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Binding Protein of Somatomedin A in Serum from Men and Rats

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Synopsis

After gel chromatography of human and rat serum at pH 7.4, all endogenous somatomedin A was recovered in the high molecular weight range. The largest peak was found in the γ-globulin (II) region and the next largest peak found in the albumin region (III). The amounts of somatomedin A in the peak II region increased in serum from acromegales and decreased in serum from growth hormone deficient patients.

Four radioactive peaks were observed after gel chromatography of serum incubated with 125I-somatomedin A. Only the two peaks corresponding to peaks II and III out of the four peaks were displaced by adding 50 μg of partially purified cold somatomedin A. The radioactivity of peak II decreased in sera from growth hormone deficient patients and increased after growth hormone administration.

These observations support the hypothesis that the growth hormone regulates not only somatomedin A but also its carrier protein.

Somatomedins have been designated as factors which are growth hormone (GH) dependent, and which exert an anabolic effect on their target tissues (Daughaday et al., 1972). Numerous studies in the rat and in man have confirmed the GH dependence of somatomedins (Daughaday et al., 1959; Almqvist et al., 1961; Hall, 1972; Takano et al., 1977, 1978). Unique among polypeptide hormones, the somatomedins, including somatomedin A, C, IGF-I and II (insulin-like growth factor) and MSA (multiplication stimulating activities), circulate in the blood as a complex bound to proteins of larger molecular weight (Van Wyk et al., 1974; Hall et al., 1975; Zapf et al., 1975; Hintz and Liu, 1977). Moses et al. (1976) reported that not only MSA but also its binding proteins in rat serum were growth hormone dependent. We have studied here the growth hormone dependency of the binding protein of somatomedin A.

Materials and Methods

Blood samples

Venous blood samples were drawn, after an overnight fast, from three acromegales, five pituitary dwarfs and three normal subjects. The diagnoses were confirmed by clinical and laboratory findings. In the case of a patient under hGH treatment, the blood samples were drawn before and one day after im injection of 2 mg of hGH. For the rat serum, venous blood samples were withdrawn under ether anaesthesia and pooled sera were used for the study. Normal rat serum was obtained at the age of 28 days. Hypophysectomy was performed at 28 days of age and blood samples were taken 24 hr and 12 days after hypophysectomy. The hypophysectomized rat was administered 80 μg of hGH at the age of 40 days and the blood sample was taken 8 hr after ip injec-
tion. This amount of hGH was reported to increase the serum level of somatomedin A 8–24 hr after injection (Takano et al., 1977, 1978).

Somatomedin A and GH preparations

The somatomedin A was purified by Dr. L. Fryklund at Recip Polypeptide Laboratory, AB Kabi, Stockholm. The somatomedin A used for labelling was a highly purified preparation and was considered chemically homogeneous. It had a specific activity of 1,000 U/mg as determined by a sulfate incorporation bioassay in chick pelvic leaflets (Hall, 1972). A less pure preparation of somatomedin A with a specific activity of 78 U/mg was used for the displacement on the column.

The growth hormone preparation used for the study was a highly purified human growth hormone, Crescormon®B, obtained from AB Kabi/Recip, Stockholm, Sweden. This preparation has the potency of 2 IU per mg.

Gel-chromatography

Descending gel chromatography was performed on a column of Sephadex G-200 (2.6 x 41.0 cm) equilibrated and eluted with 0.005 M Tris-Buffer, pH 7.4 at 4°C. The column was calibrated with blue dextran 2000 (Pharmacia) and bromphenol blue as markers. Four or five milliliters of serum were applied to the column and eluates were collected in fractions of 2.2 ml. Each fraction was lyophilized and then diluted ten fold with water for the determination of somatomedin A by radioreceptor assay.

Half a milliliter of serum was incubated with 125I-somatomedin A (1,000,000 CPM) for 2 hr at room temperature. The incubated mixture was then applied to a Sephadex G-200 column (2.6 x 33 cm), equilibrated and eluted with 0.005 M Tris-Buffer, pH 7.4 at room temperature. Eluates were collected in fractions of 2.2 ml and the protein content and radioactivity of each fraction were determined by absorbance and γ-counter, respectively.

Radioreceptor assay

Radioreceptor assay for somatomedin A was performed as described earlier (Hall et al., 1974; Takano et al., 1975, 1976). In all instances, a symmetrical 4-point design was used with two different volumes of the local reference serum and of the unknown samples. One unit (U) of somatomedin A is defined here as the somatomedin A contained in one ml of this pooled serum.

