Communications to the Editor

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PARTIAL PURIFICATION AND BIOLOGICAL ACTIVITY OF THE PRODUCT OF CHEMICALLY SYNTHESIZED HUMAN GROWTH HORMONE GENE EXPRESSION IN ESCHERICHIA COLI

Tadashi Oshizawa,[a] Tadao Terao,[a] Hiroshi Sato,[a] Toshio Kimura,[a] Hideo Fukuda,[a] Tsuyoshi Tanimoto,[a] Shingo Niimi,[a] Jiro Kawamura,[a] Takao Hayakawa,*,[a] Eiko Otsuka,[b] and Morio Ikehara[c]

National Institute of Hygienic Sciences,[a] Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158, Japan, Faculty of Pharmaceutical Sciences, Hokkaido University,[b] Sapporo 060, Japan, and Faculty of Pharmaceutical Sciences, Osaka University,[c] Suita, Osaka 565, Japan

The product of chemically synthesized human growth hormone gene expression in Escherichia coli was partially purified and proved to be identical with human growth hormone in size, in mobility on non-denaturing polyacrylamide gel electrophoresis, in behabior on HPLC, in immunological properties, and in biological activity in hypophysectomized rats.

KEYWORDS——chemically synthesized gene; human growth hormone gene; human growth hormone; growth hormone activity; tibia test

Recently a gene coding for human growth hormone (hGH) has been totally synthesized by chemical means.[1] The gene consists of 584 base pairs, the longest gene so far synthesized chemically. A recombinant plasmid carrying the hGH gene produced the polypeptide in E. coli. It was identical with human growth hormone in size and in immunological properties. In the present work, the induced polypeptide was partially purified from the E. coli extract and its biological activity in hypophysectomized rats was examined.

The hGH gene in E. coli (HB 101) carried by plasmid phGH-1 was expressed as described previously.[1] Collected E. coli cells (about 21 g wet weight) were suspended in 100 ml of 50 mM Tris-HCl, pH 8.0 containing 50 mM EDTA and egg white lysozyme (1 mg/ml). They were incubated at 0°C for 30 min with gentle stirring. Then 20 ml of 150 mM Tris-HCl, pH 7.5 containing 280 mM $MgCl_2$, 4 mM $CaCl_2$ and DNase I (50 μg/ml) was added. This was incubated at 0°C for 30 min, then centrifuged at 15,000 rpm for 30 min. The precipitate was washed with a small volume of 50 mM Tris-HCl, pH 8.0. The supernatant and the wash were combined and then fractionated by ammonium sulphate precipitation. The major portion of the induced polypeptide, as analyzed by SDS-polyacrylamide gel electrophoresis,[2] was found in the 60% saturated ammonium sulphate pellet (protein contents:460 mg). The pellet (400 mg of protein) was then dissolved in a small volume of 20 mM Tris-HCl, pH 7.5, and dialyzed against the same buffer at 4°C. The dialysate was charged on a DE-52 column (1.6 x 40 cm) equilibrated with 20 mM Tris-HCl, pH 7.5, and then eluted with a stepwise increase of NaCl concentration. Each eluate (9.5 ml/fraction) was analyzed by radioimmunoassay using an EIKEN-hGH-I kit from Eiken Immunochemical for the double-antibody method. The elution profiles are shown in Fig. 1. The major portion of the immunoreactive hGH was found in fractions 46-51. Analysis of the fractions by SDS-polyacrylamide gel electrophoresis[2] showed that the major component in these fractions had a molecular weight of 22K, as expected for hGH. The fractions 46-51 were pooled and concentrated by using a short DE-52 column followed by ammonium sulphate precipitation. The resulting precipitate was dissolved in a

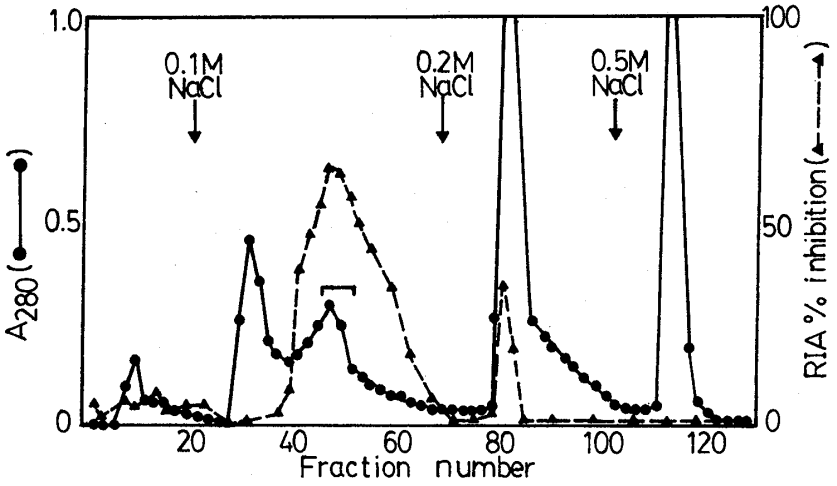

Fig. 1. The Elution Profile of a DE-52 Column Chromatography

small volume of 20 mM Tris-HCl, pH 7.5, containing 0.5 M NaCl and 0.02% NaN_3. This was dialysed against saline at 4°C for 3 h for use in further characterization.

