High affinity insulin-like growth factor-binding proteins (IGFBP-1 to -6) are a family of structurally homologous proteins that induce cellular responses by insulin-like growth factor (IGF)-dependent and -independent mechanisms. The IGFBP-3 receptor, which mediates the IGF-independent growth inhibitory response, has recently been identified as the type V transforming growth factor-β receptor (TβR-V) (Leal, S. M., Liu, Q. L., Huang, S. S., and Huang, J. S. (1997) J. Biol. Chem. 272, 20572–20576). To characterize the interactions of high affinity IGFBPs with TβR-V, mink lung epithelial cells (Mv1Lu cells) were incubated with 125I-labeled recombinant human IGFBPs (125I-IGFBP-1 to -6) in the presence of the cross-linking agent disuccinimidyl suberate and analyzed by 5% SDS-polyacrylamide gel electrophoresis and autoradiography. 125I-IGFBP-3, -4, and -5 but not 125I-IGFBP-1, -2, and -6 bound to TβR-V as demonstrated by the detection of the ~400-kDa 125I-IGFBP/TβR-V cross-linked complex in the cell lysates and immunoprecipitates. The analyses of 125I-labeled ligand binding competition and DNA synthesis inhibition revealed that IGFBP-3 was a more potent ligand for TβR-V than IGFBP-4 or -5. Most of the high affinity 125I-IGFBPs formed dimers at the cell surface. The cell-surface dimer of 125I-IGFBP-3 preferentially bound to and was cross-linked to TβR-V in the presence of disuccinimidyl substrate. IGFBP-3 did not stimulate the cellular phosphorylation of Smad2 and Smad3, key transducers of the transforming growth factor-β type I/type II receptor (TβR-I/TβR-II) heterocomplex-mediated signaling. These results suggest that IGFBP-3, -4, and -5 are specific ligands for TβR-V, which mediates the growth inhibitory response through a signaling pathway(s) distinct from that mediated by the TβR-I and TβR-II heterocomplex.

High affinity insulin-like growth factor-binding proteins 1–6 (IGFBP-1 to -6) are a family of structurally homologous ~24–43-kDa proteins composed of three defined domains including a nonconserved central domain flanked by conserved cysteine-rich N- and C-terminal domains (1–3). Recently, several low affinity IGFBPs with sequence homology to the N-terminal domains of the high affinity IGFBPs have been identified and referred to as IGFBP-7 to -10 (4).

High affinity IGFBPs are produced by a variety of cell types and tissues (1–3). They coordinate and regulate the biological activities of IGF-I and IGF-II by serving as transporter proteins or carriers and by scavenging IGFs from IGF receptors (1–3). High affinity IGFBPs have also been shown to induce cellular responses in an IGF-independent manner (5–15). These IGF-independent actions of high affinity IGFBPs are believed to be mediated by specific cell-surface receptors or membrane proteins (6, 16–18). The IGFBP-3 receptor, which mediates the IGF-independent growth inhibitory response, has been recently identified as the type V TGF-β receptor (TβR-V) (19).

The TβR-V is a 400-kDa non-proteoglycan membrane glycoprotein (20). It is a Ser-specific protein kinase and co-expresses with type I, type II, and type III TGF-β receptors (TβR-I, TβR-II, and TβR-III) in most cell types (21–23). The TβR-V is a low affinity TGF-β receptor with $K_d$ of ~0.4 nM for TGF-β1 and TGF-β2, and ~5 nM for TGF-β3 (23, 24). Nevertheless, several lines of evidence suggest that TβR-V is important in mediating TGF-β-induced growth inhibitory responses. These include the following: 1) cells lacking TβR-V but expressing TβR-I, TβR-II, and TβR-III do not exhibit the growth inhibitory response to stimulation by exogenous TGF-β, although exogenous TGF-β is able to induce transcriptional activation of plasminogen activator inhibitor 1 and fibronectin in these cells (24); 2) TβR-V mediates the growth inhibitory response in the absence of TβR-I or TβR-II, but both TβR-I and TβR-II are required for maximal growth inhibition (24); and 3) the cells lacking TβR-V have been found to be carcinoma cells, whereas all normal cell types studied express TβR-V (19, 21, 24). This implies that the loss of TβR-V, which mediates negative growth regulation, may contribute to malignancy of certain carcinoma cells (19, 21, 24).

