Purification and Properties of Chicken Growth Hormone

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Abstract Growth hormone (GH) was extracted and purified from the chicken anterior pituitaries and its properties were investigated. The procedure used in the present study consisted of alkaline extraction, ammonium sulfate precipitation, ion-exchange chromatography on Amberlite IRC-50, gel filtration on Sephadex G-75 and isoelectric focusing. The chicken GH obtained had a significant activity in increasing the width of the epiphyseal cartilage of tibia in the hypophysectomized chicken. The preparation was estimated to have a molecular weight of 25,800 by the sodium dodecyl sulfate polyacrylamide gel electrophoresis, a pl value of 7.93 by the isoelectric focusing and a cross-reactivity of about 2% with rat GH. Amino acid composition was very similar to those of chicken GH preparations described in the previous reports.


The physiological role and the chemical properties of growth hormone (GH) have been extensively studied in many species of mammals. Although the existence of GH in chicken pituitary gland has been suggested by several investigators1-3), there were only a few reports4-6) concerning purification of GH and little is known about the role of GH. Harvey and Scanes6) have purified chicken GH from the anterior pituitaries and have first developed the specific radioimmunoassay for the chicken GH. The authors also attempted to purify GH from chicken anterior pituitary glands and investigated its properties.

Materials and Methods

Chicken GH was purified according to the methods described by Farmer et al.4) and Harvey and Scanes6) with a slight modification. Anterior pituitaries were collected from male and female broilers from a local killing plant (Ijichi Poultry Farm Co. Ltd., Kagoshima) and stored at -20°C until used. Unless otherwise described, all procedures for obtaining GH preparation were carried out at 4°C. The molecular weight was estimated by the sodium dodecyl sulfate polyacrylamide gel electrophoresis in comparison with the mobilities of materials of known molecular weight (Markers: BDH molecular weight marker mixtures, BDH Chemical Ltd., England). The polyacrylamide gel disc electrophoresis was carried out in 7.5% gel at pH 4.5 and 8.3. The isoelectric focusing of chicken GH was performed in a column of 110 ml at 4°C
for 48 hr with carrier ampholyte covering the pH range 3–10. The amino acid composition of chicken GH was estimated by the amino acid analyzer after hydrolyzing in 6 N HCl at 110°C for 24 hr. The immunological cross-reactivity of chicken GH to rat GH was examined by radioimmunooassay with the monkey antiserum against rat GH. The GH activity was estimated by the effect on the increase in the width of the epiphyseal cartilage of tibia in the hypophysectomized chicken. White Leghorn male chicks were hypophysectomized at three weeks of age by the transbucal method originated by Hill and Parkes7). The GH preparations obtained by the present procedure were dissolved in 0.9% saline solution and injected subcutaneously into the hypophysectomized chicks once a day (daily dose: 50 μg) for 5 or 10 days. The injections were begun after a postoperative period of 10 days. The birds were killed 24 hr after the final injection and the width of the epiphyseal cartilage of tibia was measured by the method described by Greenspan et al.8). Ovine GH (HIN-GH-S11) was also used to compare with the chicken GH.

Results

Purification of chicken GH

Extraction of GH from the frozen pituitaries was done with 10 volume of 0.9% saline solution, pH 9.0 adjusted with Ca(OH)₂, using the Ultra Turrax homogenizer. After stirring for 3 hr, the homogenate was centrifuged and ammonium sulfate was added to the supernatant to 0.5 saturation and then the solution was adjusted to pH 7.0 with NaOH. After standing overnight, the precipitate was collected by centrifugation and dialyzed against water, followed by 0.3 M phosphate buffer (pH 6.0). The fraction was applied to an Amberlite IRC-50 column equilibrated with the same buffer. As shown in Fig. 1, the adsorbed fraction (FA-2) was eluted with 1 M NaCl after the non-adsorbed fraction (FA-1) was eluted. The FA-2 fraction had a significant effect on the epiphyseal cartilage width of tibia in the hypophysectomized cockerels, but the
Purification of Chicken GH

