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Detection of keratan sulfate by immunological methods in commercial chondroitin sulfate preparations

Takuo Nakano*, Lech Ozimek

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada

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ABSTRACT

Chondroitin sulfate (CS), a well known nutraceutical, and keratan sulfate (KS) are glycosaminoglycans involved in the structure of cartilage proteoglycan, aggrecan. Since CS is extracted from cartilage, there may be a possibility that purified CS is contaminated with small amount of KS. A total of 15 samples, including four samples of CS as laboratory reagents, one sample of CS as a food additive and ten samples of dietary supplements containing CS were examined to detect KS in these samples by using immunodiffusion and enzyme-linked immunosorbent assay (ELISA) with anti-KS monoclonal antibody (IgM). With the exception of three samples of CS as laboratory reagents, all samples were found to contain varying amounts of KS. It was concluded that both the immunodiffusion, a quick one-step method, and ELISA for quantification, are reliable methods to detect KS contamination in CS products.

1. Introduction

Chondroitin sulfate (CS) is an acidic polysaccharide (or glycosaminoglycan, GAG) comprised of repeating disaccharide units of N-acetylgalactosamine and glucuronic acid (Lamari & Karamanos, 2006; Nakano, Betti, & Pietrasik, 2010; Rodén, 1980; Wight, Heinegård, & Hascall, 1991). The disaccharide unit is usually sulfated at either the C-4 or C-6 position of N-acetylgalactosamine. Keratan sulfate (KS) is also a GAG comprised of repeating disaccharide units of N-acetylglucosamine and galactose. Both CS and KS are involved in the structure of aggrecan, which is a major proteoglycan abundant in cartilage accounting for 5–10% of the tissue wet weight (Heinegård & Oldberg, 1989). In bovine cartilage aggrecan, approximately 100 CS chains with molecular mass of 10–25 kDa and 30–60 KS chains with molecular mass of 4–8 kDa are covalently attached to the core protein as GAG side chains (Hascall, 1977). In the extracellular matrix of articular cartilage, aggrecans with highly hydrophilic polyanionic CS chains interact with GAG hyaluronic acid to form large aggregates, which is believed to contribute to the resilience of load-bearing tissue (Mow, Ratcliffe, & Poole, 1992). Cartilage tissues are also known to contain dermatan sulfate proteoglycans, decorin and biglycan (Rosenberg et al., 1985) and KS proteoglycan, fibromodulin (Heinegård et al., 1986) as minor constituents accounting for 1–2% of the total mass of cartilage proteoglycans (Heinegård & Oldberg, 1989). KS in fibromodulin (KS-I) is distinguished from most of KS in aggrecan (KS-II) by the structure of linkage to the core protein, in that KS-II is O-glycosidically linked to serine or threonine, whereas KS-I is N-glycosidically linked to asparagine (Barry et al., 1995; Plaas, Neame, Nivens, & Reiss, 1990).

CS extracted and isolated from other GAGs present in cartilage has a wide range of applications in food, cosmetic and pharmaceutical industries. Oral administration of CS was reported to be beneficial in maintaining healthy articular cartilage or treatment of osteoarthritis (Uebelhart et al., 2004; Volpi, 2009), and thus dietary supplements containing CS has been popular products seen in health food market.

Several groups of researchers (Adebowale, Cox, Liang, & Eddington, 2000; Ji, Roman, Zhou, & Hildreth, 2007; Sakai, Otake, Toida, & Goda, 2007; Sim et al., 2005, 2007; Volpi & Maccari, 2008) have determined the content of CS or its disaccharide composition in commercially available samples of dietary supplements. Volpi (2009), in a review of the quality of commercial CS preparations, reported presence of hyaluronic acid as a contaminant in dietary supplement samples. This author, however, did not discuss the possibility of contamination with KS (derived from aggrecan and fibromodulin) or dermatan sulfate (from decorin and biglycan) in CS preparations. There is unlikely any report of dermatan sulfate contamination in CS preparations. However, Møllar, Møllar-Pedersen, Damsgaard, and Poulsen (1995) reported KS contamination in a commercial preparation of CS from shark cartilage (see below for the method of detection). More recently,

* Corresponding author. Tel.: +1 780 492 3830; fax: +1 780 492 4265.
E-mail address: takuonakano@hotmail.com (T. Nakano).

