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# Purification and characterization of type II collagen from chick sternal cartilage

Hui Cao, Shi-Ying Xu *

*School of Food Science and Technology, Southern Yangtze University, P.O. Box 98, No. 1800, Lihu Road, Wuxi, 214122 Jiangsu, China*

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**Abstract**

Type II collagen was purified from sternal cartilage of the chick using a combination of pepsin digestion, NaCl precipitation and DEAE-sepharose CL 6B ion exchange chromatography. Pepsin-solubilized type II collagen of higher stability can be obtained with the extraction time of 32 h, 0.5% pepsin concentration at 20 °C. The purified preparation showed a single peak on RP-HPLC and a single band (α-chain) and its dimers (β-chains) on SDS–PAGE with a subunit Mr of 110 kDa. The amino acid composition of the type II collagen derived from chick cartilage was closer to that of reference Sigma–Aldrich type II collagen which contains more imino acid. Analysis by differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) revealed that type II collagen from chick sternal cartilage retains more intermolecular crosslinks during the purification process. Collagen purified from chick sternal cartilage was typical type II collagen and may find applications in functional foods.


*Keywords:* Type II collagen; Purification; Sternal cartilage; Characterization; Secondary structure

## 1. Introduction

Type II collagen is the main structural component of cartilage and, together with other tissue-specific collagens and proteoglycans, provides the tissue with its shock-absorbing properties and its resiliency to stress (Gelse, Poschl, & Aigner, 2003). Type II collagen with specific molecular structure is used in various food applications (clarification agent, emulsifier, or whipping agent). Its usage extends even further to other industrial (shampoo and lipstick) and pharmaceutical applications (tissue engineering material, microencapsulation, or tablet coating). Today, there is an increasing demand for type II collagen as research suggests that type II collagen can suppress Rheumatoid arthritis (RA) and promote healthy joints as superior dietary supplement products (David, Alexander, & Andrew, 1977; David & Roselyn, 1993; Takashi, Akio, & Satoshi, 1998).

Type II collagen, traditionally, has been extracted from bovine or porcine articular cartilage. Chick sternal cartilage containing a high amount of collagen, one of the by-products of chick manufacturing industry is recognized as a potential source of type II collagen. However, most chick sternal cartilage is conventionally used to produce animal feed or is directly discharged into estuaries resulting in environmental pollution. Thus, new strategies must be explored to find a way of upgrading the processing of waste to value added products such as type II collagen.

Type II collagen has been extracted from articular cartilage. The result indicates that the functional properties of type II collagen are highly influenced by its molecular structure (Miller, 1971; Rigo, Hartmann, & Bairati, 2002). In general, the telopeptide of type II collagen is thought to be responsible for causing an immunogenic response when introduced into xenogenic hosts (Takaoka, Koezuka, & Nakahara, 1991). To eliminate this problem, pepsin has been applied to solubilize collagen and remove telopeptides. Ramesh and Sehgal (1991) described the procedure that involved suspending tissue (200 g wet weigh) in

* Corresponding author. Tel./fax: +86 510 85884496.
*E-mail address:* syxu@sytu.edu.cn (S.-Y. Xu).

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1.5 L of 0.5 M acetic acid mixed with 100 mg pepsin and incubation at 1 °C for 48 h with stirring. Vasantha, sehgal, and Rao (1988) used 0.001 M hydrochloric acid for preparation of telopeptide-poor collagen by treatment with pepsin (approximate ratio of enzyme to collagen was 1:400) at 20 °C with intermittent stirring for 5 days. The main difficulty, however, with all these techniques, which involve various different digestion conditions of time, temperature and pepsin concentration, is that they can not ensure the quality of the pepsin-solubilized type II collagen isolated from cartilage. Circular dichroism (CD) is particularly useful for analyzing collagen and associated degradation products, who are able to assign the secondary structure of type II collagen (Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003; Usha & Ramasami, 2005).