Radioiodination of somatomedin A

The somatomedin A used for labelling was purified by Dr. L. Fryklund at Recip Polypeptide Laboratory, AB Kabi, Stockholm, and had a biological activity of 1,000 U/mg as determined by chick bioassay for somatomedin A. Somatomedin A was iodinated by the lactoperoxidase procedure (Thorrell and Johansson, 1971), purified by carboxymethyl-cellulose column in 0.1 M ammonium acetate, pH 4.0, and eluted with a pH (5–6.8) gradient (Rechler et al. 1978). The specific radioactivity of the preparations used for these experiments was 30–40 μCi/μg.

Absorbance was measured at 280 nm. Paper electrophoresis were performed by standard laboratory methods.

Results

Endogenous somatomedin A by gel chromatography

After separating a normal serum by gel chromatography at neutral pH, most of the somatomedin A was recovered in the high molecular weight range. At least two high molecular weight forms of somatomedin A were found on Sephadex G-200, corresponding to 43 and 70 percent bed volume. (Fig. 1). These two peaks belong to the γ-globulin region (peak II) and the albumin region (peak III), respectively, as determined by electrophoresis.

When separating sera from patients with an acromegaly and a pituitary dwarfism on the same gel column, similar histograms of somatomedin A were obtained (Fig. 2). Most of the somatomedin A was recovered in the high molecular weight form and the amount of somatomedin A in the γ-globulin region was the largest in serum from acromegaly followed by normal and pituitary dwarfs. The ratio of the amounts of somatomedin A in the γ-globulin and albumin regions (II/III) was 3.3 and 3.7 in two normal subjects, 7.4 and 6.2 in two acomegaly, and 1.7 and 2.3 in two pituitary dwarfs, respectively.

In normal rat serum, most of the somatomedin A also appeared in the high molecular weight form. The elution pattern on Sephadex G-200 was similar to that obtained with human sera (Fig. 3). After hypophysectomy, the serum levels of somatomedin A decreased from 9.13 to 0.51 U/ml with the
Fig. 1. Gel chromatography of serum from normal subjects. Four milliliters of serum were applied to a column of Sephadex G-200 equilibrated with Tris-HCl buffer (0.005 mol/liter, pH 7.4) at 4°C. Eluates were collected in fractions of 2.2 ml. Absorbance was measured at 280 nm. Histograms show somatomedin A in four tubes. Recovery of somatomedin over the column was 50.4%. The arrows (↓) indicate the peaks of protein I, II and III from left to right.

Fig. 2. Gel chromatography on Sephadex G-200 of sera from patients with an acromegaly (above) and a pituitary dwarfism (below). Original serum contained somatomedin A 2.62 and 0.31 U/ml and the total recovery of somatomedin A over the column were 54.9 and 63.0%, respectively. The arrows (↓) indicate the peaks of protein I, II and III from left to right.

Fig. 3. Gel chromatography on Sephadex G-200 of rat sera. Five milliliters of pooled normal rat serum and two milliliters of hypophysectomized rat serum were applied to the column. Original serum contained somatomedin A 9.13 and 0.51 U/ml and the total recovery of somatomedin A over the column were 76.1 and 70.6%, respectively. The arrows (↓) indicate the peaks of protein I, II and III from left to right.
decreased peak of somatomedin A in the γ-globulin region.

**125I-somatemedin A binding to sera**

Normal rat serum was incubated with 125I-somatemedin A and chromatographed on Sephadex G-200 (Fig. 4). Radioactivity appeared in four peaks corresponding to protein peaks of macroglobulins (peak I), γ-globulins (peak II), albumin (peak III) and the somatomedin region (peak IV). When 125I-somatemedin A was incubated with buffer and chromatographed on Sephadex G-200, almost all the radioactivity appeared in the same position of the previous peak IV (data not shown). Therefore, the radioactivity in peaks I, II and III indicates the combined form of 125I-somatemedin A and some components from serum. Only the binding to γ-globulin region was abolished in the presence of 50 µg unlabelled somatomedin A. Although the bindings of 125I-somatemedin A to proteins of the macroglobulin and albumin region were not displaced by unlabelled somatomedin A, the possibility of some specific binding of somatomedin A to these proteins has not been excluded.

The patterns of 125I-somatemedin A binding to pooled normal rat sera, sera from hypophysectomized and GH treated rats were compared. The binding of 125I-somatemedin A to the peak II region, which represented the major specific binding protein for somatomedin A in normal rat sera, was decreased in sera obtained 24 hr after hypophysectomy and it was very low in sera obtained 12 days after hypophysectomy (Fig. 5). In this serum, 125I-somatemedin A bound to the peak III region. The radioactivity of the peak II region reappeared 8 hr after 80 µg of growth hormone treatment to hypophysectomized rats (Fig. 5).