**Table 1.** Effect of daily injections of the fractions on the width of the epiphyseal cartilage of tibia in the hypophysectomized cockerels.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Fraction</th>
<th>No. of chickens</th>
<th>Epiphysial cartilage width (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>FA-1</td>
<td>5</td>
<td>34±2</td>
</tr>
<tr>
<td>I</td>
<td>FA-2</td>
<td>4</td>
<td>46±7**</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>5</td>
<td>32±3</td>
</tr>
<tr>
<td>II</td>
<td>FB-2</td>
<td>5</td>
<td>49±4*</td>
</tr>
<tr>
<td>II</td>
<td>Ovine GH</td>
<td>3</td>
<td>44±1</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>6</td>
<td>42±4</td>
</tr>
</tbody>
</table>

Values are mean ± S.D.  
(a: Injections for 5 days (total dose: 250 μg/bird).  
(b: Injections for 10 days (total dose: 500 μg/bird).  
*, **: Significantly different from the saline at 5% or 1% level respectively.

FA-1 fraction had no effect (Table 1). The FA-2 fraction was concentrated and dialyzed against 0.05 M NH₄HCO₃ (pH 8.0). The fraction was subjected to a column on Sephadex G-75 equilibrated with 0.05 M NH₄HCO₃. As shown in Fig. 2, two fractions (FB-1 and FB-2) were obtained by the gel filtration. Since the FB-2 fraction with a Ve/Vo value of 1.88 had a significant activity in increasing the epiphyseal cartilage width of tibia (Table 1), this fraction was concentrated with the collodium membrane filter. Under the same condition as above, the re-gel filtration of FB-2 fraction on a Sephadex G-75 column was carried out and a single peak was obtained. After concentration, the fraction was applied to the isoelectric focusing. As shown in Fig. 3, the GH preparation was separated into the major peak and the minor one, their isoelectric points being pH 7.93 (FC-2) and 6.97 (FC-1) respectively. The major peak with pI 7.93 was considered as GH according to the previous report5).

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**Fig. 2.** Column chromatography of FA-2 fraction on Sephadex G-75 in 0.05 M NH₄HCO₃ (pH 8.0). Column size: 2.1×99 cm, Flow rate: 30 ml/hr, Fract. size: 3 ml.
Fig. 3. Isoelectric focusing of the FB-2 fraction. Isoelectric focusing was carried out for 48 hr at 900 V and 4°C in a 110 ml column size using ampholite covering the pH range of 3–10.

Fig. 4. Disc electrophoretic patterns of chicken GH (FC-2) at pH 4.5 and 8.3 in 7.5% gel stained with Amido Schwartz.

Properties of the chicken GH preparation

The disc electrophoretic patterns are shown in Fig. 4. The purified GH (FC-2) gave the two bands and the migrations of them in a gel were very similar to those reported previously by Farmer et al. The molecular weight of GH was determined to be about 25,800 by the sodium dodecyl sulfate polyacrylamide gel electrophoresis (Fig. 5). The amino acid composition of the present preparation is shown in Table 2, together with those of the chicken GH preparations reported by Farmer et al. and Harvey and Scanes. The proportion of amino acid composition of the present prep-
Purification of Chicken GH

**Fig. 5.** Determination of the molecular weight of the chicken GH (FC-2) by SDS-polyacrylamide gel electrophoresis. Standard marker proteins were: monomer protein (a), MW 14,300; dimer protein (b), MW 28,600; trimer protein (c), MW 42,900; chicken GH (d).