This paper describes the effect of temperature, time and pepsin concentration on the yield and secondary structure of type II collagen, with the aim of producing extracted protein with minimal changes to its functional properties. Further, some biochemical characterizations of type II collagen from chick sternal cartilage are also assessed.

## 2. Materials and methods

### 2.1. Materials

Chick sternal cartilage was provided by Nanjing YuRun Co., Ltd. (Nanjing, China), and stored in refrigerator at −20 °C until use. Resins of DEAE-sepharose CL 6B were purchased from Pharmacia Biotech (Uppsala, Sweden). Standard protein (e.g., myosin heavy chain 200 kDa, Camodulin-binding protein, 130 kDa, Rabbit Phosphorylase b, 97.4 kDa; bovine serum albumin, 66.2 kDa; rabbit actin, 43 kDa) for sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) were obtained from Shanghai Huamei Biotech (Shanghai, China). Standard type II collagen and pepsin (EC 3.4.23.1) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were in reagent grade or higher.

### 2.2. Pretreatment of chick sternal cartilage

The sternal cartilage of chicks were cleaned to remove adhering tissue and washed thoroughly with water. The cartilage was cut into small pieces and defatted with chloroform–methanol (2:1, v/v). After the pieces of tissue were cleaned with deionized water, then the chloroform–methanol-free pieces were stored at −20 °C until use.

### 2.3. Preparation of pepsin-solubilized type II collagen

#### 2.3.1. Digestion test

Sternal cartilage was homogenized at 10,000 rpm for 10 min using 1000 ml 0.2 M NaCl in 0.05 M Tris–HCl (pH 7.5). The mixture was then extracted using 1.0 M NaCl in 0.05 M Tris–HCl (pH 7.5) at 4 °C for 24 h. After the extracts were aggregated by centrifugation at 8000*g* at 4 °C, the digestion was tested by mixing precipitation with pepsin to assess the effect of temperature (4 °C, 20 °C and 37 °C ), times (16 h, 32 h and 48 h) and the ratio of enzyme to precipitation (1:100, 1:200 and 1:400) on the yield and secondary structure of type II collagen. The resulting viscous solution was centrifuged at 10,000*g* for 30 min to remove insoluble substances. NaCl was added to a final concentration of 0.9 M, and the collagen was allowed to precipitate for 16 h. The precipitated collagen was dissolved in 0.5 M acetic acid (pH 2.5) and aggregated by dialysis against 0.02 M phosphate buffer (pH 7.4), then lyophilized. The lyophilized collagen was stored in desiccator placed in a refrigerator (4 °C), until used.

#### 2.3.2. The yield of pepsin-solubilized type II collagen

The yield of pepsin-solubilized type II collagen with different digestion conditions was monitored by the content of Hydroxyproline. The percentage (%) of hydroxyproline in the collagen was determined using the method of Reddy and Enwemeka (1996).

#### 2.3.3. The secondary structure of pepsin-solubilized type II collagen

Circular dichroism (CD) spectra were applied to assess the secondary structure of pepsin-solubilized type II collagen from the different digestion conditions. The type II collagen was diluted using 0.05 M acetic acid and then the solution placed into a quartz cell with a path length of 1 mm. CD spectra measurements were performed and the wavelengths 250–190 nm with a scan speed of 100 nm/min at an interval of 1.0 nm. A reference spectrum containing acetic acid was also recorded. The CD spectra of the samples were obtained after subtracting the reference spectrum. The data were accumulated three times.

### 2.4. Purification of type II collagen

#### 2.4.1. DEAE-sepharose CL 6B ion exchange chromatography

The lyophilized pepsin-solubilized type II collagen was dissolved in 0.05 M acetic acid and dialyzed overnight at 4 °C against 100 volumes of 0.2 M NaCl (0.05 M Tris–HCl, pH 7.5). During dialysis, the solution within the dialysis tubing remained clear. Following dialysis, 3 ml of extract were loaded onto the 1 × 20 cm column of DEAE-sepharose CL 6B equilibrated with 0.2 M NaCl (0.05 M Tris–HCl, pH 7.5). The flow rate of column was maintained 0.6 ml/min. The column effluent was monitored and recorded at 280 nm. After application of the sample to the column, elution with 0.2 M NaCl (0.05 M Tris–HCl, pH 7.5) was continued until no further ultraviolet-absorbing material was detected in the effluent. At this time, the eluting solvent was changed to 1.0 M NaCl (0.05 M Tris–HCl, pH 7.5) and elution with the latter buffer was continued until an additional peak was eluted from the column. The column was reequilibrated with the starting buffer and was ready for reuse.

