Identification of serum GH-binding proteins in the goldfish (Carassius auratus) and comparison with mammalian GH-binding proteins

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Abstract
The present study constitutes the characterization of a specific, high-affinity GH-binding protein (GHBP) in the serum of a teleost, the goldfish (Carassius auratus). GH-binding assay and ligand blotting techniques were employed to identify GHBPs in goldfish serum and hepatocyte culture medium. The binding characteristics and apparent molecular weights (M_r) of goldfish GHBPs were also compared with those of rabbit and rat. LIGAND analysis identified a single class of high-affinity and low-capacity binding sites for iodinated recombinant carp GH (rcGH) in the goldfish serum, with an association constant (K_a) of 20·1 \times 10^9 M^{-1} and a maximum binding capacity (B_{max}) of 161 fmol ml^{-1} serum. A single class of binding sites for iodinated recombinant sea bream GH and bovine GH (bGH) was also found in goldfish serum, but with a much lower affinity than that of rcGH. The binding affinity for iodinated bGH in rabbit and rat sera was found to be similar to that reported previously. Ligand blotting revealed multiple forms of GHBPs in sera of goldfish, rabbit and rat with M_r ranging from 70 kDa to 400 kDa and 27 kDa to 240 kDa under non-reducing and reducing conditions respectively. A prominent band with M_r of 66 kDa and a minor band with M_r of 27 kDa were observed to occur in sera from all three species under reducing conditions. Iodoacetamide promoted the shedding of three GHBPs with M_r of 25, 40 and 45 kDa from the cultured goldfish hepatocytes. The appearance of all bands was completely inhibited by the presence of excess unlabeled rcGH. Our results provide clear evidence that a GHBP exists in the goldfish and indicate that more information on teleost GHBPs is needed if the physiology of growth in teleosts is to be fully understood.

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Introduction
Circulating growth hormone-binding proteins (GHBP) have been identified and characterized in the serum of a number of mammalian species, including the mouse (Peeters & Friesen 1977, Smith et al. 1989), rabbit (Ymer & Herington 1985), rat (Baumbach et al. 1989, Amit et al. 1990, Massa et al. 1990), dog and pig (Lauteric et al. 1988), sheep (Amit et al. 1992, Davis et al. 1992), goat (Jammes et al. 1996), guinea pig (Ymer et al. 1997), and human (Baumann et al. 1986, Herington et al. 1986). In mouse and rat, GHBP is derived by translation of an alternatively spliced growth hormone receptor (GHR) mRNA lacking the appropriate transmembrane and intracellular domains of the GHR (Baumbach et al. 1989, Smith et al. 1989). In humans and rabbits, GHBP is considered to be largely generated from proteolytic cleavage of the membrane-anchored receptor (Barnard & Waters 1997). The mechanistic details of proteolytic shedding of the human and rabbit GHRs remain unclear, although a recent study in the IM-9 lymphocye culture (Alele et al. 1998) indicated the involvement of a metalloprotease.

In other vertebrate groups, studies on GHBPs are very limited. There are reports of serum GHBPs in avian (Vasilatos-Younken et al. 1991, Davis et al. 1992), a reptilian (Sotelo et al. 1997), and a teleost (Sohm et al. 1998) species. The goal of the present study was to investigate the GHBPs in the circulation of a teleost, the goldfish (Carassius auratus). GH-binding assay and ligand-blotting techniques were employed to identify GHBPs in goldfish serum and from cultured hepatocytes. The binding characteristics and molecular sizes of goldfish GHBPs were also compared with those of rabbit and rat. Our results provide clear evidence that a GHBP exists in the goldfish, as in other vertebrates, and indicate that the physiological role of GHBP in teleosts needs to be investigated in future studies.

