RESOLUTION OF A CROSS-REACTING ANTI-HUMAN GROWTH HORMONE SERUM INTO A MONOSPECIFIC ANTIBODY POPULATION

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1. Introduction

Antisera raised in animals against human growth hormone (HGH) have previously been shown to cross-react with human placental lactogen (HPL) [1]. Recently, a large homology in amino-acid sequence between HGH and HPL was described [2, 3]. However, differences in the magnitude of the biological activity of the two hormones were observed [4]. The striking similarities between a pituitary and placental protein is intriguing and lead us to investigate whether in this model system for cross-reacting antisera monospecific antibody populations directed against HGH only can be isolated.

We wish to report a resolution of antibodies in an anti-HGH serum into cross-reacting and monospecific populations by affinity chromatography using a specific HPL–immunoadsorbent.

2. Materials and methods

Antiserum to HGH used throughout this study was obtained from a single rabbit immunized with HGH (NIH-GH-HS1268A). NIH-GH-H1207A was used for labelling and as standard. The experiments were carried out with immuno-grade HPL (HPL 196A). Iodination of HGH and HPL was performed by a modification of a method reported previously [5]. We used Sepharose for the attachment of HPL by a modification of methods described recently [6–8]. For the preparation of HPL–Sepharose, 10 ml bed volume Sepharose 6B was activated at pH 11 with 350 mg CNBr at room temperature for 10 min, then washed and incubated at pH 9 and 4° with 10 mg of a highly purified preparation of HPL. The course of the reaction was followed by observing the decrease in 280 nm absorbance of the supernatant with time. HPL tracer was used in some experiments and the decrease in radioactivity of the supernatant with time was recorded. Within two hours more than 70 percent of HPL was attached to the Sepharose. A coupling of better than 95 percent was achieved in all experiments after 12 hr. The immunoadsorbent was thoroughly washed until HPL was no longer detectable in the washing medium and stored in 0.01 M phosphate buffer (pH 7.4), 0.145 M NaCl and 0.02% NaN_3 at 4°.

Anti-HGH serum was diluted 1:10 and passed through a column packed with HPL–Sepharose. The column was washed with buffer and adsorbed antibodies were eluted with 3 M KSCN at neutral pH. The eluted fractions were dialized against buffered saline. The binding capacities of all fractions were determined by a modification of a radioimmunoassay system reported previously [9] using ^{125}I-HGH and ^{125}I-HPL as tracer. Cross reaction was checked by plotting a dose response curve. The data were obtained with a solid-phase radioimmunoassay described recently [13].

3. Results and discussion

Antisera raised in animals against protein antigen usually show a mixture of serum antibodies against different determinants on the immunizing protein [10]. Immunization with a protein which shares common amino acid sequences with a homologous protein elicits cross-reacting antibodies. Human growth hormone (HGH) is a protein of the anterior pituitary and consists of 188 amino acid residues of known sequence [11]. During pregnancy a polypeptide hormone termed human placental lactogen (HPL) is synthesized by the human placenta [12]. HGH and HPL have similar molecular weights and amino acid composition. Eight of twenty tryptic peptides, including the *C*-terminal peptides of HGH and HPL are identical [3]. The similar biological and immunological properties of the two polypeptide hormones are based on the chemical similarity between the two.

An antiserum to HGH was obtained from a single rabbit and showed a strong cross-reaction with HPL. Biphasic titration curves of this anti-HGH serum were obtained by checking its binding capacity at serial dilutions against ^{125}I-HGH and ^{125}I-HPL in a radioimmunologic system. Furthermore, this antiserum showed a biphasic dose response with HPL in a radioimmunoassay system. This biphasic curve is characterized by an initial displacement of ^{125}I-HGH by HPL and then a prolonged plateau where increasing doses of HPL fail to displace the tracer. These two observations suggested to us the presence of two populations of antibodies against HGH, one cross-reacting and the other showing no cross-reaction with HPL.