#### 2.4.2. NaCl precipitation

The fraction of unretained DEAE-sepharose were pooled and concentrated using 3 M NaCl precipitation. The precipitate was collected by centrifugation, redissolved in 0.5 M acetic acid, dialyzed against a large volume of the same solvent, and then lyophilized.

#### 2.4.3. Polyacrylamide gel electrophoresis

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed for the determination of purity of type II collagen as described by Laemmli (1970), using a 5% (w/v) stacking gel and a 7.5% (w/v) separating gel. The samples were prepared by mixing the purified collagen at a 1:1 (v/v) ratio with distilled water containing 10 mM Tris–HCl pH 6.8, 2.5% SDS, 10% glycerol, 5% β-mercaptoethanol and 0.002% bromophenol blue. Gels were stained with protein Coomassie brilliant blue R250. The molecular weight of the collagen was estimated using a high molecular weight calibration kit as marker.

#### 2.4.4. Reversed-phase high performance liquid chromatography (RP-HPLC)

Prior to HPLC analysis, the purified type II collagen was diluted to 4.5 mg/ml using 0.5 M acetic acid (pH 2.5) and filtered through a 0.25 μm cellulose acetate membrane filter. Chromatography was performed on an Agilent 1100 HPLC system (Aglient, Palo Alto, CA, USA) with an ultraviolet detector. The reversed phase column was a 4.6 mm × 250 mm ZORBAX 300 SB (Aglient, Wilmington, DE, USA) with an injection volume of 20 μl. The mobile phase consisted of two solvent: (A) 5% acetonitrile and 0.05% trifluoroacetic acid (TFA) and (B) 80% acetonitrile (v/v). The separation was performed using the linear gradient of A–B (v/v). The flow rate was maintained 1 ml/min, and an absorbance was monitored at 220 nm.

### 2.5. Biochemical properties of type II collagen

#### 2.5.1. Amino acid analysis

For amino acid analysis, the collagen purified from chick sternal cartilages was hydrolyzed with 6 M hydrochloric acid for 24 h at 120 °C. The resulting mixture was analyzed by an Agilent 1100 HPLC system (Aglient, Palo Alto, C, USA) following online derivatisation with O-phthalaldehyde and 9-fluorenylmethoxycarbonyl (Sigma Chemical Co., St. Louis, MO, USA) for proline.

#### 2.5.2. Denaturation temperature ($T_d$)

The $T_d$ was determined by means of a differential scanning calorimetry (Perkin–Elmer Co., Norwalk, CT, USA). Purified type II Collagen and intact cartilage were immersed in deionized water at 4 °C for 16 h. The wet samples were wiped with filter paper to remove excess water and hermetically sealed in aluminum pans. A heating rate of 5 °C/min was applied from 20 to 90 °C and the endothermic peak of the thermogram was monitored.

#### 2.5.3. Fourier transform spectroscopy (FTIR)

FTIR spectra were obtained from discs containing 2 mg samples in approximately 100 mg potassium bromide (KBr) with a Fourier transform IR instrument (Nicolet Nexus, Thermo Electron Co., Madison, WI). A spectral range of 4000–400 $cm^{-1}$ (2.5–25 μm) was analyzed and registered in the transmission mode with resolution of 2 $cm^{-1}$.

