Partial Purification and Quantification of Insulin-like Growth Factor-I from Red Deer Antler

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Deer antler tissue contains the most rapidly growing bone in the animal kingdom. Thus, it is likely that growing antler tissue is a rich source of local paracrine bone-stimulating factors. Growth factors, at least the insulin-like growth factor (IGF), control the bone-remodelling process. In this study, we tried to isolate and purify IGF-I from fresh antler tissue by the routine isolation and purification of protein. The purification involved ammonium sulfate precipitation, DEAE-Sepharose CL-6B ion-exchange chromatography, CM-Sepharose CL-6B ion-exchange chromatography, and Sephadex G-50 chromatography. Purified fractions from each step were analyzed by high-performance liquid chromatography (HPLC), SDS polyacrylamide gel electrophoresis (SDS-PAGE), Dot-blot, and Western-blot methods. Furthermore, the quantification of partially purified IGF-I was calculated by enzyme-linked immunosorbent assays (ELISA) using antibody to human recombinant IGF-I. SDS-PAGE analysis of the final fraction yielded two molecular bands and the signal band was at 12 kDa on the Western-blot film. This purified IGF-I fraction showed a peak at retention time of eight min. The quantity of IGF-I in 20 g deer antler tissue as starting weight was calculated using a standard curve to be 2910 ng/ml, and total IGF-I amount is 0.291 g. The results show that IGF-I, which can be found in deer antler, can be partially purified and quantified by classic protein isolation methods.

Key words – Deer antler, insulin-like growth factor (IGF), DEAE-Sepharose CL-6B, CM-Sepharose CL-6B, Sephadex G-50, HPLC, ELISA

Introduction

The regulation of growth is a complex process involving the interaction of a wide variety of systems. Insulin-like growth factors (IGFs) are ubiquitously expressed and are important mitogens that affect cell growth and metabolism [12]. Most growth-promoting effects of IGF are mediated by the IGF receptors. The mitogenic effects of IGF are mediated mainly through interactions with the type I IGF receptor, which, like the insulin receptor, is a receptor with tyrosine-kinase activity. IGF binding to the receptor results in the activation of the tyrosine-kinase system and regulation of cellular growth, differentiation, and apoptosis [2]. Therefore, the IGFs and their cognate receptors and binding proteins (IGFBPs) play a central role in the endocrine regulation of growth and development in mammals.

IGFs, including IGF-I and IGF-II, are evolutionarily ancient hormones/growth factors that are present in all vertebrates and are essential for normal growth and development [4]. IGF-I, a 7.6 kDa, 70-amino-acid polypeptide with three internal disulfide bonds, regulates cell proliferation and differentiation in a variety of cell types by endocrine, autocrine, and paracrine mechanisms. All of the growth-promoting actions of growth hormone (GH) are mediated by IGF-I [19]; the physiological actions of both IGFs are mediated by interactions with cell-surface receptors, which have protein kinase activity, and the actions of IGF-I are also controlled by six types of IGF-binding proteins [16]. IGF-I was first isolated from human serum [18]. Previous studies have isolated and partial characterized similar IGFs in various body fluids and tissues of numerous species such as IGF from fetal rat calvariae [5], IGF-like peptides from Cohn fraction IV of human plasma [21], and IGF-binding protein in rainbow trout [4], and biological activity of insulin-like growth factor-I has been identified in chicken serum [1,3].

The deer antler represents the most rapidly growing form of bone in mammalian tissues. It has an annual cycle of regeneration and grows very rapidly from the tip at up to 1 cm/day in red deer for a 90-120-day period [8]. Some of the mediators supporting this rapid growth are thought

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to be the local growth factors and growth-factor receptors. These growth factors, at least the insulin-like growth factor (IGF), have already been shown to be important for the rapid growth and differentiation of antler tissue [6]. It has been shown that several growth factors are expressed in developing antlers, including increased levels of IGF-I [7]. IGF-I was also suggested to be an antler-stimulating hormone that affects cartilage growth. This was demonstrated by measuring the plasma levels of IGF-I, which are significantly increased during the velvet antler growing phase [20]. The growing antler tip is composed of fibroblasts that divide and differentiate into chondroblasts and eventually osteoblasts. As IGF-I stimulates cartilage proteoglycan synthesis in vitro, it has been suggested that the extracellular matrix has an important role in cellular development and maturation, particularly for chondroblasts [6].

The objective of our study was to purify IGF-I, one of the biological activities of known deer antler growth factors, and produce evidence for the presence of IGF-I in antlers by purification and quantification.