To define the function of TβR-V, we developed specific peptide antagonists that showed higher affinity to TβR-V than to other TGF-β receptor types (25). The structural and functional analyses of these peptide antagonists revealed that a W/RXXD motif is essential for the antagonist activity. Multiple conjugation of the peptide antagonists to carrier proteins conferred TGF-β agonist activity in growth inhibition but not in transcriptional activation (25). These results prompted us to identify structurally unrelated TGF-β agonists that possess the W/RXXD motif. IGFBP-3 was the first TGF-β agonist identified (19). IGFBP-3 possesses a putative TGF-β active site motif (WCVD) near its C terminus (1–3).

Because IGFBP-3 is structurally homologous to other high affinity IGFBPs and because four of six high affinity IGFBPs (IGFBP-3 to -6) possess the putative TGF-β active site motif...
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(VCVD) near their C termini (1–3), we hypothesized that at least some of these IGFBPs might bind to TβR-V, which may mediate the IGF-independent activities of these IGFBPs. To test this hypothesis, we characterized the interactions of IGFBP-1 to -6 with TβR-V in mink lung epithelial cells (Mv1Lu cells). In this communication, we show that IGFBP-3, -4, and -5 but not IGFBP-1, -2, or -6 bind to TβR-V as demonstrated by 125I-labeled ligand affinity labeling of TβR-V in Mv1Lu cells. IGFBP-4 and -5 bind to TβR-V with lower affinities than that of IGFBP-3. The cell surface-associated dimeric form of IGFBP-3 exhibits a preference for binding to TβR-V. We also demonstrate that IGFBP-3-induced growth inhibition mediated by TβR-V does not involve the stimulated phosphorylation of Smad2 and Smad3.

**EXPERIMENTAL PROCEDURES**

Materials—Na[125I] (17 Ci/mg), [methyl-3H]thymidine (67 Ci/mmol), and [32P]orthophosphate (500 mCi/mmol) were purchased from ICN Biochemicals, Inc. (Costa Mesa, CA). High and low molecular mass protein standards, recombinant human IGF-1, avidin-agarose, and other chemicals were purchased from Sigma. Disuccinimidyl suberate (DSS) and sulfo-NHS-biotin were obtained from Pierce. Anti-Smad2, anti-Smad3, and goat IgG were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Recombinant human TGF-β1, IGFBP-2, IGFBP-4, IGFBP-5, and IGFBP-6 were obtained from Australl Biologics (San Ramon, CA). Recombinant nonglycosylated human IGFBP-3 (expressed in Escherichia coli) was provided by Celsrix Pharmaceutical, Inc. (Santa Clara, CA). 125I-Labeled IGFBPs (125I-IGFBPs) and antiserum to TβR-V were prepared according to our published procedures (19). The TGF-β1, peptide antagonist, β1-(41–65), was synthesized as described previously (25). Mv1Lu cells were grown and maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal calf serum (FCS).

125I-IGFBP Affinity Labeling of Mv1Lu Cells—Mv1Lu cells grown to confluence in 35-mm-diameter Petri dishes were incubated with 1 nM 125I-IGFBPs (specific activity, 10–90 mCi/mmol) in the presence of various concentrations (1–100 nM) of unlabeled IGFBPs with and without 10 μM MβC (41–65) in binding medium (125 mM NaCl, 5 mM KCl, 5 mM MgSO4, 2 mM CaCl2, 50 mM Hepes, pH 7.5) at 0 °C for 4 h. After 125I-IGFBP affinity labeling in the presence of DSS (19), the 125I-IGFBP/TβR-V complex was cross-linked with DSS and identified by 5% SDS-PAGE under reducing conditions and autoradiography.