## 3. Results and discussion

### 3.1. Preparation of pepsin-solubilized type II collagen

The pepsin is applied to remove the telopeptide of the collagen because the natural polymer of telopeptide-poor collagen is of low antigenicity, biocompatible, biodegradable, and less toxic. There are several factors affecting the characteristics of telopeptide-poor collagens including the ratio of pepsin to cartilage, temperature, and digestion time (Takaoka et al., 1991). We determined the yield and secondary structure of obtained type II collagen under different extraction conditions, the results are summarized in Tables 1 and Fig. 1.

The temperature, pepsin concentration and times of digestion have a significant effect on the yield of type II collagen (Table 1). The yield for type II collagen obtained, was increased with increase in extraction times, temperature and pepsin concentration. The highest yield 62.5% of type II collagen was obtained with the extraction times for 32 h and 0.5% pepsin concentration at 37 °C.

Table 1
Concentration, temperature and times of pepsin digestion effect on the yield and secondary structure of pepsin-solubilized type II collagen[d]

| | Type II collagen yield (%) | Ellipticity (m deg) | | Secondary structure | |
|---|---|---|---|---|---|
| | | 221 nm | 198 nm | β-sheet (%) | Random coil (%) |
| *Concentration (w/v)*[a] | | | | | |
| 1 | 60.2a | 12.1a | −98.7a | 44.3a | 55.7a |
| 0.5 | 59.7a | 13.0a | −103.7a | 44.2a | 55.8a |
| 0.25 | 54.7 b | 12.8a | −99.7a | 46.6a | 53.4a |
| *Temperature (°C)*[b] | | | | | |
| 4 | 53.6a | 13.2a | −112.1a | 46.5a | 53.5a |
| 20 | 58.2a,b | 11.9a | −95.7a,b | 43.4a,b | 56.6a |
| 37 | 62.5b | 11.4a | −57.2b | 39.1b | 60.9b |
| *Times (h)*[c] | | | | | |
| 16 | 50.7a | 13.2a | −112.1a | 45.6a | 54.4a |
| 32 | 56.1a,b | 12.0a,b | −97.5a,b | 44.1a,b | 55.9a |
| 48 | 58.3b | 10.9b | −55.3b | 39.7b | 60.3b |

[a] The concentration of pepsin digestion was 0.25%, 0.5% and 1% (w/v). (32 h, 20 °C).

[b] The temperature of pepsin digestion was 4 °C, 20 °C and 37 °C, respectively. (0.5% pepsin (w/v), 32 h).

[c] The times of pepsin digestion 16 h, 32 h and 48 h, respectively (0.5% pepsin (w/v), 20 °C).

[d] Results are presented as the means ($n = 3$), where mean within a column followed by different letters are significantly different ($P < 0.05$).