Materials and methods

Protein extraction

Fresh red deer *(Cervus elaphus L.* 1758) antlers were collected from an anesthetized four-year-old red deer stag from a local deer farm during the late spring (Jinshan, Korea).

Fresh antler tissues (tip) (20 g samples) were homogenized in a Waring blender. The homogenized antler tissues were crushed in 500 ml of 0.02 M NaAc-HAc (acetic acid—sodium acetate) buffer (pH 5.5) with a mortar and pestle. The suspension supernatant was filtered and concentrated by dialyzing in polyethylene *8000* (Sigma, USA) to 100 ml and stored at -20°C.

Ammonium sulfate precipitation with solid ammonium sulfate

To identify the ammonium sulfate fraction with the highest IGF-I concentration, ammonium sulfate gradient precipitation was performed by bringing it to 30% saturation with solid ammonium sulfate (Daejung, Japan) [10]. The solution was stirred for at least 1 hr at 4°C and allowed to stand at 4°C overnight. The turbid solution was centrifuged at 12,000×g, 4°C for 15 min, the pellet was dissolved in 0.05 M Tris-HCl buffer (pH 9.0), and the supernatant was brought to 40% saturation by the addition of

solid ammonium sulfate and allowed to stand at 4°C overnight. The same steps were repeated by adding solid ammonium sulfate to obtain final saturated concentrations of 50%, 60%, 70%, and 85%. The mixture was then centrifuged at 10,000×g for 20 min. The final supernatant of 80% saturation was discarded and the pellet redissolved in 0.05 M Tris-HCl buffer (pH 9.0) and extensively dialyzed using 12-14-kDa dialysis tubing (Spectra/Por1, USA) to remove SO_4^{2-} and NH_4^+. The dialyzed solution was analyzed by Western-blot assay and prepared for further purification.

DEAE-Sepharose CL-6B ion-exchange chromatogram

DEAE-Sepharose CL-6B ion-exchange resin (Pharmacia, Sweden) was pre-equilibrated in 0.05 M Tris-HCl buffer (pH 9.0) until the liquid reached the same pH value. The total protein (2.6 g) collected from ammonium sulfate precipitation was applied onto a 5×30 cm column (Bio-Rad, USA). The column was washed with two volumes of the starting buffer at a flow rate of 1 ml/min and protein separation elution was achieved by stepwise increasing the NaCl concentration to 0 M, 0.2 M, 0.4 M, 0.6 M, 0.8 M, 1 M, and 1.2 M in 0.05 M Tris-HCl buffer (pH 9.0) until the substance was completely removed. 10-ml fractions were collected at 4°C. The Bradford method (Bio-Rad, USA) was used to measure protein concentrations at 595 nm absorption. Fractions were analyzed by 12% SDS-PAGE and Dot-blot assays. More concentrated samples were freeze-dried to powder form by a lyophilizer and stored at -20°C.

CM-Sepharose CL-6B ion-exchange chromatogram

Fractions collected from the first column detected by Dot-blot assays were combined and loaded on the second column (42×50 cm) with CM-Sepharose 6B-CL (Pharmacia, Sweden). The columns had been equilibrated previously with 0.05 M NaAc-HAc buffer (pH 4.5). The column was eluted with 0.05 M NaAc-HAc buffer (pH 4.5) containing a linear gradient of 0.1-0.5 M NaCl. The eluate was collected in 5-ml fractions. The protein concentration in the fractions was estimated by absorbance at 595 nm with the Bradford method, analyzed by 12% SDS-PAGE, HPLC, and Western-blot assays. Collected fractions were concentrated by a lyophilizer and stored at -20°C.

Sephadex G-50 chromatography

The lyophilized powder obtained and analyzed by

SDS-PAGE and Western-blot assays was dissolved in 50 mM NaAc-HAc (pH 5.0) buffer to a total volume of 1 ml and centrifuged at 10,000×*g* for 15 min. The solution was loaded onto a column (1.2×120 cm) with a Sephadex G-50 gel filtration (Sigma, USA), eluted with 50 mM NaAc-HAc (pH 5.0) at a flow rate of 0.6 ml/min. The elution was collected in 6-ml fractions and the Bradford method was used to measure protein concentration at 595 nm absorption. Fractions were separated by 12% SDS-PAGE, HPLC, and Western-blot assay, freeze-dried using a lyophilizer and stored at -20°C.

