Purification of Chicken Growth Hormone from Pituitary Residue after Extraction of Glycoprotein Hormones

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Hideaki Tojo and Kiyohiko Ogawa
(*Laboratory of Animal Reproduction*)
(Received for Publication August 30, 1975)

Introduction

The studies on the purification and characterization of growth hormone (GH) have been restricted almost in mammals.

Papkoff and Hayashida[6] attempting fractionation of GH from duck and turtle pituitaries, showed by agar-gel diffusion and radio-immunoassay that the both GH fractions were identical to the immunoreactive material in pituitary extract.

Recently, Farmer *et al.*[2] succeeded in the purification of avian GHs from anterior pituitaries (duck, pigeon, turkey and chicken), and reported that all four preparations were active in the tibia test.

Hitherto, there have been only a few reports concerning fractionation and purification of chicken GH, and the biological action of GH was scarcely clarified in the chicken.

We have obtained the acetone-dried pituitary residue produced after the extraction of glycoprotein hormones in another experiment.

The present study was performed to purify chicken GH from the pituitary residue, by using the method reported to be effective in the purification of horse GH[4].

Materials and Methods

All operations were carried out at 4°C.

The acetone-dried residue (after glycoprotein extraction) was added in 2 volumes of 0.1 N NaOH. The mixture was stirred for 15 min, the pH was adjusted to 10.5 with 4 N HCl and the stirring was continued overnight. The mixture was centrifuged after being adjusted to pH 7.0, and the residue was re-extracted at pH 10.5 for 1 hr.

The extracts were combined and 10 N KOH was added, with stirring until the solution was 0.3 M in potassium ion. The solution was centrifuged after the adjustment to pH 8.5 with glacial acetic acid.

The protein in the supernatant fluid was precipitated by adding an equal volume of 96% (v/v) ethanol, with stirring.

The precipitate (crude GH) was centrifuged and washed with cold ethanol and ether and dried *in vacuo*.

Further purification of crude GH was conducted by DEAE-cellulose column chromatography. The crude GH was mixed with water and 2 N NaOH was added drop-

wise with stirring until it was dissolved. It was dialyzed against 0.01 M tris-buffer (pH 8.0). After the removal of insoluble material by centrifugation, the soluble protein was applied to a column of DEAE-cellulose (Whatman DE-32) pre-equilibrated with 0.01 M tris-formate buffer (pH 8.0).

The unadsorbed protein was eluted by gradient elution with 0.3 M tris-formate buffer (pH 8.0). The adsorbed protein was eluted with 1 M ammonium acetate.

The tubes of effluent were pooled into fraction 1 and 2. Each fraction was adjusted to pH 5.5 with glacial acetic acid and the protein was precipitated by drop-wise addition of an equal volume of cold ethanol, with stirring.

After 48 hrs, the precipitate was centrifuged with cold ethanol and ether and dried *in vacuo*.

The biological activity of the fraction was assayed by the tibia test[3]. Female Wistar rats were hypophysectomized at 28 days of age and used 14 days postoperatively for the bioassay.

The preparations were intraperitonealy injected once daily for 4 days. Those rats were sacrificed on the fifth day, and then the epiphyseal cartilage plate width was measured.

Results and Discussion

The weight-yields of preparations produced in the present study were shown at Table 1. Large volumes of the crude GH were obtained from the acetone-dried residue. The fraction 1 and 2 were obtained by chromatography of column on DEAE-cellulose. The former was obtained by the gradient elution with 0.3 M tris-formate buffer (pH 8.0) and the latter was obtained by the additional elution of 1 M ammonium acetate.

As shown, two fractions were almost equally produced from the crude GH in both trials, but fraction 2 was yielded somewhat more than fraction 1.

The yields of the both fractions from the pituitary powder were far less in comparison with those from the horse pituitary by Hartree *et al.*[4] The reason may be due to a species-difference between the materials, or to a loss in purificating-process in the present study.

Fig. 1 shows the result of column chromatography of the crude GH on DEAE-cellulose. The peak of fraction 1 was smaller than that of fraction 2. As shown, the chromatography in the present study coincides well with that of horse GH reported by

Table 1. Weight yields of preparations produced from chicken anterior pituitary powder.

Preparation	Yield		
		Trial 1	Trial 2
Pituitary powder	23.2750 (g)		
Acetone-dried residue	17.3013 (g)		
Crude GH	2.2551 (g)	0.3450 (g)*	1.2934 (g)*
Fraction 1		6.0 (mg)	15.2 (mg)
Fraction 2		6.2 (mg)	17.6 (mg)

* Volume of the starting material used in chromatography on DEAE-celulose.