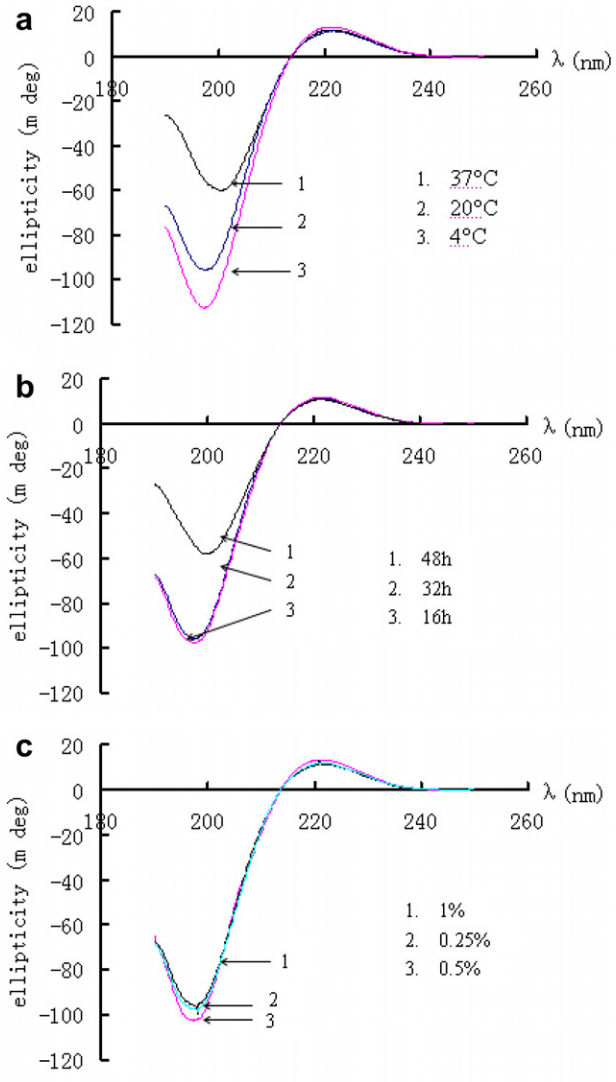


Fig. 1. CD spectra of pepsin-solubilized type II collagen treated with various conditions: (a) temperature; (b) time and (c) concentration.

CD-spectra of protein solutions provide information about secondary structure content of proteins (Kelly & Price, 1997; Usha, Maheshwan, Dhathathreyan, & Ramasami, 2006). Fig. 1 shows the CD spectra of the pepsin-solubilized type II collagen under different extraction conditions. All collagen samples showed a rotatory maximum at 221 nm (positive band), minimum at 198 nm (negative band) and a consistent cross over point (zero rotation) at about 212 nm, which was characteristic of triple helical conformation of the protein (Usha & Ramasami, 2004). The ellipticity of type II collagen deduced from CD spectra are given in Table 1. It is evident that in the presence of increasing time and temperature, there was a decrease in ellipticity at 221 nm and an increase at 198 nm, while the increase of pepsin concentration did not alter the ellipticity significantly. This can be interpreted as being due to decomposition of the collagen triple helical structure with increase in digestion temperature and time. However, it has been reported that on complete denaturation, the positive peak at 221 nm disappears completely and the negative band is found red shifted (Kwak, Jefferson, Bhumralkar, & Goodman, 1999). In this investigation, there was neither significant change in the red shift of the negative band nor any disappearance of the positive band at 221 nm. The results indicated that partially denatured collagen gave CD spectra with increasing digestion times and temperature.

The secondary structure of the pepsin-solubilized type II collagen with different extraction condition is given in Table 1. The digestion temperature and time have a significant effect on the secondary structure of type II collagen. Extraction at 37 °C resulted in 60.9% random coil compared to 53.5% at 4 °C and extraction for 48 h resulted in 60.3% random coil compared to 54.4% for 16 h. Although an increase in digestion time and temperature give higher collagen yields, the longer times and higher temperatures also result in a higher change of collagen secondary structure. This is not desirable since it causes a higher destruction of secondary structure of collagen resulting in altered functional properties. It seemed, therefore, that the pepsin-solubilized type II collagen of higher stability can be obtained with the extraction times for 32 h, pepsin concentration for 0.5% and temperature of 20 °C.

### 3.2. Purification determination of type II collagen

The purification procedures of type II collagen consisted of chromatography techniques and NaCl precipitation as outlined above in Section 2.4. DEAE-sepharose CL 6B chromatography of neutral-salt extracts of cartilage results in the fractionation of the total extract into two major components. The effluent from both peaks were dialyzed against water and lyophilized, then analyzed for hydroxyproline content. The results of the analyses showed that the collagen in the extracts, which had been eluted with 0.2 M NaCl in the first unretained fraction from the DEAE column, contained abundant hydroxyproline. On the other hand, the fraction eluted with 1.0 M NaCl did not contain any hydroxyproline. These results indicate that DEAE-sepharose CL 6B chromatography under the conditions outlined above provided a rapid and efficient method for separating collagen from proteoglycans in cartilage extracts. This is in agreement with the results of Miller (1973).