Detection of fractions by RP-HPLC

Fractions collected from CM-Sepharose CL-6B ion-exchange and Sephadex G-50 chromatography were detected by Western-blot analysis. The fractions with the immunoactivity were combined and subjected to RP-HPLC on a C18 column (SHIMADZU, Japan, ODS 0.5 μm, 0.46×25 cm). Elution was performed with a linear gradient of 20-40% ACN in 0.1% TFA-acidified water for 30 min at a flow rate of 0.8 ml/min. The sample was monitored by UV absorbance at 280 nm.

Dot blotting of IGF-I

The fractions collected from DEAE-Sepharose 6B-CL column were analyzed by Dot-blot assays to filter the fractions with IGF-I according to the Bio-Dot microfiltration apparatus instructions (Bio-Rad, USA). A nitrocellulose membrane (Millipore, USA) was pre-soaked in 1×Tris-buffered saline (TBS) binding/wash buffer (20 mM Tris-HCl, pH 7.5, and 0.5 M NaCl). The wet membrane was loaded onto a Dot-blot manifold, and the wells to be used were washed again with a binding/wash buffer under vacuum. Samples of 500 μl were loaded into the wells and the entire sample was allowed to filter through the membrane by gravity. A blocking solution (1% bovine serum albumin in TBS) was added to each well and gravity filtration allowed to occur until the blocking solution was completely drained from each well. The membrane was vacuum-washed three times with a solution of TTBS (0.05% Tween 20 in TBS) and a 1:100 dilution of specific IGF-I polyclonal immunoglobulin G (IgG; Santa Cruz Biotechnology, USA) was added to each well and gravity filtered. This was followed by vacuum washing three times with TTBS and incubated with 1:3000 anti-rabbit IgG hoseradish-peroxidase-linked whole second antibody (Amersham, USA).

After washing, the membrane was removed from the microfiltration apparatus and analyzed by chemiluminescence using the enhanced chemiluminescence (ECL) Advance Western-blot detection kit (Amersham, USA).

SDS-PAGE and Western-blot

During the purification, the purity and the amount of protein were monitored by 12% SDS-PAGE (Mini Protean II; Bio-Rad). Gels were stained with Coomassie Brilliant Blue R-250 or silver. For Western-blot analysis, proteins were electro-transferred to a nitrocellulose membrane pre-soaked with transfer buffer (Millipore, USA) at 90 mA for 2.5 hr. The membrane was blocked overnight at 4°C with an ECL blocking agent containing 5% skimmed milk (Amersham, USA) in 1×TTBS (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 0.5% Tween 20). Immunodetection was performed by incubation with a 1:500 dilution of specific IGF-I polyclonal IgG (Santa Cruz Biotechnology, USA), which is an affinity-purified polyclonal antibody raised against a peptide that maps to the N-terminus of goat IGF-I, for 2 hr at room temperature. This was followed by incubation for 1 hr at room temperature with ECL anti-rabbit IgG hoseradish-peroxidase-linked whole second antibody (Amersham, USA) at 1:3000 dilution in a blocking solution. The membranes were washed in TTBS after incubation with each antibody. Antigens were detected by chemiluminescence using an ECL Advance Western-blot detection kit (Amersham, USA).

Quantification of insulin-like growth factor-I by enzyme-linked immunosorbent assay (ELISA)

After eluted from Sephadex G-50, the fractions with IGF-I-binding activity by Western-blot analysis were freeze-dried to powder form and weighed, and 50 mg was the dissolved in 1 ml distilled water. Wells of ELISA plates (human IGF-I immunoassay, R&D Systems, USA) were coated with 150 μl assay diluent and 50 μl of isolated fraction and human recombinant IGF-I standard (6, 3, 1.5, 0.75, 0.375, 0.188, or 0.094 ng/ml), and incubated for 2 hr at 4°C. Each well was washed three times with the wash buffer and 200 μl IGF-I conjugate was added to each well and incubated for 1 hr at 4°C. The wash steps were repeated and 200 μl substrate solution was added to each well and incubated for 30 min at room temperature in the dark. After incubation, 50 μl stop solution was added to each well and the optical density (OD) at 450 nm was determined within

30 min. A standard curve was generated and the concentration of insulin-like growth factor-I was calculated from the standard curve.

Results

Precipitation with solid ammonium sulfate

Precipitation of the antler protein extract was performed with solid ammonium sulfate from 30% to 80% saturated concentration. Western-blot detection showed that only the precipitation fractions with 40%, 70%, and 80% saturated solid ammonium sulfate had IGF-I-binding immunoactivity (Fig. 1A). These three fractions were combined for chromatographic purification.