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Table 1
Results of immunodiffusion analysis.

Sample no.	Product	Source	Immunoreaction	Keratanase treatment
1	CS (laboratory reagent)	Porcine rib cartilage	–	nd[a]
2	CS (laboratory reagent)	Sturgeon cartilage	–	nd
3	CS (laboratory reagent)	Shark cartilage	–	nd
4	CS (laboratory reagent)	Bovine tracheal cartilage	+	–
5	CS (food additive)	No information on the label	+	–
6	CS/glcosamine-sulfate	Bovine cartilage	+	–
7	CS/glcosamine-sulfate	Bovine tracheal cartilage	+	–
8	CS/glcosamine-sulfate	Bovine cartilage	+	–
9	CS/glcosamine-HCl	Bovine, porcine and avian cartilages	+	–
10	CS/glcosamine-HCl	No information on the label	+	–
11	CS/glcosamine-sulfate	Bovine cartilage	+	–
12	CS/glcosamine-sulfate	Bovine cartilage	+	–
13	CS/glcosamine-sulfate	Bovine cartilage	+	–
14	CS/glcosamine-sulfate	Bovine cartilage	+	–
15	CS/glcosamine-HCl	Bovine cartilage	+	–
16	Standard KS	Human costal cartilage	+	–
17	Standard C4S	Sturgeon notochord	–	nd
18	Standard C6S	Cranial cartilage of sturgeon	–	nd
19	Glcosamine-HCl	Shrimp/crab exoskeleton	–	nd
20	Glcosamine-sulfate	Shrimp/crab exoskeleton	–	nd

+: Positive result with precipitin line formation.
–: Negative result with no visible precipitin line formation with 40 μg of CS sample/well or with approximately 40 μg of dietary supplement sample containing average 12 μg SGAG per well.
[a] Not determined.
See text for other details.

Pomin, Piquet, Pereira, and Mourão (2012) analyzed samples of CS formulations for oral administration prepared from shark and bovine cartilages, and reported presence of significant amounts of KS in samples prepared from shark cartilage but not from bovine cartilage. These authors suggested bovine tissues as a preferable source of CS. Since both CS and KS chains are covalently attached to the same core protein of cartilage aggrecan (see above), there may be a possibility of the presence of KS as a contaminant in CS preparations, if purification processes are not efficient. To detect KS, Pomin et al. (2012) used several techniques including agarose gel electrophoresis, strong anion exchange HPLC, digestion with specific GAG lyases including keratanase and chondroitin AC lyase, and nuclear magnetic resonance spectroscopy.

An alternative method to detect KS is the use of antibody specific to this GAG. It turned out that immunodiffusion is a very applicable method to detect KS in CS preparation with small amounts of sample due to its simplicity, specificity, and straightforward application (Nakano, Pietrasik, Ozimek, & Betti, 2012; Srichamroen, Nakano, Pietrasik, Ozimek, & Betti, 2013). Møllar et al. (1995) used an enzyme-linked immunosorbent assay (ELISA) with anti-KS monoclonal antibody (5D4) to detect KS in a low grade commercial CS (>120 kDa) from shark cartilage. As far as we know, however, there has been no published information available on the application of immunological method to detect KS in commercial samples of dietary supplements containing CS.

KS free CS as a research chemical is needed as a standard CS for either quantitative or qualitative analysis of this GAG. Dietary supplement containing KS free CS is also needed to determine the effect of oral administration of CS on the serum concentration of KS, a metabolite of cartilage proteoglycan, which may be affected in patients suffering from osteoarthritis (Thonar et al., 1985).

Analysis of KS may be important for the quality control in CS producing factories, and thus development of a simple sensitive method to detect KS in CS preparation is needed. This study was undertaken to determine whether KS can be detected by using immunodiffusion and ELISA methods in commercially available samples of CS and dietary supplements containing CS.

