A MONOSPECIFIC ANTIBODY POPULATION IN CROSS-REACTING ANTI-HUMAN PLACENTAL LACTOGEN SERUM

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1. Introduction

Several years ago a close structural similarity between human growth hormone (HGH) and human placental lactogen (HPL), also known as human chorionic somatomammotropin (HCS), was proposed on the basis of physiological and immunological data [1–3], and could be confirmed by several studies on the amino acid sequences of the two hormones [4, 5]. Recently we could show that despite this high degree of structural similarity a cross-reacting anti-human growth hormone serum may be resolved into a monospecific antibody population [6].

A very recent publication [7] indicates that the regions of homology seem to include more than 90% of all sequence positions. This finding prompts us to report our experiments on the isolation of a monospecific antibody population from a cross-reacting anti-human placental lactogen serum.

2. Materials and methods

Antiserum to HPL used throughout this study was obtained from a single rabbit immunized with HPL, which was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, USA. Immunoglobulins were prepared from this antiserum by sodium sulphate precipitation. The immunoglobulins were dissolved in 0.01 M phosphate buffer (pH 7.4), 0.145 M NaCl and 0.02% NaN_3 to a volume corresponding to that of the serum and stored deep frozen at $-20°$.
NIH-GH-H1207A and HPL 196A were used for labelling and as standard. Iodination of HGH and HPL was performed by a modification of a method reported previously [8]. The attachment of HGH to Sepharose was done as described recently for HPL [6]. The solution of immunoglobulins was diluted 1:10 and passed through a column packed with HGH-Sepharose. Elution was started with 0.01 M phosphate buffer (pH 7.4), 0.036 M NaCl and adsorbed antibodies were eluted with 3 M KSCN at neutral pH. The eluted fractions were dialyzed against buffered saline. The binding capacities of all fractions were determined by a modification of a radioimmunoassay system reported previously [9] using ^{125}I-HPL and ^{125}I-HGH as tracer. Cross-reaction was checked by plotting a dose-response curve. The data were obtained with a modification of a solid-phase radioimmunoassay system recently described [10].

3. Results and discussion

An antiserum to HPL from a single rabbit showed a strong cross-reaction with HGH. Biphasic titration curves of this anti-HPL serum were obtained when its binding capacities were checked at serial dilutions against ^{125}I-HPL and ^{125}I-HGH in a radioimmunological system (fig. 1). We found a biphasic dose-response for this antiserum by radioimmunoassay. This biphasic curve of the antiserum before chromatography on HGH-Sepharose is characterized by an initial displacement of ^{125}I-HPL by HGH. Then a prolonged plateau is observed where increasing doses of HGH fail to displace the tracer (fig. 2). These observations suggested two populations of antibodies against HPL, one cross-reacting and the other showing no cross-reaction with HGH.

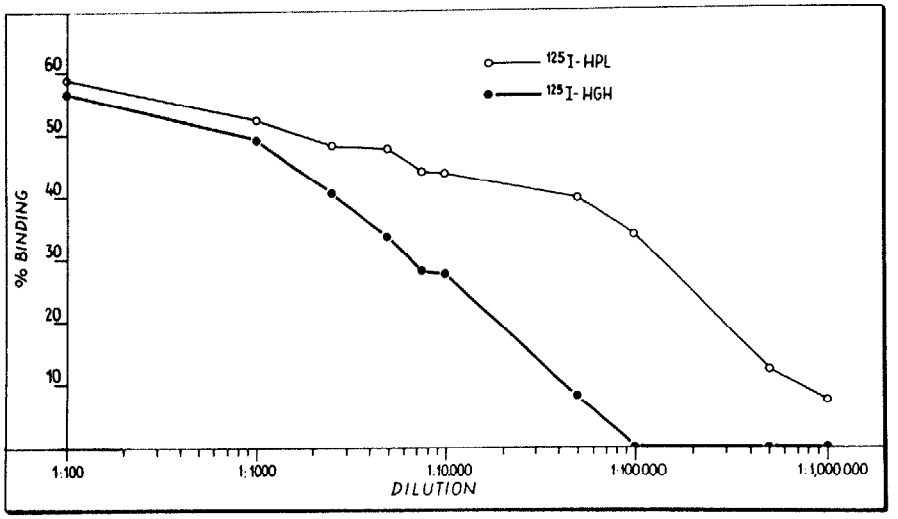

Fig. 1. Titration curves of the unpurified immunoglobulins of the anti-HPL serum.

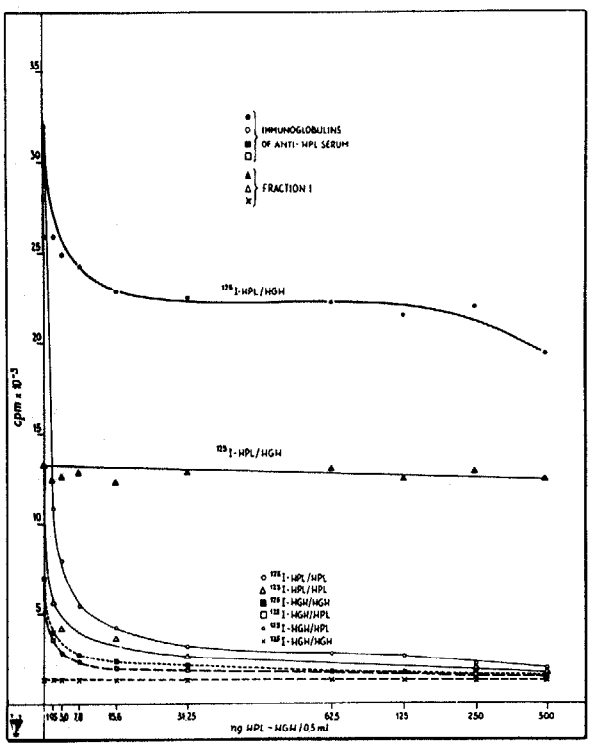

Fig. 2. Dose-response curves of the unpurified immunoglobulins of anti-HPL serum and of fraction 1.