IGF-I purification by chromatogram columns

IGF-I was partially purified by four purification steps: $(NH_4)_2SO_4$ precipitation, DEAE-Sepharose CL-6B column chromatography, CM-Sepharose CL-6B ion-exchange column chromatography, and Sephadex G-50 column chromatography. With DEAE-Sepharose CL-6B column chromatography using 0.05 M Tris-HCl buffer (pH 9.0) with a NaCl gradient from 0 to 1.2 M NaCl, the antler protein extract was eluted with six peaks as shown in Fig. 2. All the fractions collected were detected using the Dot-blot method and the fractions with the immunoactivity were combined for the CM-Sepharose CL-6B column chromatography. Proteins eluted from the CM-Sepharose CL-6B
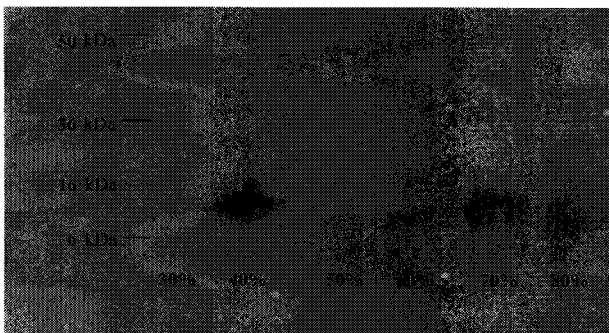

Fig. 1. Western-blot of fractions from ammonium sulfate precipitation. 40%, 60%, and 70% saturated concentration precipitation showed the blot bands. The proteins of each precipitation were separated by 12% SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was incubated with specific IGF-I polyclonal IgG, and then incubated with anti-rabbit IgG hoseradish-peroxidase-linked whole second antibody. Antigens were detected by chemiluminescence with an ECL Advance Western-blot detection kit.

column with 0.05 M NaAc-HAc buffer (pH 4.5) containing a linear gradient of 0.1-0.5 M NaCl formed four peaks (Fig. 3). Western-blot analysis showed that peak 2 and peak 3
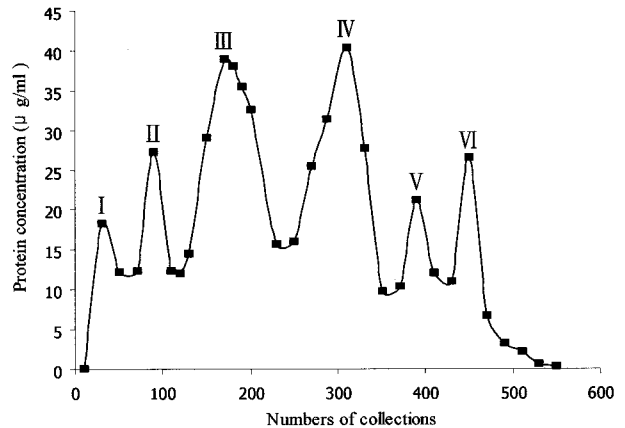

Fig. 2. Elution profile of antler protein with IGF-I from DEAE-Sepharose CL-6B ion-exchange chromatography. Fractions of ammonium sulfate precipitation with 40%, 60%, and 70% solid ammonium sulfate was loaded on the DEAE-Sepharose CL-6B column. The column was washed with 1 M NaCl in 0.05 M Tris-HCl buffer (pH 9.0) at a rate of 1 ml/min by stepwise increases in the NaCl concentration of 0 M, 0.2 M, 0.4 M, 0.6 M, 0.8 M, and 1 M NaCl. 10-ml fractions were collected at 4°C. Protein concentrations were measured at 595 nm absorption by the Bradford method. IGF-I immunoctivity was detected by the Dot-blot method.
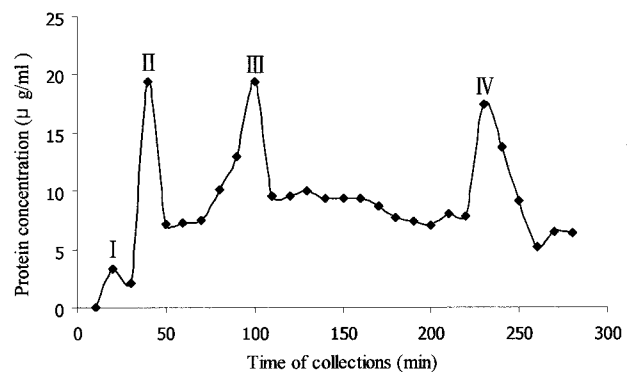