Materials and Methods
Experimental animals
Goldfish of the common or comet varieties were maintained as previously described (Zhang & Marchant 1996).
The goldfish were anesthetized by immersion in 0·005% (w/v) tricaine methanesulfonate (MS222). Blood samples were obtained from each fish by inserting a 25 gauge needle attached to a disposable syringe into the caudal vasculature. The blood was centrifuged at 10 000 g and serum was collected and stored at −20 °C. Serum from female New Zealand White rabbits and male Wistar rats was kindly provided by Drs K Prasad and A Richardson (Departments of Physiology and Anatomy, University of Saskatchewan). All animals were used in accordance with guidelines established by the Canadian Council on Animal Care.

Hormones and reagents

Recombinant carp (Cyprinus carpio) GH (rcGH) and sea bream ( Sparus aurata) GH (rsbGH) were kindly provided by Dr A Gertler (The Hebrew University of Jerusalem, Israel). The rcGH used in the present study was the form containing five cysteine residues (Fine et al. 1993). Bovine GH (bGH) and rat prolactin (rPRL) were gifts from the National Hormone and Pituitary Program (NIADDK, Baltimore, MD, USA). Carp prolactin (cPRL) was a gift from Dr R E Peter (University of Alberta, Edmonton, Alberta, Canada). Electrophoresis equipment and reagents were purchased from BioRad Co. (Mississauga, Ontario, Canada). Ultrogel AcA54, iodoacetamide, cell culture reagents, and all other chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Iodination and binding studies

Iodination of the GHs was performed as previously described, using the lactoperoxidase method (Zhang & Marchant 1996). The specific activity of the labeled hormones was routinely more than 100 µCi µg⁻¹ when determined using self displacement (Zhang & Marchant 1996) in liver membrane receptor-binding assay. Binding of goldfish, rabbit and rat sera with iodinated bGH to rabbit and rat serum was found to occur at pH 7·2, but was only slightly reduced (<1%) at pH 6·5, with no alteration in the shape of the 125I-labeled bGH displacement curve (data not shown). Thus, an assay buffer pH of 6·5 was selected in order to allow direct comparisons to be made between rcGH and bGH in all species.

Goldfish hepatocyte culture and iodoacetamide treatment

Preparation of goldfish hepatocytes was modified from Krumschnabel et al. (1994). Goldfish were killed in excess anesthetic (0·05% MS222). Portions of liver were removed under sterile conditions and washed in HEPES buffer (120 mM NaCl, 4·8 mM KCl, 1·2 mM KH2PO4, 24 mM NaHCO3, and 15 mM HEPES, pH 7·5) containing 0·1 mg/ml kanamycin. The liver was then minced and incubated with 0·1% collagenase for 2 h at 26 °C in an atmosphere of 95% air/5% CO2. Digested tissue was filtered through a 75 µm nylon mesh, centrifuged at 50 g for 45 s, and the supernatant removed by aspiration. Sedimented hepatocytes were washed three times in