2. Materials and methods

2.1. Materials

Samples of CS as laboratory reagents (Table 1) included chondroitin sulfate A from porcine rib cartilage and bovine tracheal cartilage, both obtained from Sigma–Aldrich Canada Ltd., Mississauga, ON, Canada, and chondroitin sulfate A from sturgeon notochord and chondroitin sulfate C from shark cartilage, both obtained from Seikagaku Corporation, Tokyo, Japan. A sample of CS as a food additive (Table 1) was obtained from a supplier in the United States. All CS samples were in the form of powder each in a sealed bottle.

A total of 10 samples of dietary supplements containing CS (each from different supplier) (Table 1) were obtained from health food stores in Edmonton, Canada. Most of CS in these samples was derived from bovine cartilage as shown in the table. They were in the form of capsuled powder (five samples) or gel (one sample) and tablets (four samples). Samples of dietary supplements including glucosamine-HCl and glucosamine-sulfate both in the form tablets were obtained from health food stores in Edmonton, Canada. All tablets were powdered using a motor and pestle.

Standard GAGs including chondroitin 4-sulfate (C4S) from sturgeon notochord, chondroitin 6-sulfate (C6S) from sturgeon cranial cartilage, KS from bovine nasal cartilage and hyaluronic acid from human umbilical cords were gifts from Drs. M. B. Mathews and J. A. Cifonelli, University of Chicago, USA. Ascites fluid containing anti-KS monoclonal antibody, AH12 is an IgM raised against bovine fibrocartilage CS proteoglycan containing KS (Nakano, Imai, Koga, Dodd, & Scott, 1993). By using an ELISA inhibition assay, Nakano et al. (1993) reported a much higher antigenicity in KS-peptide prepared from bovine fibrocartilage CS proteoglycan compared to bovine corneal KS (KS-I) [concentrations for 50% inhibition or IC_{50} being >70 times lower in KS-peptide than in corneal KS under the experimental condition used by Nakano et al. (1993)]. AH12 has been used for determinations of KS by immunodiffusion (Nakano et al., 1993, 2012; Srichamroen et al., 2013), immunohistochemical localization (Nakano et al., 1993; Nakano, Sim, Imai, & Koga, 1996; Nakano, Imai, Koga, & Sim, 1996; Sunwoo, Nakano, Hudson, & Sim, 1998) and ELISA (Nakano & Sim, 1995; Nakano & Scott, 1996).

CS peptide containing KS (CS-KS-peptide), used as a plate coating antigen in ELISA, was prepared from bovine nasal cartilage as previously described (Nakano, Nakano, & Sim, 1998). In this method, the GAG peptides were extracted from tissues by incubation in 0.1 M sodium acetate at pH 4.5 and 37 °C, and the CS-KS-peptide released by autolysis was separated by anion exchange chromatography on diethylaminoethyl (DEAE)-Sephacel (GE Healthcare, Baied'Urfè, PQ, Canada). The CS-KS-peptide obtained was considered to be a suitable plate coating antigen because preliminary studies showed that results of KS determination by ELISA in a crude CS preparation were consistent between two experiments, one of which was carried out with the CS-KS-peptide as a plate coating antigen and the other with bovine aggrecan, a commonly used plate coating antigen. Preparation of aggrecan requires more time consuming expensive work than does preparation of CS-KS-peptide, which lacks peptide of hyaluronic acid binding region of aggrecan.

2.2. Cellulose acetate electrophoresis

Electrophoresis of CS and dietary supplements was carried out on cellulose acetate strips (2.5 cm × 15.2 cm, Sepraphore III, Pall Corporation, Ann Arbor, MI, USA) in 0.1 M potassium phosphate buffer, pH 7.0 (Hata & Nagai, 1972). Two samples, each containing 3–5 μg GAG dissolved in 5 μL of water, were applied to a 2.5 cm wide strip giving a 1.25 cm wide space per sample. Electrophoresis was performed by applying a constant current of 1 mA cm^{-1} for 1 h at room temperature. After electrophoresis, the strips were stained in 0.5% (w/v) Alcian blue 8GX in 5% (v/v) acetic acid and destained in deionized water.