Fig. 3. Elution profile of antler protein with IGF-I from CM-Sepharose CL-6B ion-exchange chromatography. Fractions with IGF-I immunoactivity detected by Dot-blot from DEAE-Sepharose CL-6B ion-exchange column chromatography were combined and loaded onto the CM-Sepharose CL-6B ion-exchange column. The column was eluted with a 0.05 M NaAc-HAc buffer (pH 4.5) containing a linear gradient of 0.1-0.5 M NaCl. 5-ml fractions were collected. The protein concentration in the fractions was estimated by absorbance at 595 nm by the Bradford method and analyzed by 12% SDS-PAGE, HPLC, and Western-blot assays.
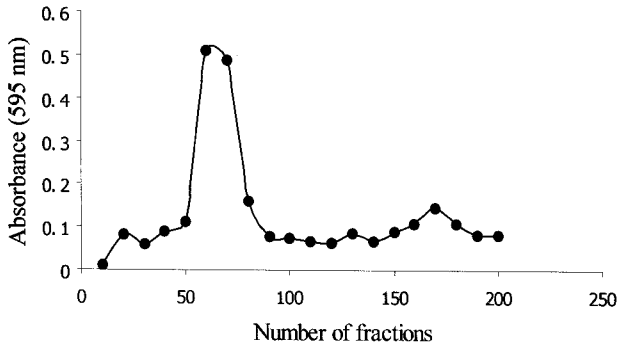
Fig. 4. Elution profile of antler IGF-I from Sephadex G-50 chromatography. Fractions 2 and 3 with IGF-I immunoactivity detected by Western-blot assay from the CM-Sepharose CL-6B ion-exchange column were combined and loaded onto the Sephadex G-50 column. Fractions were eluted with 50 mM NaAc-HAc (pH 5.0) at a flow rate of 0.6 ml/ min and 6-ml fractions were collected. The Bradford method was used to measure protein concentration at 595 nm absorption. Fractions were analyzed by 12% SDS-PAGE, HPLC, and Western-blot assays.

contained the IGF-I protein. These two fractions were combined and analyzed by HPLC to confirm the protein profile. Fig. 4 shows the elution peak from the Sephadex G-50 column with 50 mM NaAc-HAc (pH 5.0) at a flow rate of 0.6 ml/min. The signal peak was shown to contain the IGF-I protein by Western-blot analysis, in which the blot band is about 12 kDa. The HPLC protein profile showed that the fraction that contained the IGF-I protein was eluted at the retained time of eight min, which is the same retention time as the fractions from CM-Sepharose CL-6B.

SDS-PAGE and Western-blot analysis

The fractions eluted from the CM-Sepharose CL-6B ion-exchange and Sephadex G-50 column were analyzed by electrophoresis. The SDS-PAGE profile of the aliquots of the peak fractions obtained from the CM-Sepharose CL-6B ion-exchange column is shown in Fig. 5A. Further analysis of the peak fractions eluted with 50 mM NaAc-HAc (pH 5.0) from the Sephadex G-50 column showed two bands detected by silver staining (Fig. 5B). There was a distinguishable extra band, which matches the theoretical size of IGF-I. Dot-blot (Fig. 6) and Western-blot analysis (Fig. 7) revealed the presence of a band of approximately 12 kDa in molecular size that binds to the anti-goat IGF-I and is likely to be IGF-I. The fractions collected from DEAE-Sepharose 6B-CL column were detected by Dot-blot
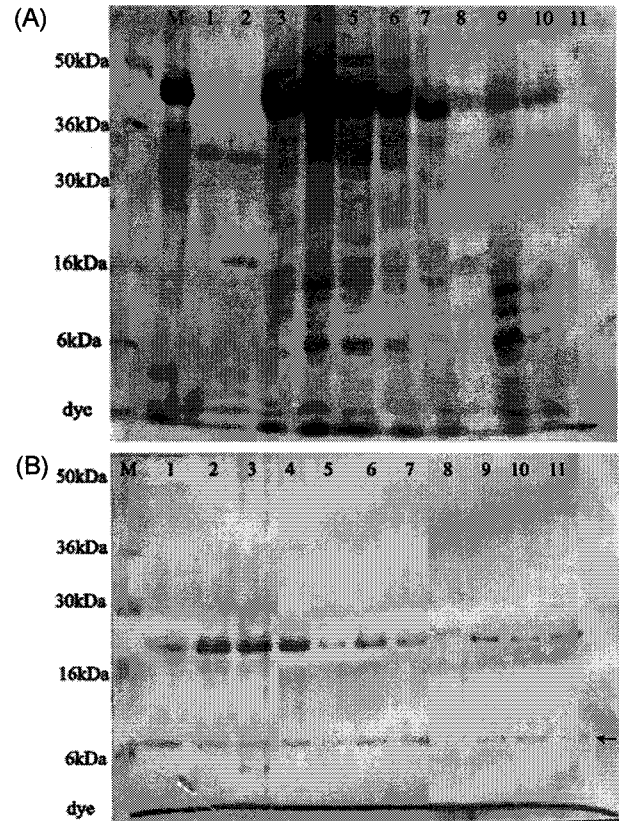