Ligand blotting of serum GHBP

Ligand blotting of GHBP from goldfish, rabbit and rat sera was performed using a slight modification of published methods (Hocquette et al. 1990, Vasilatos-Younken et al. 1991). Briefly, 20 µg serum protein was separated by SDS-PAGE on a 7·5% gel under both reducing and non-reducing conditions (Laemmli 1970). Under the reducing conditions, serum proteins were treated with 100 mM dithiothreitol (DTT) or 5% (v/v) β-mercaptoethanol prior to separation. Prestained molecular weight standards (Bio-Rad, Richmond, CA, USA) were separated in another lane of the gel. Separated proteins were transferred to nitrocellulose (0·45 µm pore size) using a BioRad mini transfer unit with Towbin buffer (25 mM Tris, 192 mM glycine, and 20% methanol, at pH 7·4). The nitrocellulose membrane was washed with 3% Nonidet P-40 in Tris saline (10 mM Tris, 150 mM NaCl, pH 7·4) for 30 min and placed in blocking buffer (Tris saline containing 2% skim milk powder, 1% BSA, and 0·1% Tween–20, pH 7·4) for 2 h. The nitrocellulose membrane was then incubated with approximately 200 000 c.p.m. tracer in 50 µl assay buffer in the presence or absence of excess unlabeled GH (10 µg/ml) for 24 h at room temperature. Finally, the nitrocellulose membrane was washed, dried, and exposed to X-ray film (Kodak X-Omat AR5 or Biomax MS1) for 4 days at −70 °C.
HEPES buffer and resuspended in a serum-free culture medium (Dulbecco’s modified Eagles’ medium/medium 199, 3:1) containing 0·1 mg/ml kanamycin. Prior to cell seeding, the number of viable cells was counted on a hemocytometer using the dye exclusion method. Only cell preparations with a viability greater than 98% were used for the subsequent experiments. The protein content of hepatocytes was determined using the method of Lowry et al. (1951). Approximately 1 × 10⁶ cells were plated in a 2·5 cm culture dish and incubated in a humidified atmosphere of 95% air/5% CO₂. Cell attachment occurred approximately 40 min after seeding. The attachment efficiency of goldfish hepatocytes was routinely 80% or greater. After cell attachment, fresh medium was added and renewed every 2 days thereafter.

In order to release GHBPs in the culture medium, goldfish hepatocytes cultured for 3 days were treated with 20 mM iodoacetamide for 1·5 h at 26 °C. Similar iodoacetamide treatment has been reported to release a maximum amount of GHBPs into the culture medium from IM-9 lymphocytes (Trivedi & Daughaday 1988, Alele et al. 1998). At the end of the incubation, the medium was collected and any remaining cells were removed by centrifugation. Medium protein (20 µg) was separated by SDS-PAGE on a 12% gel (Laemmli 1970) under both reducing and non-reducing conditions. Separated proteins were then subjected to ligand blotting as described above.

Results

GH-binding studies

Binding of ¹²⁵I-rcGH to goldfish serum was dependent on incubation pH, time, and temperature. At the optimal pH of 6·5, equilibrium was reached 3 h after incubation at room temperature (data not shown). The fraction of ¹²⁵I-rcGH, ¹²⁵I-bGH and ¹²⁵I-rsbGH that would bind to an infinite concentration of serum protein was calculated to be approximately 80% of the total radioactivity added. Therefore, total binding was corrected prior to LIGAND analysis as described previously (Zhang & Marchant 1996). Typical elution profiles for the total and non-specific binding of ¹²⁵I-rcGH to goldfish serum are shown in Fig. 1. Three peaks were observed for the total or non-specific binding profile (Fig. 1). Peak I in the total binding profile represented specific GHBPs fractions which were inhibited by the presence of excess unlabeled rcGH in the incubation mixture prior to gel filtration. The peak I in the non-specific binding profile represented fractions of non-specific binding of ¹²⁵I-rcGH to goldfish serum. Peak II and peak III represented free ¹²⁵I-rcGH and Na ¹²⁵I respectively (Fig. 1). Specific binding of ¹²⁵I-rcGH to serum GHBPs was calculated as the difference between the total and non-specific radioactivity corresponding to the peak I fractions.

LIGAND analysis of ¹²⁵I-rcGH, ¹²⁵I-bGH and ¹²⁵I-rsbGH binding to goldfish serum indicated only a single class of high-affinity and low-capacity binding sites. The Kᵣ for ¹²⁵I-rcGH binding to goldfish serum was approximately tenfold higher than that of ¹²⁵I-bGH and ninefold higher than that of ¹²⁵I-rsbGH (Fig. 2). A single class of high-affinity and low-capacity binding sites for ¹²⁵I-rcGH and ¹²⁵I-bGH was also identified in rabbit and rat serum (Fig. 2). The Kᵣ and Bₘₐₓ for iodinated rcGH, bGH and rsbGH in goldfish, rabbit and rat serum are summarized in Table 1.