When the anti-HGH serum was passed through a column of HPL–Sepharose cross-reacting populations of antibodies were adsorbed by the HPL–Sepharose and almost 100 percent of the specific anti-HGH population were recovered in fraction 1. The results are reported in percent binding of tracer of specific activity of 100 μCi/μg in fig. 1. The binding capacity of the antiserum at 1:100 dilution before chromatography on HPL–Sepharose was 69.0 percent against HGH and 64.8 percent against HPL, resp., and these data are shown on the ordinate in fig. 1. The concentration of fraction 1 was equivalent with a 1:100 dilution of the original serum. On rechromatography

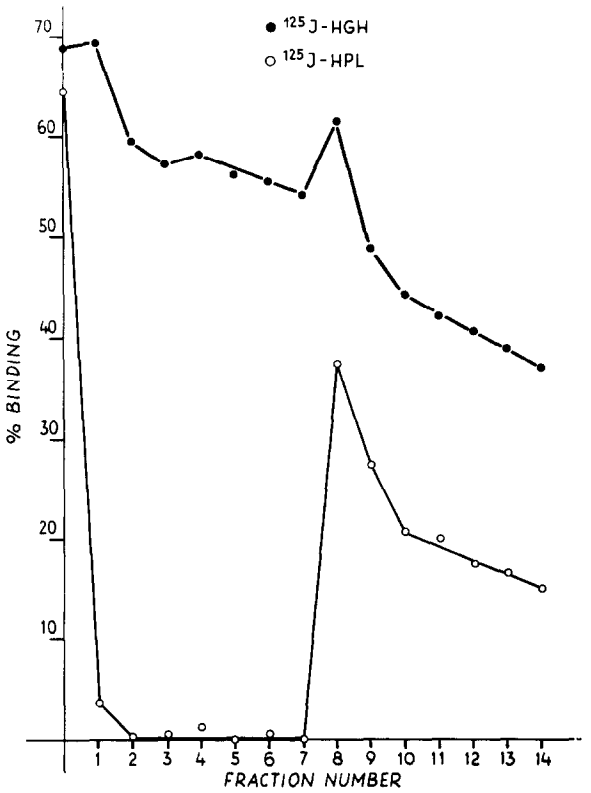

Fig. 1. Chromatography of 1.0 ml anti-HGH serum at a 1:10 dilution on 1 ml bed volume of HPL–Sepharose. Elution was started with phosphate buffered saline (see text) containing 0.5% bovine serum albumin. 10 ml each of fractions 1 through 7 were collected. Elution of bound antibodies was started at fraction 8 with 5 ml each of a 3 M KSCN solution. Percent binding of all fractions was determined by radioimmunoassay. *Ordinate*: percentage binding of the antiserum before chromatography.

of fraction 1 on HPL–Sepharose no further adsorption occurred and the bound antibody peak in fraction 8 was again completely adsorbed, ruling out both incomplete binding due to overload and non-specific adsorption. When anti-HGH serum was applied to uncoupled Sepharose no adsorption was detected either. This is further evidence for the specificity of this system. When fraction 1 was checked in a radioimmunoassay system no displacement of ^{125}I-GHG by HPL occurred. However, a strong displacement and a biphasic dose response with HPL was observed with the original antiserum in the same system under otherwise identical conditions. A single dose response

curve was obtained for unlabelled HGH and HPL, resp., when the unpurified antiserum and ^{125}I-HPL tracer were incubated. No standard curve could be obtained with fraction 1 under conditions otherwise identical to those of the latter experiments.

The following experiments will only be described and are not shown in fig. 1. Elution of adsorbed antibodies was also attempted with KSCN at serial concentrations by starting with 1 M KSCN. After five fractions of 5 ml each the concentration of KSCN in the buffer used for elution was changed to 2 M and another five fractions were collected. This was repeated for 3 and 4 M KSCN. Four distinct peaks were eluted suggesting several antibody populations with different avidities. In another experiment further elution was carried out with 1.5 M HCl after elution with 3 M KSCN was completed. A new cross-reacting antibody population could be eluted indicating the strong binding of this immunoadsorbent.

The experiments described here have shown the presence of several cross-reacting populations of antibodies in this antiserum differing in their avidities. They have permitted the rapid isolation of a monospecific anti-HGH population. Specific determinants in the HGH molecule must be responsible for the production of a monospecific antibody population, and the genes for the pituitary and placental hormones are assumed to be similar. They must contain information for specific amino acid sequences to account for monospecific antibody populations. However, the genes for the placental protein are activated during pregnancy only.

Similar experiments were done for anti-HPL serum and will be reported in detail elsewhere [14]. We are currently studying that part of the HGH molecule which gives rise to the monospecific antibody population. Experiments are in progress to determine the relationship between specificity and avidity of antibodies.

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