Fig. 5. SDS-PAGE analysis of protein purified from CM-Sepharose CL-6B ion-exchange column (A) and Sephadex G-50 column (B) chromatography. Proteins in each faction were separated by 12% SDS-PAGE. Gels were stained with Coomassie Brilliant Blue R-250 (A) and silver stain (B). Size markers at the left indicate migration positions and molecular weights of IGF-I. There was a distinguishable extra band at about 12 kDa, which matches the theoretical size of the IGF-I. M: size marker; 1-11: fraction 50-550 (A); M: size marker; 1-11: fraction 50-100 (B);

analysis; positive immunoblot spots are indicated by squares in Fig. 6. Two peaks from the CM-Sepharose CL-6B ion-exchange column, peak 2 and peak 3, were shown to be positive immunoblots (Fig. 7A), and the fraction from Sephadex G-50 column also contained a band with a molecular size of 12 kDa (Fig. 7B).

HPLC analysis

Partially purified IGF-I was detected by reverse-phase HPLC (Fig. 8). The peak appears at retention time of eight min in fractions from both the CM-Sepharose CL-6B (arrow in Fig. 8A) and Sephadex G-50 columns (arrow in Fig. 8B). Analysis of the fractions eluted from the C_{18} column indicated that IGF-I had been isolated by this series of chromatography steps.
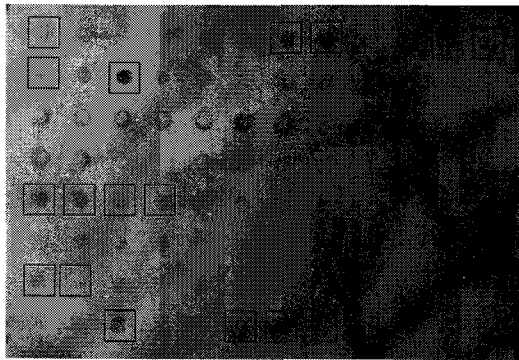
Fig. 6. Dot-blot of fractions collected from the DEAE-Sepharose CL-6B column chromatography isolation step. 500 μl fractions collected from DEAE-Sepharose 6B-CL column were loaded onto the Bio-Dot microfiltration apparatus instructions with a pre-wet nitrocellulose membrane. A blocking solution was added to each well and allowed the gravity filtration complete. A 1:100 dilution of specific IGF-I IgG was added to each well and gravity filtered. Followed by incubation with 1:3000 anti-rabbit IgG hoseradish-peroxidase-linked whole second antibody. Removed the membrane from the microfiltration apparatus and the blot was detected by chemiluminescence using the ECL Advance Western-blot detection kit. The positive spots are indicated by squares.

Enzyme-linked immunosorbent assay and quantification of IGF-I

To assess the biological binding characteristics and quantification of this purified IGF-I, we measured the level of immunoreactive IGF-I in purified fractions from the last Sephadex G-50 column by comparison with human recombinant IGF-I as a standard. A standard curve of human IGF-I concentration was made according to a serial dilution of standard human IGF-I (Fig. 9). The level of IGF-I in antlers was calculated as 2190 ng/ml using the standard curve, and the total IGF-I amount is 0.291g.