Displacement of ¹²⁵I-rcGH from goldfish serum by various unlabeled hormones is shown in Fig. 3. The Kᵣ for each hormone was estimated using the LIGAND program in order to determine the relative cross-reactivities of the various hormones with rcGH as the reference; the Kᵣ of unlabeled rcGH was estimated as 12 ± 4 × 10⁹ M⁻¹ (mean ± s.e.m., n=3). The relative cross-reactivities of rsbGH, bGH, cPRL, and rPRL were found to be 13·8, 10·9, 1·7, and 0·4% respectively.

Displacement of ¹²⁵I-bGH and ¹²⁵I-rcGH from rabbit serum by various unlabeled hormones is shown in Fig. 4. ALLFIT analysis revealed that all parameters of the bGH and rcGH displacement curves were identical in the ¹²⁵I-bGH and ¹²⁵I-rcGH displacement tests (Fig. 4). Prolactins displayed relatively little displacement of either labeled GH (Fig. 4). When ¹²⁵I-bGH was used, the relative cross-reactivities of bGH, rcGH, rsbGH, rPRL, and cPRL as determined by LIGAND analysis were found to be 100%, 99·7%, 2·0%, 0·2%, and 0·05% respectively. In contrast, in the binding assay of ¹²⁵I-rcGH to rabbit serum, the relative cross-reactivities of rcGH, bGH,
rsbGH, cPRL, and rPRL were found to be 100%, 102.6%, 2.9%, 0.22%, and 0.17% respectively.

**Ligand-blotting studies**

The serum GH-binding site consisted of multiple bands with high apparent molecular masses ($M_r$) in the goldfish (70, 80, 120, 180, 240, 360, and 400 kDa), rabbit (80, 120, 180, and 240 kDa), and rat (180 and 240 kDa) serum (Fig. 5). Sera from all three species contained the 180 kDa and 240 kDa bands. Two bands with $M_r$ of 80 kDa and 120 kDa were also found in both goldfish and rabbit sera. The $M_r$ of the labeled bands in all three species was altered under the reducing conditions. The $M_r$ of the bands under reducing conditions ranged from 27 to 160 kDa in the goldfish and rat sera and 27 to 240 kDa in the rabbit serum (Fig. 5). A prominent band with $M_r$ of 66 kDa and a minor band with $M_r$ of 27 kDa were observed to occur in sera from all three species under reducing conditions.

**Table 1** Comparison of bGH-, rcGH- and rsbGH-binding sites in rabbit, rat and goldfish sera. The $K_a$ and $B_{max}$ values were obtained by LIGAND analysis of the results presented in Fig. 2. All data are expressed as means ± S.E.M. ($n=3$).

<table>
<thead>
<tr>
<th>Source of sera</th>
<th>Hormone</th>
<th>$K_a$ ($10^9$ M$^{-1}$)</th>
<th>$B_{max}$ (fmol/mg protein)</th>
<th>$B_{max}$ (fmol/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldfish</td>
<td>rcGH</td>
<td>20.1 ± 1.8</td>
<td>4.1 ± 0.2</td>
<td>161 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>rsbGH</td>
<td>2.8 ± 0.06</td>
<td>4.1 ± 0.2</td>
<td>158 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>bGH</td>
<td>2.0 ± 0.04</td>
<td>4.0 ± 0.1</td>
<td>162 ± 6.5</td>
</tr>
<tr>
<td>Rabbit</td>
<td>bGH</td>
<td>9.1 ± 0.2</td>
<td>49.8 ± 3.2</td>
<td>3540 ± 230</td>
</tr>
<tr>
<td></td>
<td>rcGH</td>
<td>9.5 ± 0.3</td>
<td>46.6 ± 3.7</td>
<td>3310 ± 261</td>
</tr>
<tr>
<td></td>
<td>rsbGH</td>
<td>1.1 ± 0.2</td>
<td>41.8 ± 4.5</td>
<td>2970 ± 316</td>
</tr>
<tr>
<td>Rat</td>
<td>bGH</td>
<td>1.2 ± 0.1</td>
<td>83.8 ± 8.9</td>
<td>6030 ± 639</td>
</tr>
<tr>
<td></td>
<td>rcGH</td>
<td>1.1 ± 0.1</td>
<td>80.2 ± 9.8</td>
<td>5780 ± 706</td>
</tr>
<tr>
<td></td>
<td>rsbGH</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, $K_a$ and $B_{max}$ values are not available due to low (<2%) specific binding of the labeled hormone.