2.3. Sample preparation for immunodiffusion test

A sample of CS as a laboratory reagent or a food additive was weighed in duplicate, which were dissolved in water to obtain 1% (w/v) solution. A portion of each solution was first examined with immunodiffusion test. A sample showing positive immunoreaction was then serially diluted (1–0.01 mg/mL) to determine a minimum amount of sample required to show immunoreactivity. The concentration of standard KS tested ranged from 0.1 to 0.001 μg/mL.

A sample of dietary supplement (~10 mg) was weighed in triplicate in microcentrifuge tubes, to which 100 volumes of water were added. After thorough mixing, each mixture was centrifuged at 12,000 × *g* and a portion of supernatant (water soluble fraction) obtained was used for immunodiffusion test. A sample showing a positive immunoreaction was then serially diluted as described above for the sample of CS to determine a minimum amount of sample required to show immunoreactivity.

2.4. Immunodiffusion analysis

Undiluted ascites fluid containing anti-KS monoclonal antibody, AH12 (4 μL) and samples of CS as laboratory reagent or food additive [4 μL of 1% (w/v) solution] or those of dietary supplements (4 μL of water soluble fraction) were applied to wells in gels made from 1% (w/v) agar in 0.9% (w/v) NaCl containing 0.05% (w/v) sodium azide (Nakano et al., 1993). Agar-gels were incubated in a humid chamber and diffusion allowed to proceed at 37 °C for 20 h.

2.5. Keratanase digestion

To confirm that the anti-KS monoclonal antibody did not recognize any other GAG except KS, samples showing a positive immunoreactivity to AH12 were further treated with keratanase, an enzyme which specifically digests KS. Standard KS (0.025 μg) or sample of bovine tracheal CS (2.5 μg) containing KS was incubated with and without 0.2 unit of keratanase from *Pseudomonas* sp. (Seikagaku Corporation, Tokyo, Japan) in 0.02 M sodium acetate/0.02 M Tris–HCl, pH 7.3 at 37 °C for 3 h (Nakano et al., 2012). Samples of dietary supplements (2.5–10 μg) were also incubated with and without 0.2 unit of keratanase as described above. After keratanase treatment, each sample was subjected to an immunodiffusion test (see above).

2.6. Preparation of sulfated GAG (SGAG) fraction from dietary supplement

A water soluble fraction prepared by adding 20 volumes of water to a portion (200–500 mg) of sample of dietary supplement was chromatographed on a 1.5 cm × 5 cm column of DEAE-Sephacel. SGAG (CS + KS) bound to the column was then eluted with 2 M NaCl. Fractions containing SGAG, which was detected by dimethylmethylene blue dye binding method (Farndale, Buttle, & Buttle, 1986), were pooled, dialyzed against water and freeze-dried to obtain an SGAG fraction. The content of SGAG was determined by the dye binding method using C4S (see Section 2.1) as a standard GAG. The KS content in SGAG was then determined by ELISA.

2.7. ELISA

KS contents in CS and SGAG samples were estimated by an ELISA with an inhibition step using anti-KS monoclonal antibody, AH12. Microtiter plates (Costar 9018, Corning Inc., Corning, NY, USA) were precoated with 20 μg/mL concentrations of CS-KS-peptide from bovine nasal cartilage (see Section 2.1). Increasing concentrations (0.1–100 μg/mL) of CS or SGAG as competing antigen were then prepared by dissolving an appropriate amount of each sample in phosphate-buffered saline (0.14 M NaCl, 0.0027 M KCl, 0.0081 M disodium phosphate, 0.0015 M monopotassium phosphate, and 0.02% (w/v) sodium azide, pH 7.2) (PBS) followed by serial dilutions with PBS. Competing antigens (50 μL) were incubated with an equal volume of 8000-fold diluted AH12 ascites fluid in the precoated plates for 1–2 h at room temperature. Normal mouse serum (1: 8000 dilution) and increasing concentrations (5–20,000 ng/mL) of standard KS from bovine nasal cartilage were included in each assay. After incubation, plates were washed with PBS containing 0.1% (v/v) Tween-20 (PBST), and the second antibody [100 μL of a 1: 1000 dilution of alkaline phosphatase conjugated goat-anti mouse Ig (A, G, M) (Sigma–Aldrich)] was added. Following incubation as above, plates were washed again with PBST, and the alkaline phosphatase substrate, *p*-nitophenyl phosphate (100 μL of a 1 mg/mL solution in 1 M diethanolamine/0.005 M magnesium chloride/0.02% (w/v) sodium azide, adjusted to pH 9.8 with HCl) was added and incubation continued until the appropriate color had developed. The absorbance at 405 nm was then measured, and the concentration of antigen required for 50% inhibition (IC_{50}) was determined from a sigmoid plot generated using an ELISA curve-fitting tool (ReaderFit, Hitachi Solutions America, Ltd.). The concentration of KS was calculated by referring to standard KS inhibition curve.