Discussion

In this report, we describe the purification and characterization of IGF-I from the fresh antler tip and have demonstrated the presence of multiple growth factors in the growing deer antler, which supports the hypothesis that paracrine/autocrine stimulation is important for regulating antler growth. Deer velvet has long been used in traditional Chinese medicine for treating diseases, strengthening the body, and healing chronic wounds. The activities attributed to the antler extracts are similar to those of growth factors [9]. The annual growth of antlers in male deer is a unique
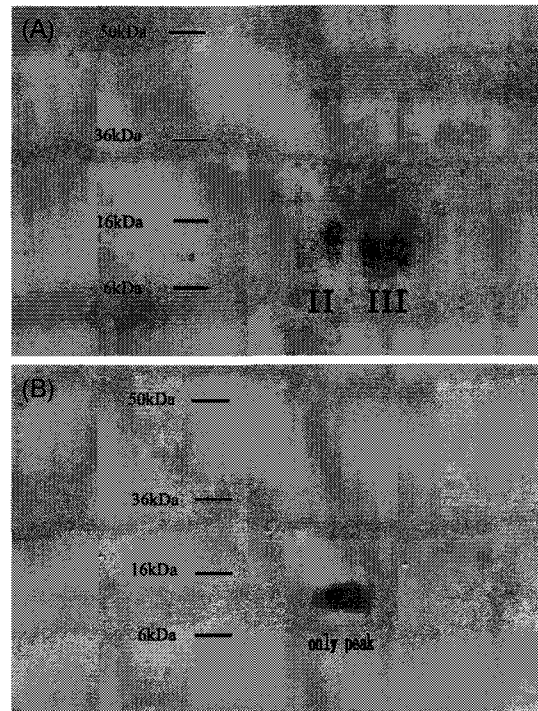

Fig. 7. Western-blot analysis of fractions collected from CM-Sepharose CL-6B and Sephadex G-50 column chromatography isolation steps. The proteins in each fraction were separated by 12% SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was incubated with specific IGF-I polyclonal IgG, and then incubated with anti-rabbit IgG hoseradish-peroxidase-linked whole second antibody. Antigens were detected by chemiluminescence with an ECL Advance Western-blot detection kit. (A) Western-blot analysis of fractions eluted from CM-Sepharose CL-6B column. The peak 2 and peak 3 showed the positive immunoblot bands (B) Western-blot analysis of fractions from the Sephadex G-50 column. The only peak showed the positive immunoblot band. Indicators at the left indicate migration positions and molecular weights of IGF-I.

example of complete bone regeneration in an adult animal [17]. The fact that it grows so rapidly indicates that the growth factors involved in this system may be expressed in large quantities, making the identification process more straightforward [13]. It has been found that, during the antler growth period, deer blood contained high levels of IGF-I and the as well as receptors to IGF-I in the antler itself which promoted growth in laboratory mouse cell lines. Extracts of the deer antlers were found to stimulate the growth of nerve fibers, induce morphologic changes during differentiation, and affect DNA synthesis in cells [11]. The presence of IGF-I, demonstrated by purification and quantification, gives hints for understanding the mechanisms that
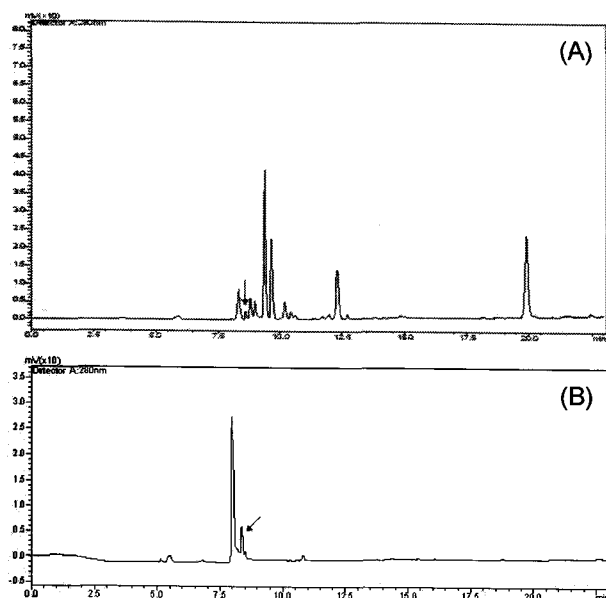
Fig. 8. Elution profile of antler IGF-I from reversed-phase HPLC. Fraction collection combinations with IGF-I immunobinding activity by Western-blot detection from CM-Sepharose CL-6B ion-exchange chromatography (A) and Sephadex G-50 chromatography (B) were applied to a C_{18} column. Elution was performed with a linear gradient of 20-40% ACN in 0.1% TFA-acidified water for 30 min at a flow rate of 0.8 ml/min. The sample was monitored by UV absorbance at 280 nm. Both column elutions yielded the same peak at a retained time of 8 min (arrows).
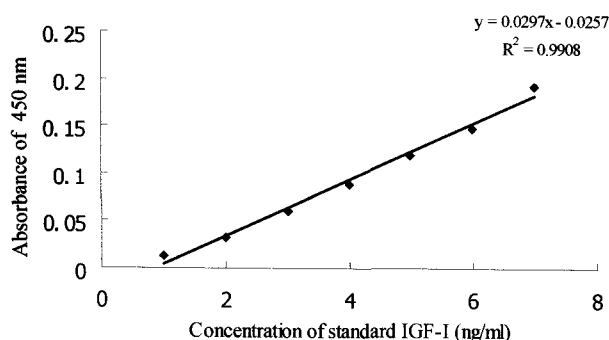