Separation of cross-reacting and monospecific antibody populations was attempted by chromatography of the anti-HPL serum on HGH-Sepharose. The results (fig. 3) are reported in percentage binding of tracer of specific activity of 100 μCi/μg. The binding capacity of the antiserum at 1:100 dilution before chromatography on HGH-Sepharose was 61.1 percent against HPL and 59.8 percent against HGH, respectively, and these data are indicated on the ordinate in fig. 3. Cross-reacting antibody populations were adsorbed by HGH-Sepharose and retarded on the column. Almost 100 percent of the specific anti-HPL population was recovered in fraction 1 as estimated from its binding capacity. The protein concentration in each fraction was determined by the absorbance at 280 nm (fig. 3). 90 Percent of the protein applied to the column was found in fraction 1. The remaining 10 percent could be eluted in fraction 36 by 3 M KSCN. Fraction 1 was rechromatographed on HGH-Sepharose and no further adsorption was detected. Chromatography of the antibody peak of fraction 36 resulted in complete adsorption on HGH-Sepharose. Anti-HPL serum was not adsorbed on uncoupled Sepharose. This rules out both incomplete binding due to overload and non-specific adsorption.

Fraction 1 was checked for cross-reaction in a radioimmunoassay system and compared with the

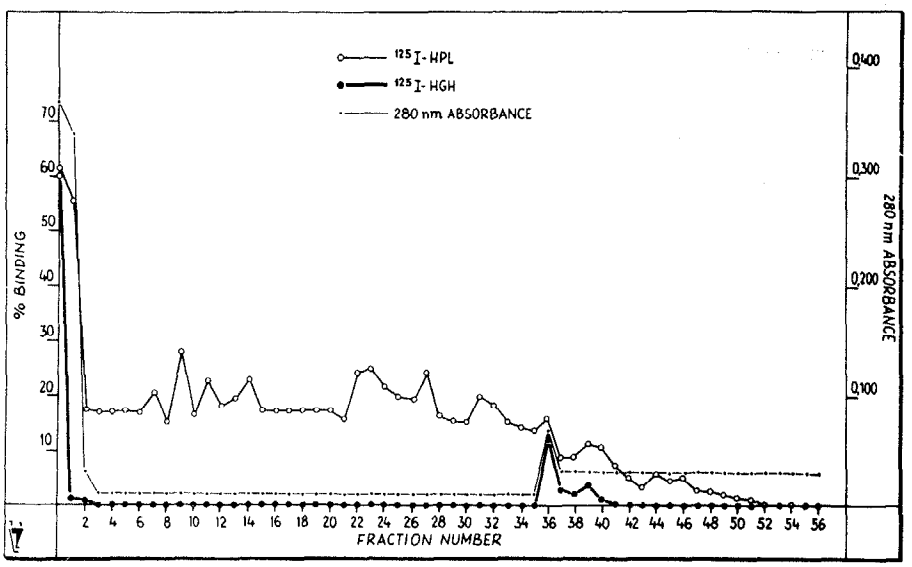

Fig. 3. Chromatography of 1.0 ml immunoglobulin solution of the anti-HPL serum at a 1:10 dilution on 1 ml bed volume of HGH-Sepharose. Elution was started with phosphate buffer (see text) and 10 ml each of fraction 1 through 35 were collected. Elution of bound antibodies was started at fraction 36 with 5 ml each of a 3 M KSCN solution. Left ordinate: percentage binding of the immunoglobulin solution before chromatography.

antiserum before chromatography on HGH-Sepharose (fig. 2). The unpurified anti-HPL exhibited 70 percent cross-reaction with HGH, but no cross-reaction was detectable in fraction 1. A strong displacement of ^{125}I-HPL by HGH and a biphasic dose-response with HGH was observed with the original antiserum in a solid-phase radioimmunoassay system. However, no displacement was noted for fraction 1 in the same system under otherwise identical conditions (fig. 2). We found a single dose-response curve with the original antiserum using ^{125}I-HGH as tracer and unlabelled HPL and HGH, respectively as standard. No standard curve could be obtained with fraction 1 under conditions otherwise identical to those of the latter experiment (fig. 2).

The experiments described here have permitted the rapid isolation of a monospecific antibody population from a cross-reacting anti-HPL serum. This is of interest since radioimmunologic estimations of HPL at early stages of pregnancy, when only small amounts of HPL are present in pregnancy serum, are hampered by the strong cross-reaction of anti-HPL sera with HGH. The second implication of our work described here involves the recent report [7] on the amino acid sequences of HPL and HGH. This indicates that the regions of homology between the two hormones include more than 90 percent of all sequence positions. This suggests that differences in immunological properties between HPL and HGH may depend on comparatively circumscribed sequence differences. Work is in progress to isolate that part of the HPL molecule which elicits the monospecific antibody population.

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