3. Results and discussion

On cellulose acetate electrophoresis, every sample of CS or dietary supplement gave a single band with its mobility similar to the mobility of standard CS (Fig. 1). There was no visible band with the mobility of KS, which was less (0.9) relative to that of CS (1.0).

Results of immunodiffusion analysis are given in Table 1, and immunoprecipitation patterns for representative samples are shown in Fig. 2. A clear precipitin line was observed between anti-KS monoclonal antibody, AH12 and bovine tracheal cartilage CS

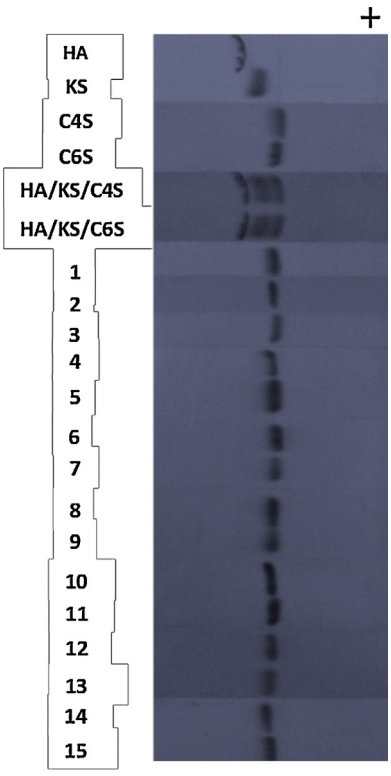

Fig. 1. Cellulose acetate electrophoresis of standard GAGs including hyaluronic acid (HA), KS, chondroitin 4-sulfate (C4S), chondroitin 6-sulfate (C6S), and mixtures of HA, KS and C4S (HA + KS + C4S) and HA, KS and C6S (HA + KS + C6S). The mobility was the fastest in C4S or C6S, intermediate in KS and the slowest in HA. The mobility of C4S was similar to the mobility of C6S. Electrophoretograms of samples 1–15 listed in Table 1 are also shown. See text for other details.

(sample no. 4) or CS for food additive (sample no. 5). The precipitin line was seen to be fused with that formed with standard KS from bovine cartilage as expected (Fig. 2). The minimum amount of sample required to show immunoreaction was 100 times lower in standard KS (0.01 μg) than in bovine tracheal cartilage CS (1 μg) or CS for a food additive (1 μg), suggesting that KS is present as a relatively minor component in each preparation of CS. No precipitin line was formed between AH12 and commercial CS from porcine rib cartilage (sample no. 1), sturgeon cartilage (sample no. 2), shark cartilage (sample no. 3), standard C4S or standard C6S.

When dietary supplements were examined, all samples gave clear precipitin lines with AH12 (Table 1), each of which was also seen to be fused with the precipitin line formed with standard KS (results not shown). The minimum amount of sample required to show immunoreaction averaged 1.6 μg, which corresponded to 0.48 μg SGAG calculated using analytical data shown in Table 2.

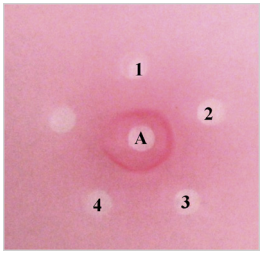

Fig. 2. Immunodiffusion reaction of anti-KS monoclonal antibody (well A) with standard KS (well 1), CS from bovine tracheal cartilage (sample no. 4, well 2), CS as a food additive (sample no. 5, well 3), and dietary supplement (sample no. 6, well 4). Gel was stained with 0.5% (w/v) Ponceau S in 7.5% (w/v) trichloroacetic acid and destained with 7.5% trichloroacetic acid followed by water. See text for other details.