Fig. 9. Standard curve for the enzyme-linked immunosorbent assay. The human IGF-I concentration standard curve was generated by a serial dilution of standard human IGF-I. Quantification of IGF-I in antlers was calculated as 2190 ng/ml using the standard curve.

control the growth of antlers, as well as other tissues in which control of the growth rate is important. Previous studies have demonstrated that both IGF-I and IGF-II are expressed in the growing antler; both type I and II IGF receptors are present in the antler tip [6], which indicates that both IGFs are involved in the growth and differentiation of

antler tissue. The presence of IGF-I was detected using RT-PCR [7], which helped clarify the role of these growth factors.

Recently, the purification of IGFs and growth-factor-binding proteins has been reported [5,15,19]. The purification of mammalian IGF and IGF-binding proteins from a variety of biological fluids and culture media has also been successful [19]. The purification procedure comprises acidification, IGF affinity chromatography, and reversed-phase HPLC. However, some modifications were incorporated in this purification from antlers. In the present study, this strategy was adapted for the purification of antler IGF-I, and a combination of cation and anion exchange column chromatography was used in the purification.

HPLC analysis of biological and environmental samples, such as plant and animal tissues often requires the removal of contaminating sample matrix effects [14]. Normally, samples with less purification, gives poor values from HPLC, like in Fig. 8A, many compounds appeared; Chromatography has shown considerable promise in the concentration and cleanup of purification from natural plant or animal samples prior to HPLC analysis. As the samples were progressively purified from one more chromatography, the value for purification is better like in Fig. 8B, the peaks appeared in the HPLC result which contained an IGF-ELISA-reactive fraction.

It is worth noting here that IGF-I activity was detected by Western-blot analysis. Although the ligand-blotting technique is the preferred and most commonly used method for detecting IGF and IGF-binding protein interactions, more sensitive methods include enzyme cross-linking assays [12]. Although the isolated IGF-I is not entirely purified, and on SDS-polyacrylamide gels, the results do not show that the single band, the high immunoactivity of IGF-I has long been demonstrated by ELISA analysis.

Enzyme linked immunosorbent assays are sensitive, flexible, easy-to-perform and fast. This technique relies on the availability of antibodies against different epitopes of the enzyme. If the purification yields quantities exceeding those necessary for immunization, the development of a sensitive ELISA assay for IGF quantification would be realistic. Therefore, the ELISA method, which has been established in this study might be the appropriate tool for the quantitative detection of IGF quantification isolated from deer antler. Furthermore, the molecular size and immunoactivity binding the human IGF-I resemble the IGF.

The rapid rate of growth at the antler tip requires major remodelling and angiogenic production. There is a current interest in the factors controlling such responses to develop novel IGF products. This study demonstrates that antlers are a valuable source of IGF, which will facilitate new discoveries and applications in this exciting field. However, to definitively identify the types of IGF-I, complete amino acid sequence data would be helpful.

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초록 : 녹용으로부터 Insulin-like Growth Factor-I 의 일부정제 및 정량

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사슴 뿔은 동물세계에서 가장 빨리 성장하는 조직이다. 따라서 성장중인 사슴 뿔은 뼈 성장을 촉진하는 인자가 풍부하게 포함된 것으로 생각된다. 이들 성장인자들 중 IGF-1은 뼈를 자라게 하는 조골세포의 대사에 중요한 역할을 한다고 알려져 있어 이를 정제하고자 하였다. IGF-1의 정제는 상대라고 불리는 신선한 사슴 뿔을 유안침전, DEAE-Sepharose CL-6B 이온교환수지, CM-Sepharose CL-6B 양이온교환수지, Sephadex G-50의 순차적인 방법으로 할 수 있었다. 각 과정마다 IGF-1의 거동을 HPLC, SDS-PAGE, Dot blot, 그리고 western blot으로 분석하였다. IGF-1의 정량은 ELISA기술로 재조합 인간 IGF-1을 이용하여 계산되었으며, 최종 분별 액은 두 개의 단백질을 보였으나, Western-blot에서 작은 분자량인 12 kDa으로 최종 판명할 수 있었다. 정제된 단백질은 HPLC에서 retention 시간 8분만에 검출되었으며, 총 농도는 2910 ng/ml 이고 중량은 0.291 g 이었다.