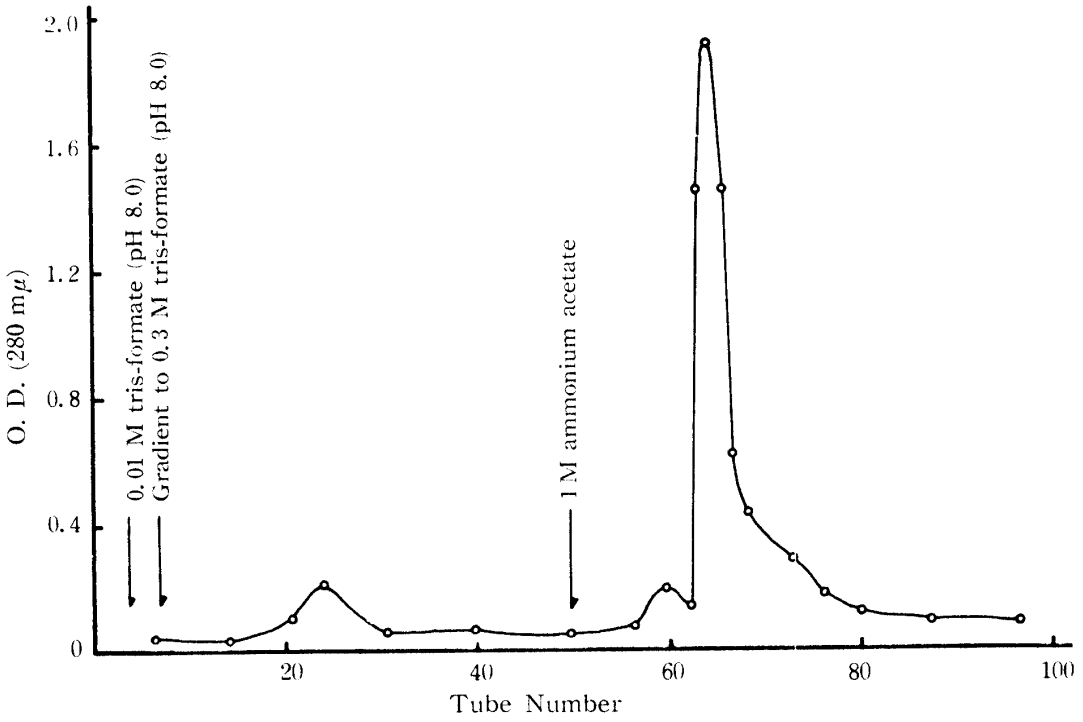

Fig. 1. Chromatography of crude chicken growth hormone on DEAE-cellulose. The column was equilibrated with 0.01 M tris-buffer (pH 8.0). Column size : 2.0×30 cm. Flow rate : 8.0 ml/hr.

Hartree *et al.*[4]

Hartree *et al.*[4] indicated that gradient elution with 0.3 M tris-formate buffer was effective in the purification of horse GH. In this study, also two fractions were successfully separated from the crude GH by DEAE-cellulose column chromatography, as well as in horse GH[4].

These fractions were assayed for biological activity by the tibia test. The total dose of the fraction was 200 μg per rat, and ovine GH (NIH-GH-Sll) was used as a comparative hormone. The results are presented at Table 2.

Ovine GH showed significant activity ($p < 0.01$) in both the body-weight increasing and the epiphyseal cartilage plate width.

Whereas the activities of chicken GH fractions were not statistically significant at

Table 2. The results of bioassay for preparations by the tibia test.

Preparation[a]	No. of rats	Body weight[b] increase (g)	Tibia width (Micra±S. D.)
Saline (Controls)	5	1.8±1.5	174.6±23.4
Ovine GH	5	7.8±2.7**	291.6±45.0**
Fraction 1	4	2.4±1.9	244.8±45.0
Fraction 2	4	2.8±2.1	216.0±46.8

a : 200 μg total dose per rat
b : Increasing in body weight from a start of injection to autopsy
** Significant difference from controls ($p < 0.01$)

the same dose as that of ovine GH. It is assumed that each fraction might indicate a significant activity, provided that it was to be given to the test animals more abundantly than in the present case.

However, it is suggested that two fractions may contain GH activity, since there is no statistical difference between the value of ovine GH and that of each fraction in both the body-weight increasing and the tibia width.

Hartree *et al.*[4] reported that horse GH produced by the present method was large volumes of low potency, when assayed by the tibia test. Moreover, Farmer *et al.*[2] indicated that four purified avian GHs (duck, pigeon, turkey and chicken) were active in the tibia test but chicken GH was especially less potent among them.

In the present study, chicken GH fractions were not so much active in the tibia test, though the purificating method was different from the method of Farmer *et al.* Thus, the low potency of chicken GH may be due to a difference in species between the preparation and the test animal for bioassay. Therefore it may be necessary to establish a original bioassay for chicken GH on the basis of the same species.

Finally, in the chicken, the biological action of GH has been scarcely clarified. Although there were some reports[1, 5, 7] investigating GH action in the chicken, all the studies were conducted by using mammalian GH. Thereafter, it is required to investigate GH action in the chicken with a purified chicken GH but not with mammalian GH.

Summary

The present study was performed to purify chicken growth hormone (GH) from the pituitary residue produced after the extraction of glycoprotein hormones.

Two fractions (fraction 1 and 2) were separated by chromatography of column on DEAE-cellulose. Fraction 1 was obtained by the gradient with 0.3 M tris-formate buffer (pH 8.0), and fraction 2 was obtained by the additional elution of 1 M ammonium acetate.

GH activity of those fractions seem to be active in the tibia test but was not statistically significant at the dose of 200 μg per test animal.

As above, the present study suggestes that it is possible to purify chicken GH from the pituitary residue produced after glycoprotein extraction.

Acknowledgments

We are grateful to the National Institute of Health, U. S. A., for supplying NIH-GH-S11.

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