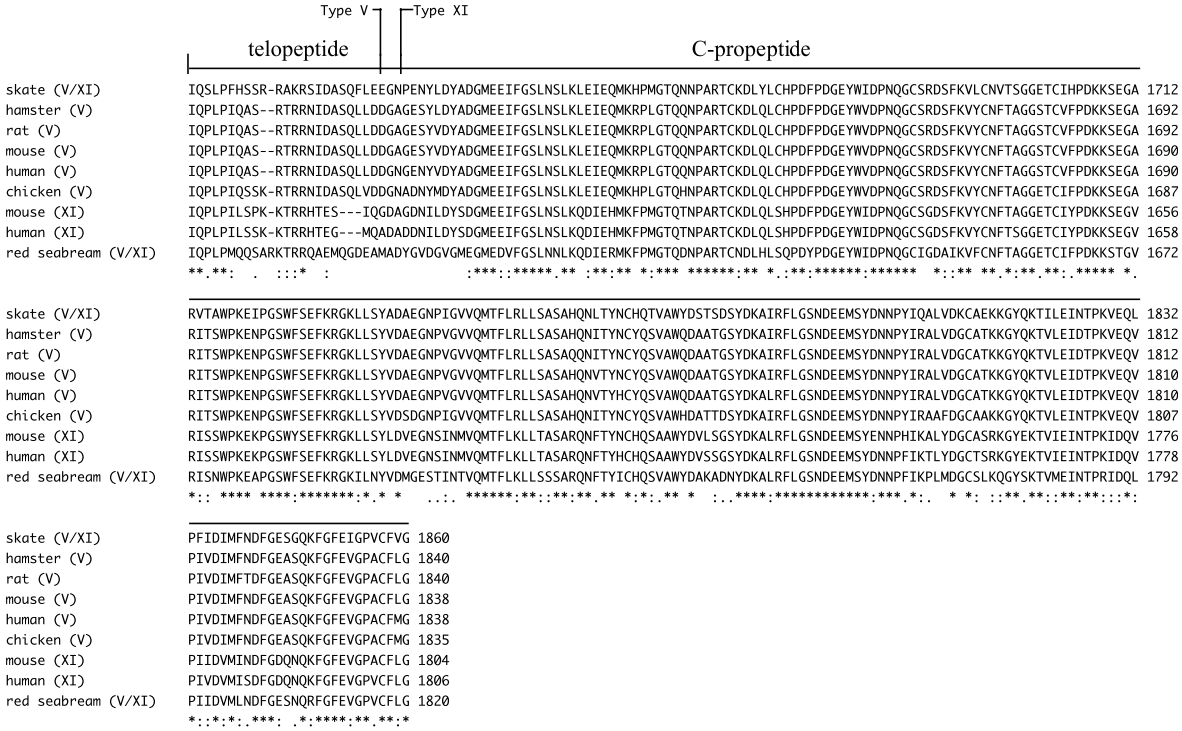

Fig. 2. Alignment of amino acid sequences of C-domains of vertebrate pro-α1(V), pro-α1(XI) and pro-α1(V/XI) chains. The dashed lines, asterisks, colons and dots are the same as in Fig. 1.

ment site for lysyl oxidase (Mayne & Burgeson, 1987c). Moreover, two Arg-Gly-Asp sequences (666–668, 684–686), known to be a potential cell-binding site (Ruoslahti & Pierschbacher, 1986), are conserved in the central triple-helical domain (data not shown). In addition, one characteristic of pro-α1(XI), pro-α2(XI) and pro-α1(V) chains is the presence of a heparin binding site, which is absent in the pro-α2(V) chain (Fichard et al., 1994; Yaoi, Hashimoto, Koitabashi, Takahara, Ito & Kato, 1990). In the skate pro-α1(V/XI) chain, sequence 918–950 of the triple helical region corresponding to the heparin binding site location, is extremely well conserved, together with other vertebrate pro-α1(V) and pro-α1(XI) chains (data not shown).

Comparison of the skate pro-α1(V/XI) chain with other vertebrate pro-α1(V) and pro-α1(XI) chains confirmed the high homology of the amino acid sequence among them (data not shown). The skate pro-α1(V/XI) chain exhibited a high level of sequence homology, in the order of triple-helical, C-terminal and N-terminal domains, as with the results obtained between human pro-α1(V) and pro-α1(XI) chains (Greenspan et al., 1991).