[methyl-3H]Thymidine Incorporation Assay—Mv1Lu cells were plated on 24-well cluster dishes at near confluence in DMEM containing 10% FCS. After 18 h at 37 °C, the cells were plated on 24-well cluster dishes at near confluence in DMEM containing 0.1% FCS. After 18 h at 37 °C, the cells were rinsed twice with 1 ml of solubilization buffer containing Triton X-100 (0.2%) and 0.5 mM NaCl followed by two more washes with salt-free solubilization buffer. Concentrated SDS sample buffer (2×) containing β-mercaptoethanol was added to the agarose beads. The bead suspension was boiled for 5 min and vortexed vigorously to release biotinylated IGFBP-3 and 125I-IGFBP-3 from the beads. The agarose beads were pelleted, and the supernatant was analyzed by SDS-PAGE and autoradiography.

[32P]Orthophosphate Metabolic Labeling and Immunoprecipitation by Anti-Smad2 and Anti-Smad3 IgGs—Mv1Lu cells were plated on 100-mm Petri dishes in DMEM containing 10% FCS at near confluence. After 16–18 h at 37 °C, cells (14 × 106 total) were rinsed twice with 1 ml of phosphate-free DMEM and incubated with 1 μCi [32P]orthophosphate for 1 h at 37 °C. Cells were metabolically labeled with 1.0 Ci/ml [32P]orthophosphate in phosphate-free DMEM containing 0.2% diazoyl FCS for 2 h at 37 °C. The TβR-V was cross-linked with DSS and identified by 5% SDS-PAGE under reducing conditions and autoradiography.

**RESULTS**

IGFBP-3, -4, and -5 Bind to TβR-V with Different Affinities—Mv1Lu cells have been used as a model cell system to investigate TβR-V and other TGF-β receptor types and TGF-β-induced cellular responses (19, 20, 24, 26). To determine the interactions of TβR-V with IGFBPs, we first performed ligand affinity labeling of TβR-V in Mv1Lu cells using 125I-labeled recombinant human IGFBP-1 to -6 (125I-IGFBP-1 to -6). After incubation of Mv1Lu cells with 5 nM 125I-IGFBP-1, -2, -3, -4, -5, or -6 at 0 °C for 3 h, the ~400-kDa 125I-IGFBP-TβR-V complex was cross-linked with DSS and identified by 5% SDS-PAGE under reducing conditions and autoradiography. As shown in Fig. 1, the 125I-IGFBP-TβR-V complex was detected in the lysates of cells that were affinity-labeled with 125I-IGFBP-3, 125I-IGFBP-4, or 125I-IGFBP-5 (lanes 1, 3, and 5). The specificity of the affinity labeling of TβR-V was confirmed by blocking with β1-(41–65), a specific TGF-β1, antibody (25) (lanes 2, 4, and 6). The 125I-IGFBP-TβR-V complex was not detected in the lysates of cells affinity-labeled with 125I-IGFBP-1, -2, or -6 (lanes 7, 9, and 11). The 125I-IGFBP-TβR-V complex was verified by its immunoprecipitation with specific antiserum to TβR-V (Fig. 2, lanes 2, 5, and 8). The immunoprecipitation of the 125I-IGFBP-3/TβR-V complex by antiserum to TβR-V was previously reported (19). It is of importance to note that all 125I-IGFBPs except 125I-IGFBP-2 formed covalently linked dimers that were stable after treatment at 100 °C for 5 min in 0.1% SDS containing β-mercaptoethanol and subsequent SDS-PAGE (Fig. 1).