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appearance of all bands was completely inhibited by the presence of 10 µg/ml of the unlabeled rcGH (data not shown).

Iodoacetamide promoted the shedding of multiple forms of GHBPs from the goldfish hepatocyte culture, whereas no GHBP was detected in the conditioned medium in the absence of iodoacetamide (Fig. 6). The GHBPs from goldfish hepatocyte culture consisted of three bands with Mr of 25, 40 and 45 kDa (Fig. 6). The appearance of these bands was completely inhibited by the presence of 10 µg/ml unlabeled rcGH (Fig. 6). The Mr of these bands was not altered under reducing conditions.

Discussion

The existence of specific GHBPs in the serum and hepatocyte culture of goldfish is confirmed by rcGH binding and ligand-blotting studies. LIGAND analysis of 125I-rcGH binding to goldfish serum indicated a single class of high-affinity and low-capacity binding sites with a Ka of 20·1 × 109 M⁻¹. In mammals, GHBPs can be divided into different types based on GH-binding affinity. Type I and type II GHBPs display low-affinity GH binding. Type I GHBPs include the mouse and rat, with a binding affinity of 1·2–3·9 × 10⁹ M⁻¹ (Amit et al. 1992). Type II GHBPs have even lower GH-binding affinity than type I GHBPs, and are found in the sheep (Amit et al. 1992, Davis et al. 1992), goat (Jammes et al. 1996), and cow (Gertler et al. 1984, Devolder et al. 1993). All type III GHBPs have high-affinity binding with GHs (4·7–9·2 × 10⁹ M⁻¹), and are present in the rabbit (Ymer & Herington 1985), dog (Lauteric et al. 1988), and horse (Amit et al. 1992).

Among all the species studied to date, goldfish serum GHBP has the highest Ka value. A recent study (Sohm et al. 1998) indicated that binding of rainbow trout GH to homologous serum was of low affinity (3·8 × 10⁸ M⁻¹), although binding of human GH to rainbow trout serum was of higher affinity (1·2 × 10⁹ M⁻¹). It is not clear why there are such large variations in the binding affinity of teleost GHBPs. One possibility is that there are different categories of serum GHBPs in teleosts, as in mammals. However, such variations may also be attributed to differences in hormone purity or bioactivity, assay conditions, or the methods used to calculate hormone-binding parameters.

In goldfish, the affinity of the serum GHBP (20·1 × 10⁹ M⁻¹) and liver membrane GHR (19 × 10⁹ M⁻¹; Zhang & Marchant 1996) for rcGH are very similar. This implies a close relationship between goldfish serum GHBP and the liver membrane GHR. A similar Ka for serum GHBP and liver membrane GHR was also found in the rabbit (Leung et al. 1992).
was significantly higher than that for 125I-bGH. Analysis of displacement of 125I-rcGH from goldfish serum by various unlabeled hormones also indicates that 125I-rcGH binding provides one explanation for the di
directly from proteolytic cleavage of the membrane GHR of an alternatively spliced GHR mRNA, rather than et al. to be 20-fold lower than that of rat liver GHR (Baxter et al. 1989, Smith et al. 1992) measured the a value for 125I-rcGH from rainbow trout (Sohm et al. 1990) was found to be considerably higher in the rabbit (205 to 1.5 M 20 fmol/ml serum or greater). Amit et al. (1990) measured the affinity constants for serum GHBP complexes in all 