Table 2
CS and SGAG contents in commercial samples of dietary supplements.

Sample no.	CS (% of dry weight)[a]	SGAG (% of dry weight)[b]
6	32.4	25.6
7	34.7	31.5
8	32.0	32.4
9	31.7	34.3
10	34.7	36.8
11	19.7	20.5
12	28.9	26.6
13	33.0	32.4
14	32.6	33.3
15	19.3	22.1

[a] Calculated from the CS content shown on the label and average dry weight ($n = 10$) of tablet or capsuled powder without gelatin capsule.
[b] Prepared by anion exchange chromatography on DEAE-Sephacel (see Section 2.6).

Glucosamine-HCl and glucosamine-sulfate showed no immunoreactions with AH12.

We then treated KS containing samples with keratanase. All samples (nos. 4–15) failed to show precipitin lines as anticipated (Table 1). This indicated that the positive immunoreactions observed were due to the presence of KS (but not other GAG) confirming the specificity of the monoclonal antibody used (Nakano et al., 1993).

In this study, monoclonal IgM, instead of monoclonal IgG, was used for immunodiffusion analysis. IgM, having ten antigen binding sites, produces larger aggregates (resulting in a denser precipitin line) with KS than does IgG, suggesting lower assay sensitivity with the latter. In the preliminary experiment, we have investigated immunoreactivity between KS containing sample (nos. 4–15, Table 1) used in this study and anti-KS monoclonal IgG (DD11), which was raised against bovine fibrocartilage proteoglycan (Nakano et al., 1993). The IgG required more antigen (>40 times) than did IgM (AH12) for precipitin line formation in all samples examined. These results suggested that anti-KS monoclonal IgG is not suitable for detection of KS with immunodiffusion technique.

Anion exchange chromatography was then carried out to separate SGAG from dietary supplements. The content of recovered SGAG was in general comparable to the content of CS calculated from the amount of CS shown on the label of each product (Table 2), and accounted for average 30% of dry matter.

KS contents in CS preparations determined by ELISA inhibition assay are given in Table 3. Consistent with the results of immunodiffusion analysis, all samples which showed antigenicity to anti-KS monoclonal antibody were found to contain varying amounts of KS. No detectable KS was seen in standard C4S and C6S, commercial CS (sample nos. 1–3), glucosamine-HCl and glucosamine-sulfate as expected. The KS content ranged from 2 to 22% of dry CS or SGAG, but majority (>80%) of samples had KS contents <10%. The higher contents (>10%) seen in nos. 5, 11 and 12 samples are comparable to KS contents (16% and 10% of total GAG) reported by Pomin et al. (2012) in CS formulations and standard CS from European pharmacopeia, respectively, both prepared from shark cartilage. The contents averaging 4.5% in the remaining samples (nos. 4, 6–10 and 13–15) are fairly comparable to the KS content (4.5%) reported in GAG-peptide extracted from bovine nasal cartilage by tissue autolysis (Nakano et al., 1998) or the KS content (8% estimated from the glucosamine/galactosamine molar ratio) reported in CS-peptide extracted from chicken cartilage by tissue autolysis (Srichamroen et al., 2013). These KS levels are, however, much higher compared to those found in CS-peptide (without detectable glucosamine or antigenicity to anti-KS antibody), which was extracted from chicken tissues by papain proteolysis followed by fractionation using anion exchange chromatography and selective precipitation with

Table 3
KS contents in CS and SGAG determined by ELISA.