The Kd of IGFBP-3 binding to TβR-V was previously estimated to be ~6 nM (19). To determine the relative affinities of IGFBP-4 and -5 to TβR-V in Mv1Lu cells, we performed competition experiments using 125I-IGFBP-3 (1 nM) as the ligand and unlabeled IGFBP-3, -4, and -5 as competitors. As shown in Fig. 3A, increasing concentrations of unlabeled IGFBP-3 quantitatively inhibited 125I-IGFBP-3 binding to TβR-V as determined by 125I-IGFBP-3 affinity labeling of TβR-V. The quantitative analysis of this inhibition revealed that unlabeled IGFBP-3 blocked the 125I-IGFBP-3 binding with an IC50 of ~6 nM, which is identical with the estimated Kd of IGFBP-3 binding to TβR-V (19) (Fig. 3B). Unlabeled IGFBP-4 and -5 weakly inhibited 125I-IGFBP-3 binding to TβR-V with an IC50 of >100 nM (Fig. 3B). We also determined the effects of various concentrations of unlabeled IGFBP-1, -2, and -6 on 125I-IGFBP-3 binding to TβR-V. Unlabeled IGFBP-1, -2, and -6 did not show any significant effect on the binding of 125I-IGFBP-3 to TβR-V.

**DISCUSSION**

The present findings demonstrate that IGFBP-3, -4, and -5 bind to TβR-V and that IGFBP-3-induced growth inhibition mediated by TβR-V does not involve the stimulated phosphorylation of Smad2 and Smad3. These results not only support the hypothesis that IGFBPs mediate the IGF-independent activities of these IGFBPs but also provide additional evidence that TβR-V is involved in the IGF-independent activities of these IGFBPs.

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at concentrations up to 100 nM (data not shown). These results suggest that IGFBP-3 binds to TβR-V with higher affinity than IGFBP-4 and -5 and that IGFBP-1, -2, and -6 are not ligands for TβR-V.

IGFBP-3 was previously shown to inhibit growth of Mv1Lu cells as measured by DNA synthesis (19). This inhibition appeared to be mediated by TβR-V because the IGFBP-3-induced growth inhibition was blocked in the presence of TGF-β1-(41–65), a TGF-β/IGFBP-3 antagonist that blocked IGFBP-3 binding to TβR-V (19). Because IGFBP-3, -4, and -5 bind to TβR-V with different affinities, we determined the relative potencies of IGFBP-3, -4, and -5 for DNA synthesis inhibition in Mv1Lu cells. As shown in Fig. 4, at 1 µg/ml (~33 nM) IGFBP-3 inhibited ~50% of DNA synthesis of Mv1Lu cells, whereas IGFBP-4 and -5 produced ~15–20% inhibition of DNA synthesis at the same concentration. The potent DNA synthesis inhibitory activity of IGFBP-3 is consistent with its high affinity to TβR-V. We also determined the effects of IGFBP-1, -2, and -6 on DNA synthesis of Mv1Lu cells. IGFBP-1, -2, and -6 exhibited ~5–10% inhibition of DNA synthesis of Mv1Lu cells at 1 µg/ml (data not shown). This inhibition may be because of scavenging endogenous IGFs from the IGF-1 receptor. IGF-1 is a weak growth factor or mitogen for Mv1Lu cells under these experimental conditions.

FIG. 1. 125I-IGFBP affinity labeling in Mv1Lu cells. Cells were incubated with 5 nM 125I-IGFBP-1 to -6 in the presence (+) and absence (−) of 10 µM β1-(41–65). After 3 h at 0 °C, the 125I-IGFBP affinity labeling was carried out and analyzed by 5% SDS-PAGE under reducing conditions and autoradiography. The arrows indicate the location of the ~400-kDa 125I-IGFBP:TβR-V complex. The asterisks denote the locations of the covalently linked 125I-IGFBP dimers.

FIG. 2. Immunoprecipitation of the 125I-IGFBP-3, -4, and -5 affinity-labeled TβR-V in Mv1Lu cells. Cells were incubated with 10 nM 125I-IGFBP-3 (A), 25 nM 125I-IGFBP-4 (B), or 25 nM 125I-IGFBP-5 (C) both with and without 10 µM β1-(41–65), a TGF-β/IGFBP-3 antagonist. After 3 h at 0 °C and affinity labeling, cell lysates were immunoprecipitated with antiserum to TβR-V or non-immune (Control serum) serum (19). The immunoprecipitates were analyzed by 5% SDS-PAGE under reducing conditions and autoradiography. The brackets indicate the locations of the 125I-IGFBP:TβR-V complexes. The asterisk denotes the location of the 125I-IGFBP-3 dimer. The arrows indicate the location of dye front.
using chloramine T. To test this possibility, we investigated the formation of the covalently linked dimer of 125I-IGFBP-3 in aqueous solution and at the cell surface of Mv1Lu cells in the absence of added cross-linking agents. As shown in Fig. 5, less than 2% of 125I-IGFBP-3 (5 nM) spontaneously formed co-