Figure 6 Autoradiogram of nitrocellulose membrane following ligand blotting of 125I-rcGH to the culture medium from goldfish hepatocytes treated with (lanes A and C) or without (lane B) iodoacetamide. Samples were separated by SDS-PAGE under non-reducing conditions; the M, of the bands was not altered under reducing conditions (data not shown). Positions of molecular weight standards (kDa) are shown on the left. All bands disappeared when the nitrocellulose membrane was incubated with labeled GH in the presence of 10 µg/ml unlabeled GH (lane C).

et al. 1987, Spencer et al. 1988), where the GHBP appears to be largely generated from proteolytic cleavage of the membrane GHR (Barnard & Waters 1997). In contrast, the Kd of the rat serum GHBP (Massa et al. 1990) was found to be 20-fold lower than that of rat liver GHR. Baxter et al. 1980). Rat serum GHBP originates from translation of an alternatively spliced GHR mRNA, rather than directly from proteolytic cleavage of the membrane GHR (Baumbach et al. 1989, Smith et al. 1989), which may provide one explanation for the difference between the Kd of the serum GHBP and liver membrane GHR in the rat.

In the goldfish GHBP assay, the Kd value for 125I-rcGH was significantly higher than that for 125I-bGH. Analysis of displacement of 125I-rcGH from goldfish serum by various unlabeled hormones also indicates that 125I-rcGH binding to goldfish serum was highly specific for teleost GHs. rsbGH was found to be less potent than rcGH, but was slightly more potent than the mammalian GH. Prolactins were considerably less potent than GHs, suggesting that the goldfish serum GHBP was specific for somatogenic hormones. This pattern of hormone specificity is similar to the goldfish GHR (Zhang & Marchant 1996), and further indicates that the goldfish GHR/GHBP is very species-specific in terms of hormone binding.

Our results also indicate that rcGH, but not rsbGH, cross-reacts highly with serum GHBP from the rabbit and rat. These results are in agreement with our previous findings for GHRs in rabbit and rat liver membranes (Y Zhang & T A Marchant, unpublished observations). High cross-reaction of rcGH with the single class of high-affinity serum GH-binding sites was also evident in the displacement of labeled bGH and rcGH from rabbit serum by various unlabeled hormones. In these experiments, displacement curves from unlabeled rcGH were similar to those of unlabeled bGH. In both the 125I-bGH and 125I-rcGH displacement tests in the rabbit, prolactins were considerably less potent than GHs suggesting that the rabbit binding sites were also specific for GHs.

It is not clear why rcGH but not rsbGH shows high cross-reactivity with mammalian GHRs (Y Zhang & T A Marchant, unpublished observations) and GHBP (present study). The rcGH used in the present study contains an extra cysteine residue at position 123, as found in the common carp (Fine et al. 1993, Law et al. 1996). This cysteine does not appear to be directly involved in binding of rcGH to the receptor (Fine et al. 1993). Goldfish also possess cDNAs for two GHs; one has cysteine at position 123, whereas this position in the other GH contains a serine (Mahmoud et al. 1996). Thus, goldfish and other cyprinid GHs may require a polar amino acid at position 123, which is not necessarily a cysteine (Mahmoud et al. 1996). Further studies will be required to fully understand the structure–activity relationship between GH and its receptor or binding protein in teleosts.