A simple RP-HPLC method was applied to determine purity of type II collagen using a ZORBAX 300 SB column (Fig. 2). Type II collagen was eluted at 10.233 min and the content was 95.4% according to RP-HPLC peak area. The results suggest that the prepared collagen essentially was free from contaminating proteins by the method used in this study for purification. This agreed with the results of Ho, Lin, and Sheu (1997). Sodium sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) is the most widely used analytical procedure for the identification of collagen chains. Fig. 3 shows the SDS–PAGE electrophoresis patterns of type II collagen from the articular cartilage, sternal

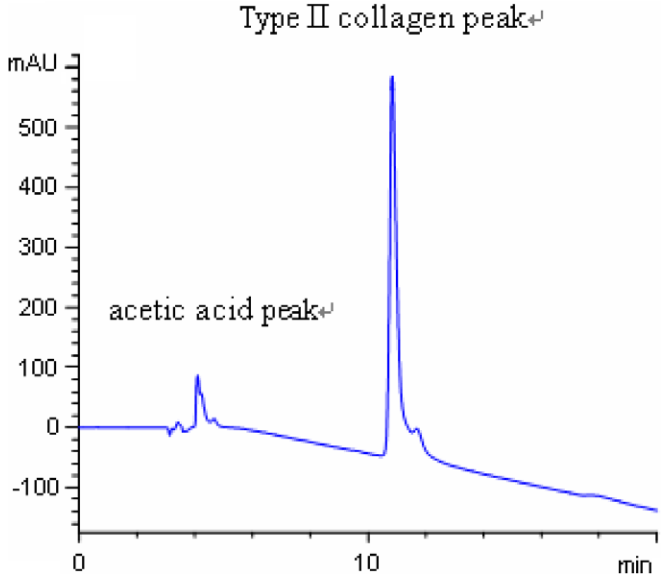


Fig. 2. RP-HPLC chromatogram for purified collagen of chick sternal cartilage.

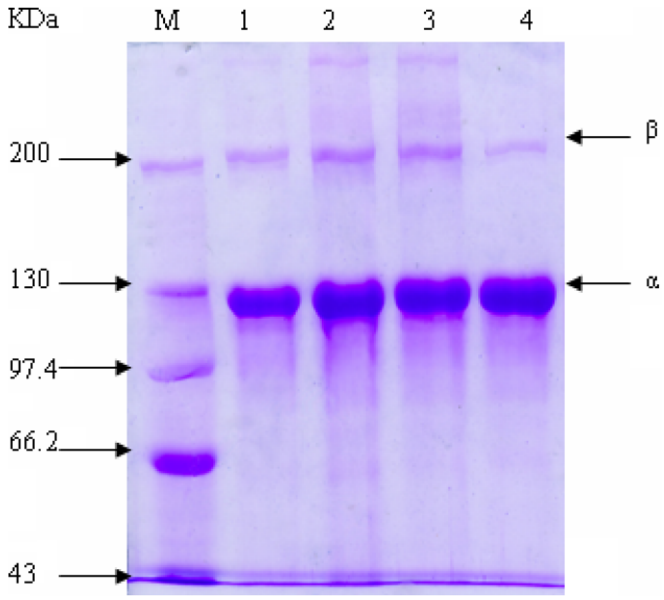


Fig. 3. SDS–PAGE profile for the Sigma–Aldrich standard type II collagen (lane 1), type II collagen purified from chick articular cartilage (lane 2), type II collagen purified from sternal cartilage (lane 4 and 3) and MW standards (lane M).

cartilage and reference Sigma–Aldrich. All these collagen samples in purified form had similar migration bands and consisted of an α-chain and their dimmers β-chains with a subunit Mr of 110 kDa. Collagen derived from chick articular cartilage has been characterized with respect to chain composition to comprise of three identical α-chains (Beck & Brodsky, 1998; Helen & Cahir, 2005; Pieper & Van der Kraan, 2002). It may be concluded, therefore, that sternal cartilage collagen is comprised of three identical α-chains with a subunit Mr of 110 kDa like articular cartilage, and collagen molecule was depicted as $[\alpha_1(II)]_3$.