Sample no.		KS (% of dry weight of CS or SGAG)
1	CS	nd[a]
2	CS	nd
3	CS	nd
4	CS	8.6
5	CS	20.3
6	SGAG	3.4
7	SGAG	2.3
8	SGAG	4.8
9	SGAG	7.3
10	SGAG	2.9
11	SGAG	21.7
12	SGAG	11.0
13	SGAG	3.4
14	SGAG	2.4
15	SGAG	5.3
16	Standard C4S	nd
17	Standard C6S	nd
18	Glucosamine-HCl	nd
19	Glucosamine-sulfate	nd

[a] Not detected. Percentage inhibitions measured at 100 μg/mL level of competing antigen were < 10% in all samples showing undetectable level of KS.

different concentrations of ethanol (Nakano et al., 2012). In contrast to the present findings, Pomin et al. (2012) fractionated CS formulations from bovine cartilage by anion exchange HPLC, and found a single peak of CS with no significant amount of KS eluting from the column. Although the purity of their CS preparations before fractionation is unknown, the possibility that KS is still in the CS fraction as a form of CS-KS-peptide cannot be ruled out (see below).

Proteolysis with exogenous proteinase (e.g. papain) is the common method to extract CS from cartilage tissues. After proteolysis, GAG-peptides including CS-peptides from CS-rich domain of aggrecan with or without KS chains and KS-peptides from KS-rich domain of aggrecan with or without CS may be the products liberated from tissues. An evidence of occurrence of GAG-peptides can be seen by the treatment of proteinase digest of tissue with alkaline borohydride (β-elimination reaction) followed by gel chromatography of the product to confirm the decrease in its molecular size as shown by Nakano et al. (2012) in their CS containing fractions. Among the GAG-peptides produced, CS-peptides without KS and KS-peptides without CS may be separated from each other by anion exchange chromatography as described by Pomin et al. (2012). However, if both CS and KS chains are covalently attached to the same core peptide (forming a CS-KS-peptide as shown in Fig. 3), the two GAGs cannot be separated from each other on anion exchange column or electrophoresis. The CS preparation (>120 kDa) from shark cartilage containing KS reported by Møllar et al. (1995) is likely in the form of large CS-KS-peptide. The size of peptide attaching to CS, which is dependent on proteolytic activity of exogenous enzyme used to release GAG-peptides from tissues (Nakano et al., 2012), may be related to the amount of CS-KS-peptides. Such peptides must be treated chemically or enzymatically for quantification

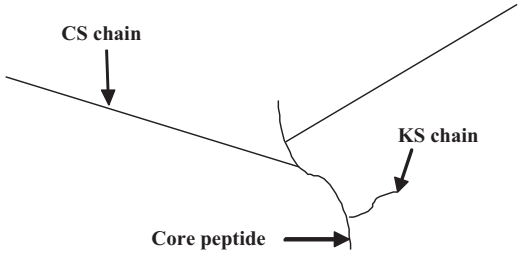

Fig. 3. A drawing of the structure of CS-KS-peptide.

of KS. This means that chromatogaraphic or electrophoretic method needs more procedures with larger amount of sample compared to immunological methods.

There may not be a single step method other than immunological method for detection of KS in CS-KS-peptide. We now suggest that both immunodiffusion and ELISA used in the present study are useful sensitive methods with small amount of sample required to detect KS in commercial preparations of CS and dietary supplements. In CS producing factories, immunodiffusion with anti-KS antibody, which requires simpler procedures than does ELISA, may be the first test as a screening tool to quickly detect KS, and then ELISA can be used to quantify KS or to confirm the result with immunodiffusion. Since the sensitivity of ELISA to determine KS is dependent on binding specificity of monoclonal antibody (Lettry et al., 2010; Wakitani, Okabe, Kawaguchi, Nawata, & Hashimoto, 2010) and chain length and sulfation of KS (Mehmet et al., 1986; Nakano et al., 1993), careful approach is needed to develop an ELISA method for quality control of commercial preparations of CS.

From the present study, we suggest that CS for laboratory use should be provided with a label indicating the content of KS, if any. For production of CS as a nutraceutical, a proper regulation concerning the content of contaminating KS should be considered in relation to the quality of dietary supplement containing CS, although little is known about the effect of KS as a dietary supplement on joint articular cartilage metabolism.

4. Conclusion

To our knowledge, this is the first report of immunodiffusion analysis with monoclonal IgM in commercial CS preparations. We have shown that the frequency of KS contamination is surprisingly high in commercial preparations of CS and dietary supplements containing CS. Immunodiffusion is a quick single-step procedure to detect KS in these products. KS can be subsequently quantified via ELISA if the need arises.

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