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>6.3</td>
<td>6.2</td>
<td>8.4</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.8</td>
<td>2.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.3</td>
<td>5.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Aspartic</td>
<td>11.7</td>
<td>10.4</td>
<td>10.5</td>
</tr>
<tr>
<td>Threonine</td>
<td>6.7</td>
<td>5.2</td>
<td>5.8</td>
</tr>
<tr>
<td>Serine</td>
<td>6.4</td>
<td>6.5</td>
<td>6.4</td>
</tr>
<tr>
<td>Glutamic</td>
<td>14.6</td>
<td>13.3</td>
<td>11.4</td>
</tr>
<tr>
<td>Proline</td>
<td>5.2</td>
<td>7.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Glycine</td>
<td>4.2</td>
<td>6.7</td>
<td>10.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>7.4</td>
<td>7.8</td>
<td>8.0</td>
</tr>
<tr>
<td>1/2 Cystine</td>
<td>ND</td>
<td>1.6</td>
<td>ND</td>
</tr>
<tr>
<td>Valine</td>
<td>5.5</td>
<td>5.2</td>
<td>6.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.9</td>
<td>1.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.2</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>13.1</td>
<td>10.5</td>
<td>11.3</td>
</tr>
<tr>
<td>Thyrosine</td>
<td>3.5</td>
<td>1.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.1</td>
<td>4.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>—</td>
<td>0.7</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are calculated as percentage to total composition analyzed. I: The present preparation. II: Farmer et al.5). III: Harvey and Scan5). ND: Not determined.

Separation was very similar to those of the previous reports5,6). This chicken GH had a slight cross-reactivity (about 2.0%) to rat GH as shown in Fig. 6.
Discussion

There were only a few reports concerning purification of avian GH. PAPKOFF and HAYASHIDA attempted to purify GH from turtle and duck pituitaries and compared the two substances in respect to the amino acid composition and the cross-reactivity against rat GH. FARMER et al. first reported the disc electrophoretic pattern and the amino acid composition of the chicken GH. HARVEY and SCANES showed that the chicken GH had a molecular weight of 23,300 and pI value of 7.5. The chicken GH obtained in this study was similar to those reported by FARMER et al. and HARVEY and SCANES in terms of disc electrophoretic pattern, amino acid composition, molecular weight and pI value. The present chicken GH had a slight cross-reactivity to rat GH as reported by FARMER et al. The two protein bands with different migration in a polyacrylamide gel were observed although only a single peak was obtained by the re-gel filtration on Sephadex G-75. The reason remains unknown. FARMER et al. also reported the two protein bands in the disc gel of the purified chicken GH.

It was shown that the present preparation significantly increased the epiphyseal cartilage of tibia of the hypophysectomized cockerel. The increase in width of the epiphyseal cartilage of tibia is generally accepted to be a precise end point for estimating GH activity and hypophysectomized rats or mice are commonly used for the tibia test. No study has been reported, however, using the hypophysectomized chicken as the assay animal. On the other hand, several attempts to investigate the role of GH in the chicken have not been successful. In this study also, the ovine GH had no effect on the epiphyseal cartilage width of tibia in the hypophysectomized chicken in spite of a large dose as much as 500 μg. GREENSPAN et al. reported that the total dose level of 20 μg of mammalian GH showed a significant response in the hypophysectomized rats. Therefore, the species specificity in the biological activity seems more remarkable in GH than in any other pituitary hormone. For this reason, the GH activity of the present preparation was examined by using the same species “hypophysectomized chicken” instead of rat. As the results, it was evidenced that the purified GH...
Purification of Chicken GH

Chicken GH obtained in the present study had a biologically significant GH activity. This chicken GH preparation is considered to be available for the further investigations on physiological role of GH in the chicken.

Acknowledgement

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References


鶏成長ホルモンの精製とその性状

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鶏の下垂体前葉から成長ホルモンを分離精製し、その性状について調べた。精製方法は、アルカリ抽出、破壊
処理、イオン交換樹脂（Amberlite IRC-50）によるクロマトグラフィー、Sephadex G-75 によるゲル拡散およ
び等電点電気泳動法を用いて行なった。得られた鶏成長ホルモンは、下垂体前葉除去雄ヒナの類骨骨端軟骨盤
を有効に増加させる作用を示し、また本実験で得られた鶏成長ホルモンは、SDS ポリアクリルアミドゲル電気
泳動法により分子量 25,800、等電点電気泳動法によりpl 7.93 およびラット成長ホルモンとの間に約 2% の相
互性をそれぞれ有することが認められた。さらに、アミノ酸分析の結果、この成長ホルモンは、これまでに報告
されている鶏成長ホルモンと極めて類似したアミノ酸組成を有することが認められた。

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