### 3.3. Biochemical properties of type II collagen

#### 3.3.1. Amino acid composition

Bella, Brodsky, and Berman (1995) reported that all collagens contained a unique triple–helical conformation as the common structural element; the triple helix consisted of three left-handed polyproline II-like chains supercoiled in a right-hand manner a common axis. The stabilization of this conformation requires the occurrence of glycine residues at every third position in the sequence and the presence of a high content of imino acids. Our work detected the composition of amino acid of the chick cartilage to resemble that from the reference Sigma–Aldrich type II collagen and articular cartilage in the high content of glycine, hydroxyproline and proline residues, with 310, 117 and 115 residues per 1000 amino acids residues respectively, and small amounts of tyrosine, cysteine, histidine and methionine residues with 5, 18 and 10 residues per 1000 amino acids residues respectively (Table 2). Tryptophan residues were absent in all samples because of the hydrolysis of 6 M hydrochloric acid. From these results it can be concluded that glycine, which constitutes about 1/3 of all residues in collagen, would be present as every third residue in the sequence, and that high amounts of proline and hydroxyproline (Hyp) could be accommodated while maintaining planar peptide bonds. These assumptions led to the construction of the correct model of sternal cartilage collagen as a (Gly-X-Y) n pattern, where X and Y are frequently Pro or Hyp, respectively. Indeed, it is the most commonly found triplet in collagen chains space (Baum & Brodsky, 1990; Camilla, Karl, Jiann-Jiu, & Christopher, 1996; Nagai & Suzuki, 2000).

Table 2
Comparison of amino acid composition of type II collagen from reference Sigma–Aldrich, sternal cartilage and articular cartilage

| | Sigma residues/1000 residues | Articular collagen residues/1000 esidues | Sternal collagen residues/1000 residues |
|---|---|---|---|
| Aspartic acid/ asparagine | 47 | 45 | 46 |
| Glutamic acid/ glutamine | 94 | 88 | 85 |
| Serine | 25 | 24 | 22 |
| Histidine | 4 | 4 | 4 |
| Glycine | 313 | 309 | 310 |
| Threonine | 30 | 31 | 26 |
| Alanine | 102 | 99 | 104 |
| Arginine | 53 | 50 | 52 |
| Tyrosine | 5 | 4 | 5 |
| Cysteine | 17 | 18 | 18 |
| Valine | 22 | 19 | 19 |
| Methionine | 2 | 8 | 10 |
| Phenylalanine | 15 | 14 | 15 |
| Isoleucine | 13 | 10 | 11 |
| Leucine | 31 | 28 | 27 |
| Lysine | 15 | 12 | 14 |
| proline | 94 | 116 | 115 |
| Hydroxyproline | 118 | 121 | 117 |

### 3.3.2. Denaturation temperature ($T_d$)

Denaturation temperature has been used as an effective guideline for assessing the stability of type II collagen. The $T_d$ was determined to be about 43.8 °C for purified type II collagen and about 44.1 °C for intact cartilage (Table 3). The minimal difference between denaturation temperature of purified type II collagen and intact cartilage indicate that purification process has little influence on stability of the collagen. The values of $\Delta H$ for intact cartilage and purified collagen were 0.5 J/g and 0.3 J/g, respectively. The values are not in direct agreement with the $T_d$ because the enthalpy changes associated with the collagen denaturation processes depend on the positional preferences of ionized residues in Gly-X-Y and the formation of hydrogen bonds in the inner coil-coiled α-chains (Rossi, Zanaboni, Cetta, & Tenni, 1997; Usha & Ramasami, 2004).

### 3.3.3. Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy has been used to study changes in the secondary structure of collagen (Friess & Lee, 1996). The IR spectra of purified type II collagen from sternal cartilage obtained is presented in Fig. 4. The main absorption bands are amide A (3309 $cm^{-1}$), amide I (1658 $cm^{-1}$), amide II (1552 $cm^{-1}$) and the amide III (1240 $cm^{-1}$).