The phylogenetic analysis is performed in order to clarify whether the skate pro-α1(V/XI) chain is close to the pro-α1(V) or the pro-α1(XI) chain. The phylogenetic analyses of, not only N- and C-terminal domains, but also full-length, suggest that the skate pro-α1(V/XI) chain could be an intermediate form of pro-α1(V) and pro-α1(XI) chains (data not shown). However, the skate pro-α1(V/XI) chain was included in the same cluster as the pro-α1(V) chains, whereas the red seabream pro-α1(V/XI) chain was included in the same cluster with pro-α1(XI) chains when the phylogenetic tree was constructed by limiting to fibrillar procollagen α1 chains of human and mouse (Fig. 3).

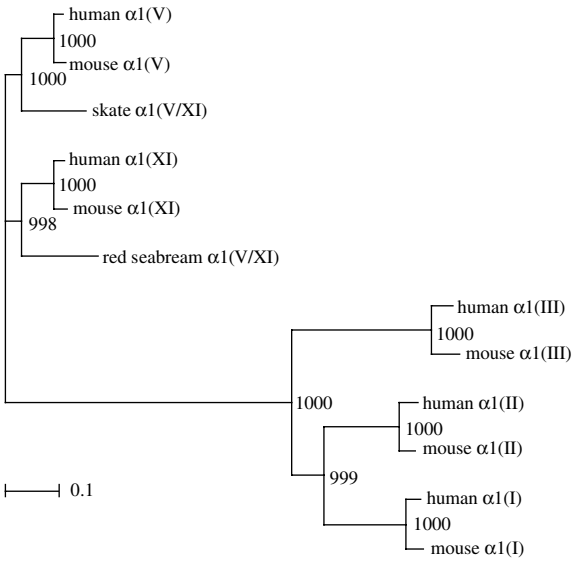

Fig. 3. Phylogenetic tree based on full-length of the skate and red seabream pro-α1(V/XI) chains, together with human and mouse fibrillar procollagen α1 chains. The scale bar shows substitution of one amino acid residue per 10 residues. The phylogenetic tree was constructed by joining amino acid sequences of various species. Numbers at nodes indicate bootstrap values for 1000 runs.

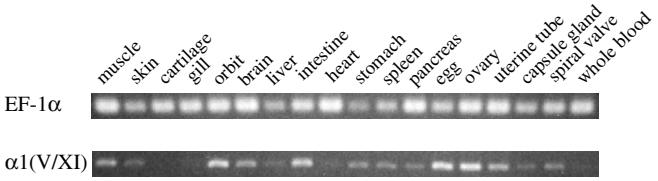

Fig. 4. Expression of the skate pro-α1(V/XI) chain mRNA. Total RNA from various tissues from the adult skate was used to generate cDNA for PCR amplification, using the skate pro-α1(V/XI) chain-specific primers, together with EF-1α (accession no. AB214933), as a positive control.

Fig. 4 shows the expressions of the pro-α1(V/XI) chain mRNA in various tissues of the skate. Amplified bands by RT-PCR were clearly detected in the muscle, skin, orbit, brain, liver, intestine, stomach, spleen, pancreas, egg, ovary, uterine tube, shell gland and spiral valve, except for cartilage, gill, heart and whole blood. PCR products were further defined by cloning and sequencing. As expected, all products were identical to a part of the skate pro-α1(V/XI) chain. This result indicates that the skate pro-α1(V/XI) chain is expressed widely in various tissues, and is consistent with biochemical and immunocytochemical studies which have indicated a wide tissue distribution of Type V collagen, in contrast to Type XI collagen, which has been found mainly in cartilaginous tissues (Mayne & Burgeson, 1987a, 1987b).

The skate pro-α1(V/XI) chain, comprised of 1860 amino acids, is the longest fibrillar procollagen chain characterized to date. Moreover, the skate pro-α1(V/XI) chain can be classified as pro-α1(V) chains rather than pro-α1(XI) chains, by conserved structural properties, as well as broad expression pattern. Further studies are now in progress to elucidate the expression properties of the skate pro-α1(V/XI) chain during embryonic development.