The product thus obtained and methionyl hGH from Kabi Ind. had identical behavior on non-denaturing polyacrylamide gel electrophoresis at pH 8.5 (data not shown), SDS-polyacrylamide gel electrophoresis (Fig. 2) and high performance liquid chromatography (Fig. 3). The biological activity of the induced polypeptide was tested by the increase in the width of the proximal epiphyseal cartilage of the tibia of hypophysectomized rats, the most sensitive and specific test known for the determination of growth hormone activity.[3-4] A group of hypophysectomized Wistar-Imamichi female rats were injected daily for 4 days with test solutions (5 or 20 μg of the induced polypeptide, 5 or 20 miliunits of methionyl hGH, or 0.5 ml of saline per day). One tibia was removed after the last injection, fixed in acetone, and stained immediately with silver nitrate. The width of the cartilage plate was measured with a calibrated eye piece micrometer and the mean width and the standard error for each group were determined. The results are shown in Table I. The induced polypeptide as well as methionyl hGH caused a marked increase in the width of the uncalcified portion of the proximal epiphyseal cartilage of the tibia. The effects of the hormones on the animals were dose dependent and the relationship of dose to response was similar in both hormones. The specific activity of induced peptide was calculated to be about 1 IU/mg. In the hypophysectomized rat weight

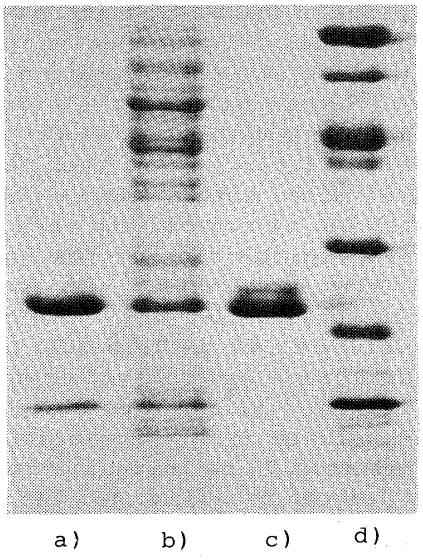

Fig. 2. SDS-Polyacrylamide Gel Electrophoresis

a) The product (fractions 46-51 of the DE-52 column chromatography in Fig. 1),
b) crude extract of E. coli,
c) methionyl hGH (Kabi), and
d) molecular weight protein standards containing lysozyme, soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, bovine serum albumin, and phosphorylase B.

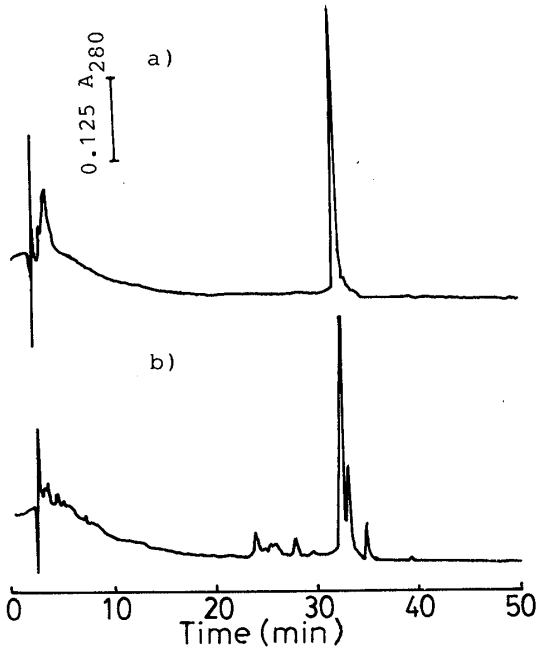

Fig. 3. High Performance Liquid Chromatography of Methionyl hGH and the Product (Immunoreactive Fractions of the DE-52 Column Chromatography in Fig. 1).

Samples were injected onto a Syn-Chropak RP-P (250 x 4.1 mm ID) column at flow rate of 1 ml/min and eluted with a linear gradient from 0% to 100% acetonitrile containing 0.1% trifluoroacetic acid for 50 min.
a) Methionyl hGH (Kabi),
b) the product (fractions 46-51 of the DE-52 column chromatography in Fig. 1).

Table I. The Effect of Methionyl hGH (Kabi) and the Product (Immunoreactive Fractions of the DE-52 Column Chromatography in Fig. 1) on the Width of the Proximal Epiphysis

Preparation	Total dose (4 days)	No. of rats	Width of tibial epiphyseal cartilage plate (μm)	
			Mean ± S.E.	Difference from control
Saline		9	193.7 ± 9.7	
Methionyl hGH	20 mIU	9	266.5 ± 6.1	72.8
	80 mIU	9	295.2 ± 4.4	101.5
The product*	20 μg	9	271.2 ± 5.8	77.5
	80 μg	9	294.5 ± 5.6	100.8

* Fractions 46-51 of the DE-52 column chromatography in Fig. 1.

increase test, which is another index of the activity of hGH, the induced peptide caused biological effects similar to methionyl hGH.

From these results it is evident that the product of chemically synthesized human growth hormone gene expression in Escherichia coli was indistinguishable from methionyl hGH in several physicochemical features and it was as active as the hGH.

Further purification and thorough analyses of the product are in progress and will be reported elsewhere.

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