Absorption peak of amide A for purified type II collagen (3309 $cm^{-1}$) had a slight shift to lower wave number compared to other proteins (3400–3440 $cm^{-1}$). The hydrogen bonds formed between N–H groups and other groups, which stabilize the helix structure, are considered to be the major factors for the shift of wave number (Muyonga, Cole, & Duodu, 2004).

Table 3
The denaturation temperature ($T_d$) and total denaturation enthalpy ($\Delta H$) for purified type II collagen and intact cartilage[a]

| Samples | $T_d$ (°C) | $\Delta H$ (J/g) |
|---|---|---|
| Intact cartilage | 44.1 | 0.5 |
| Type II collagen | 43.8 | 0.3 |

[a] Values are means from duplicate determination.

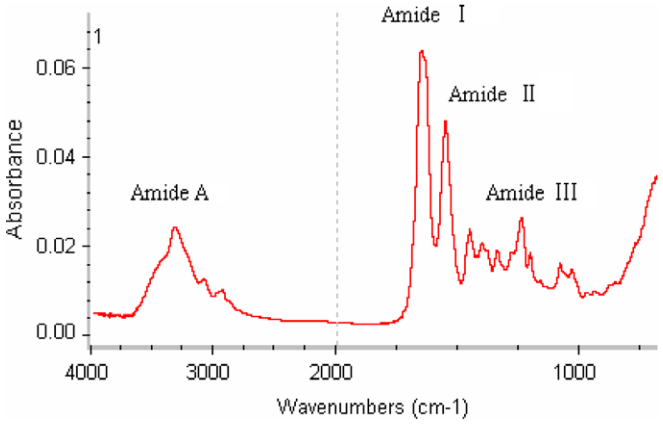


Fig. 4. The FTIR spectra of purified type II collagen.

The amide I band, between 1600 and 1700 $cm^{-1}$, is the most useful for infrared spectroscopic analysis of the secondary structure of proteins (Surewicz & Mantsch, 1988). The bands around 1630, 1660 and 1675 $cm^{-1}$ have been assigned to imide residues, intermolecular crosslinks and β-turns, respectively (Prystupa & Donald, 1996), while the random coils state is reported to show at lower wave numbers (Payne & Veis, 1988). The purified type II collagen from chick sternal cartilage was found to exhibit high intensity peaks at around 1658 $cm^{-1}$. It seemed, therefore, that type II collagen extracted retained more intermolecular crosslinks during the purification process compared to the intact cartilage.

The bands in spectral region between 1200 and 1350 $cm^{-1}$ are sensitive to the collagen molecular conformation and is also called "fingerprint" region, because changes in bands of this region are attributed to particular tripeptides $(Gly\text{-}Pro\text{-}Hyp)_n$ of collagen. The collagen band of sternal cartilage assignment for the region between 1200 and 1350 $cm^{-1}$ were: $CH_2$ deformation at 1339 $cm^{-1}$; $C(CH_2)$ twisting at 1315 $cm^{-1}$; CN stretching and NH deformation at 1240 $cm^{-1}$. This is in agreement with the result of Bachmann, Gomes, and Zezell (2005).

## 4. Conclusions

Based on the digestion test, it may be concluded that time, temperature and pepsin concentration are very important for yield and secondary structure of type II collagen. Although an increase in time, temperature and pepsin concentration give higher collagen yield, longer time and higher temperature also resulted in a higher change in the secondary structure of collagen. Based on the electrophoretic profile and amino acid composition, it may be concluded that collagen from chick sternal cartilage, like articular cartilage, is type II collagen which is comprised of three identical α-chains, and the collagen molecule is depicted as $[\alpha_1(II)]_3$. The minimal difference between denaturation temperature of purified type II collagen and intact cartilage indicate that purification process has little influence on stability of the collagen. The FTIR spectra indicate that type II collagen from chick sternal cartilage retains more intermolecular crosslinks during the purification process. Data generated by this study indicates that sternal cartilage has potential as a source of type II collagen.

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