Similar results were obtained in human serum. The pattern of 125I-somatemedin A binding to a normal human serum is shown in Fig. 6. The binding of 125I-somatemedin A to the protein of the albumin region is larger than that of the γ-globulin region. This is the opposite pattern to that obtained from normal rat serum. The binding 125I-somatemedin A to these regions was abolished in the presence of 50 µg unlabelled somatomedin A. The binding of 125I-somatemedin A to the γ-globulin region increased in serum from an acromegaly (Fig. 7) and decreased in serum from a pituitary dwarf (Fig. 7). This decreased peak was increased 24 hr after hGH injection to the pituitary dwarf (data not shown).

The ratio of the binding of the total radioactivity to the protein did not differ significantly in any kind of human serum (N=6, m=26.3 (SD=4.3)% of the total activity). The amounts of 125I-somatemedin A bound to peak II and the sum of the amount of that bound to peak I plus II plus III were calculated. The ratio (II/I+II+III) was 13.2 and 21.7% in two normal subjects, 26.4 and 40.6% in two acromegalias and 18.0 and 17.9% in two pituitary dwarfs, respectively. When calculated, the ratios
Fig. 5. Sephadex G-200 (2.6×32 cm) elution pattern of pooled serum obtained 12 days after hypophysectomy and that obtained from hypophysectomized rat 8 hr after intraperitoneal injection of 80 μg of human growth hormone, incubated with 125I-somatmedin A. The arrows (↓) indicate the four peaks of radioactivity.

(II/I+II+III) of different rat sera obtained from normal rats, rats 24 hr and 12 days after hypophysectomy, and hGH treated rat were 60.8, 45.0, 22.9 and 43.0%, respectively. This ratio decreased after hypophysectomy and increased after hGH injection. The ratio of the binding of total radioactivity to these three regions did not differ significantly (N=4, m=22.6 (SD=1.4)%).

Discussion

By the determination of endogenous somatomedin A after gel chromatography, it is reconfirmed that all somatomedin exists in the high molecular weight form in human serum (Van Wyk et al., 1969, 1971; Van den Brande et al., 1971; Hall, 1972). We also observed that all the somatomedin A in rat serum existed in the high molecular weight form and no somatomedin A was detected in the range of 7000, which is the molecular weight of purified somatomedin A (Hall, 1972). We reported that normal rat serum contained 5 to 6 times the amount of somatomedin A in humans, and the half-life of somatomedin A was shorter than that found in human serum (Takano et al., 1978). We thought first that these differences between rat and human serum were partly dependent on the increased amount of free somatomedin A in rat serum. However this idea was denied by our current observations.
It is generally accepted that the levels of somatomedin are high in serum from acromegalies and low in serum from pituitary dwarfs (Daughaday et al., 1959; Almqvist et al., 1961; Hall, 1972; Van den Brande & Du Caju, 1974; D’Ercole et al., 1977). After gel chromatography on Sephadex G-200, the ratio of the amounts of somatomedin A in the γ-globulin and the albumin region (II/III) increased in acromegaly and decreased in hypopituitarism in comparison with normal subjects. From these findings it is clear that the amounts of somatomedin A bound to peak II was increased mostly in acromegalies and decreased in pituitary dwarfs, and they decreased in serum obtained after hypophysectomy in rats. These results support the hypothesis that growth hormone regulates not only the generation of somatomedin, but also that of the carrier protein in peak II (Moses et al., 1976; Kaufmann et al., 1978; Hall et al., 1979).

The growth hormone dependency of the binding protein was further confirmed by the study of gel filtration of serum incubated with 125I-somatemedin A. Since two peaks (II and III) out of the four radioactive peaks were displaced by cold somatemedin A, there seemed to be at least two forms of binding protein existing in serum. The amount of radioactivity in peak II was larger in serum from acromegalies and smaller in that from hypopituitary dwarfs than normal serum and it increased after growth hormone treatment. The amounts of radioactivity in peak II decreased with time after hypophysectomy in rats and increased 8 hr after 80 μg of growth hormone treatment. These observations, including the gel filtration pattern of endogenous somatomedin A, suggest that the binding protein in peak II is growth hormone dependent. This is a similar observation to that found by Moses et al. (1976) using 125I-MSA. The fourth peak of the radioactivity corresponds to somatomedin A with a molecular weight of about 7,000. This big peak indicates that most of the 125I-somatemedin A does not bind to the binding protein. This lack of binding might be explained if labelling changes the affinity of somatomedin A to the binding protein. However we could find clear changes of the pattern of radioactivity after chromatography. The difference between somatomedin A binding to the whole serum and to peak II and III is now under investigation.
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