In the present study, the Bmax value for serum GHBP was found to be considerably higher in the rabbit (3300 fmol/ml serum) and rat (6000 fmol/ml serum) than that in the goldfish (160 fmol/ml serum). Amit et al. (1992) measured the affinity constants for serum GHBP in a number of mammalian species, including rabbit, rat, mouse, sheep, cow, horse, cat, monkey, and human, and reported that the Bmax varied between 140 fmol/ml serum and 19 200 fmol/ml serum (Amit et al. 1992). Among other species studied to date, turtle GHBP was found to have a Bmax of 1080 fmol/ml serum (Sotelo et al. 1997), whereas the Bmax values for serum GHBP from rainbow trout (Sohm et al. 1998) and chicken (Davis et al. 1992) were reported to be remarkably higher, ranging from 1.3 x 105 to 1.5 x 106 fmol/ml serum or greater. The physiological significance for such large variations in Bmax value between different species remains unknown.

Ligand blotting also identified GHBP in goldfish serum and culture medium of goldfish hepatocytes. Goldfish, rabbit and rat sera contained GHBP of large Mr, migrating from 120 kDa to 360 kDa. These large GHBP complexes in rabbit and rat sera have previously been identified by gel filtration (Ymer & Herington 1985, Baumbach et al. 1989, Amit et al. 1990, Massa et al. 1990). In the present study, DTT or β-mercaptoethanol treatment resulted in the reduction of the Mr of the serum GHBP complexes in all species, suggesting that the large bands observed by ligand

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blotting contain disulfide bonds. Similar results have been reported for human serum GHBP (Hocquette et al. 1990).

Large $M_r$ GHBPs were also evident in rainbow trout serum (Sohm et al. 1998), where 130 and 150 kDa complexes were identified by cross-linking of $^{125}$I-hGH to rainbow trout serum. However, the small $M_r$ GHBPs in goldfish serum could not be detected by cross-linking of $^{125}$I-rcGH to goldfish serum (Y Zhang & T A Marchant, unpublished data). Previous studies utilizing cross-linking techniques were also unable to detect small $M_r$ GHBPs in rabbit serum (Leung et al. 1987, Baumann & Shaw 1990). Thus, ligand blotting rather than cross-linking seems to be more appropriate for examining the complete range of serum GHBPs.

In the goldfish, the smallest $M_r$ of serum GHBP under reducing conditions was found to be 27 kDa. A 27 kDa GHBP was also identified in serum of chickens and turkeys (Vasilatos-Youken et al. 1991). Previous studies on the primary structure of GHR in rat (Baumbach et al. 1989) and mouse (Smith et al. 1989) have indicated that the $M_r$ of the extracellular domain of the GHRs is approximately 30 kDa. Thus, the 27 kDa GHBP observed in the present study may represent the extracellular portion of the goldfish GHR. The release of small sized GHBPs from cultured goldfish hepatocytes by iodoacetic acid suggests that a mechanism of proteolytic cleavage of membrane GHRs may also be involved in the generation of goldfish GHBP. However, additional studies will be required to determine the nature of the large $M_r$ GHBPs in the goldfish.

The goldfish GHBPs resemble GHBPs from other species by displaying a specific high-affinity and low-capacity binding for GH, presence of multiple forms of GHBPs in the circulation, and a close relationship with hepatic GHRs. Although circulating GHBPs have been found in all species tested to date, the precise physiological role of GHBPs remains unclear (Barnard & Waters 1997). We have previously established and validated a goldfish GH receptor-binding assay (Zhang & Marchant 1996). Together, these results indicate that the goldfish will be a very useful model for studies on the physiological interaction between GH, GHR, and GHBP in teleosts.

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Krumbschabel G, Schwarzbaum PJ & Wieser W 1994 Coupling of mide suggests that a mechanism of proteolytic cleavage of the extracellular domain of the GHRs is $M_r$ of the extracellular domain of the GHRs is approximately 30 kDa. Thus, the 27 kDa GHBP observed in the present study may represent the extracellular portion of the goldfish GHR. The release of small sized GHBPs from cultured goldfish hepatocytes by iodoacetic acid suggests that a mechanism of proteolytic cleavage of membrane GHRs may also be involved in the generation of goldfish GHBP. However, additional studies will be required to determine the nature of the large $M_r$ GHBPs in the goldfish.

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