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References

Bernard, M. P., Myers, J. C., Chu, M. L., Ramirez, F., Eikenberry, E. F., & Prockop, D. J. (1983). Structure of a cDNA for the pro-α2 chain of human type I procollagen. Comparison with chick cDNA for pro-α2(I) identifies structurally conserved features of the protein and the gene. *Biochemistry, 22*, 1139–1145.

Bernard, M., Yoshioka, H., Rodriguez, E., Van der Rest, M., Kimura, T., Ninomiya, Y., et al. (1988). Cloning and sequencing of pro-α1 (XI) collagen cDNA demonstrates that type XI belongs to the fibrillar class of collagens and reveals that the expression of the gene is not restricted to cartilagenous tissue. *The Journal of Biological Chemistry, 263*, 17159–17166.

Chernousov, M. A., Rothblum, K., Tyler, W. A., Stahl, R. C., & Carey, D. J. (2000). Schwann cells synthesize type V collagen that contains a novel α4 chain. Molecular cloning, biochemical characterization, and

high affinity heparin binding of α4(V) collagen. *The Journal of Biological Chemistry, 275*, 28208–28215.

de Wet, W., Bernard, M., Benson-Chanda, V., Chu, M. L., Dickson, L., Weil, D., et al. (1987). Organization of the human pro-α2(I) collagen gene. *The Journal of Biological Chemistry, 262*, 16032–16036.

Dion, A. S., & Myers, J. C. (1987). COOH-terminal propeptides of the major human procollagens. Structural, functional and genetic comparisons. *Journal of Molecular Biology, 193*, 127–143.

Fichard, A., Kleman, J. P., & Ruggiero, F. (1994). Another look at collagen V and XI molecules. *Matrix Biology, 14*, 515–531.

Fitch, J. M., Gross, J., Mayne, R., Johnson-Wint, B., & Linsenmayer, T. F. (1984). Organization of collagen types I and V in the embryonic chicken cornea: Monoclonal antibody studies. *Proceedings of the National Academy of Sciences of the United States of America, 81*, 2791–2795.

Gordon, M. K., Marchant, J. K., Foley, J. W., Igoe, F., Gibney, E. P., Nah, H. D., et al. (1999). Complete primary structure of the chicken α1(V) collagen chain. *Matrix Biology, 18*, 481–486.

Greenspan, D. S., Cheng, W., & Hoffman, G. G. (1991). The pro-α1(V) collagen chain. Complete primary structure, distribution of expression, and comparison with the pro-α1(XI) collagen chain. *The Journal of Biological Chemistry, 266*, 24727–24733.

Hwang, J. H., Mizuta, S., Yokoyama, Y., & Yoshinaka, R. (2007). Purification and characterization of molecular species of collagen in the skin of skate (*Raja kenojei*). *Food Chemistry, 100*, 921–925.

Kimura, T., Cheah, K. S., Chan, S. D., Lui, V. C., Mattei, M. G., van der Rest, M., et al. (1989). The human α2(XI) collagen (COL11A2) chain. Molecular cloning of cDNA and genomic DNA reveals characteristics of a fibrillar collagen with differences in genomic organization. *The Journal of Biological Chemistry, 264*, 13910–13916.

Linsenmayer, T. F., Fitch, J. M., Schmid, T. M., Zak, N. B., Gibney, E., Sanderson, R. D., et al. (1983). Monoclonal antibodies against chicken type V collagen: Production, specificity, and use for immunocytochemical localization in embryonic cornea and other organs. *Journal of Cell Biology, 96*, 124–132.

Loidl, H. R., Brinker, J. M., May, M., Pihlajaniemi, T., Morrow, S., Rosenbloom, J., et al. (1984). Molecular cloning and carboxyl-propeptide analysis of human type III procollagen. *Nucleic Acids Research, 12*, 9383–9394.

Mayne, R., & Burgeson, R. E. (1987a). Structure and function of collagen types. In D. R. Eyre & J.-J. Wu (Eds.), *Type XI or 1α2α3α collagen* (pp. 261–281). New York: Academic Press.

Mayne, R., & Burgeson, R. E. (1987b). Structure and function of collagen types. In J. H. Fessler & L. I. Fessler (Eds.), *Type V collagen* (pp. 81–103). New York: Academic Press.

Mayne, R., & Burgeson, R. E. (1987c). Structure and function of collagen types. In K. Kuhn (Ed.), *The classical collagens: Types I, II, and III* (pp. 1–42). New York: Academic Press.

Mendler, M., Eich-Bender, S. G., Vaughan, L., Winterhalter, K. H., & Bruckner, P. (1989). Cartilage contains mixed fibrils of collagen types II, IX, and XI. *Journal of Cell Biology, 108*, 191–197.

Mizuta, S., Hwang, J. H., & Yoshinaka, R. (2002). Molecular species of collagen from wing muscle of skate (*Raja kenojei*). *Food Chemistry, 76*, 53–58.

Mizuta, S., Hwang, J. H., & Yoshinaka, R. (2003). Molecular species of collagen in pectoral fin cartilage of skate (*Raja kenojei*). *Food Chemistry, 80*, 1–7.

Myers, J. C., Loidl, H. R., Seyer, J. M., & Dion, A. S. (1985). Complete primary structure of the human α2 type V procollagen COOH-terminal propeptide. *The Journal of Biological Chemistry, 260*, 11216–11222.

Niyibizi, C., & Eyre, D. R. (1989). Identification of the cartilage α1(XI) chain in type V collagen from bovine bone. *Federation of European Biochemical Societies Letters, 242*, 314–318.

Ruoslahti, E., & Pierschbacher, M. D. (1986). Arg-Gly-Asp: A versatile cell recognition signal. *Cell, 44*, 517–518.

Sandell, L. J., Prentice, H. L., Kravis, D., & Upholt, W. B. (1984). Structure and sequence of the chicken type II procollagen gene. Characterization of the region encoding the carboxyl-terminal telopeptide and propeptide. *The Journal of Biological Chemistry, 259*, 7826–7834.

Sato, K., Ando, M., Kubota, S., Origasa, K., Kawase, H., Toyohara, H., et al. (1997). Involvement of type V collagen in softening of fish muscle during short-term chilled storage. *Journal of Agriculture and Food Chemistry, 45*, 343–348.

Takahara, K., Sato, Y., Okazawa, K., Okamoto, N., Noda, A., Yaoi, Y., et al. (1991). Complete primary structure of human collagen α1(V) chain. *The Journal of Biological Chemistry, 266*, 13124–13129.

Touhata, K., Tanaka, H., Yokoyama, Y., Sakaguchi, M., & Toyohara, H. (2001). Structure of full-length cDNA clone for the pro-α1(V/XI) collagen chain of red seabream. *Biochimica et Biophysica Acta, 1517*, 323–326.

Vuorio, E., & de Crombrugghe, B. (1990). The family of collagen genes. *Annual Review of Biochemistry, 59*, 837–872.

Wu, Y. L., Sumiyoshi, H., Khaleduzzaman, M., Ninomiya, Y., & Yoshioka, H. (1998). cDNA sequence and expression of the mouse α1(V) collagen gene (Col5a1). *Biochimica et Biophysica Acta, 1397*, 275–284.

Yaoi, Y., Hashimoto, K., Koitabashi, H., Takahara, K., Ito, M., & Kato, I. (1990). Primary structure of the heparin-binding site of type V collagen. *Biochimica et Biophysica Acta, 1035*, 139–145.

Yata, M., Yoshida, C., Fujisawa, S., Mizuta, S., & Yoshinaka, R. (2001). Identification and characterization of molecular species of collagen in fish skin. *Journal of Food Science, 66*, 247–251.

Yoshioka, H., Inoguchi, K., Khaleduzzaman, M., Ninomiya, Y., Andrikopoulos, K., & Ramirez, F. (1995). Coding sequence and alternative splicing of the mouse α1(XI) collagen gene (Col11a1). *Genomics, 28*, 337–340.

Yoshioka, H., & Ramirez, F. (1990). Pro-α1(XI) collagen. Structure of the amino-terminal propeptide and expression of the gene in tumor cell lines. *The Journal of Biological Chemistry, 265*, 6423–6426.

Zhi, C. (1996). Simple modifications to increase specificity of the 5′ RACE procedure. *Trends in Genetics, 12*, 87–88.