valently linked dimers in binding medium (lane 1). However, approximately 20% of the 125I-IGFBP-3 associated with the cell surface was found to be covalently linked dimers (lane 2). These results indicate that the formation of the covalently linked 125I-IGFBP-3 dimer does not require the presence of cross-linking agents. These results also suggest that the 125I-IGFBP-3 dimer formation may be enhanced at the cell surface. Alternatively, the 125I-IGFBP-3 dimer may associate with the cell surface of Mv1Lu cells with an affinity higher than that of the 125I-IGFBP-3 monomer.

As described above, we demonstrated the covalently linked dimer formation of 125I-IGFBP-3 or other 125I-IGFBPs by taking advantage of the properties of covalent linking of 125I-IGFBPs prepared by the chloramine-T procedure. To prove that the dimer formation is an inherent property of IGFBP-3, we determined the formation of the IGFBP-3 dimer using an approach in which IGFBP-3 was tagged with 125I or biotin. The formation of the IGFBP-3 dimer was detected by identifying the 125I-IGFBP-biotinylated IGFBP-3 complex in the lysates of Mv1Lu cells that were incubated with a premix of 125I-IGFBP-3 and biotinylated IGFBP-3 (4:1, mol/mol). After 2.5 h at 0 °C, the cell lysates were incubated with avidin-agarose. After centrifugation, the avidin-agarose pellets were analyzed by 7.5% SDS-PAGE under reducing conditions and autoradiography. The arrows indicate the locations of the covalently linked 125I-IGFBP dimer and 125I-IGFBP-3 monomers. The relative intensities of covalently linked IGFBP-3 dimers and monomers were quantitated using a PhosphorImager.
IGFBP-3 in Mv1Lu cells. Cells were incubated with a premix of 125I-IGFBP-3 and biotinylated IGFBP-3 (4:1, mol/mol) in the presence and absence of a 100-fold excess of unlabeled IGFBP-3. After 3 h at 0 °C, the cells were washed; the cell lysates were incubated with avidin-agarose with or without ~1.6 mM biotin at 4 °C for 1 h. After centrifugation, the pellets were analyzed by 12% SDS-PAGE under reducing conditions and autoradiography. The exposure times for the autoradiograms of the medium and cell lysates were 16 and 2 h, respectively, to have comparable intensities for 125I-IGFBP-3 dimers. In the presence of the cross-linking agent DSS, most of the covalently linked dimer associated with the cell surface was cross-linked with TβR-V and putative TβR-I heterocomplex (lane 3 versus 2). These results are also consistent with our previous observation that both TβR-V and 125I-IGFBP-3 dimers were immunoprecipitated by specific antiserum to TβR-V after the 125I-IGFBP-3-affinity labeling of TβR-V in Mv1Lu cells (19).

**FIG. 6.** Complex formation of 125I-IGFBP-3 with biotinylated IGFBP-3 in Mv1Lu cells. Cells were incubated with a premix of 125I-IGFBP-3 and biotinylated IGFBP-3 (4:1, mol/mol) in the presence and absence of a 100-fold excess of unlabeled IGFBP-3. After 3 h at 0 °C, the cells were washed; the cell lysates were incubated with avidin-agarose with or without ~1.6 mM biotin at 4 °C for 1 h. After centrifugation, the pellets were analyzed by 12% SDS-PAGE under reducing conditions and autoradiography. The arrow indicates the location of 125I-IGFBP-3.

in the avidin-agarose pellets of lysates of cells incubated with a premix of 125I-IGFBP-3 and biotinylated IGFBP-3. Very little 125I-IGFBP-3 was detected in the avidin-agarose pellets of lysates of cells incubated with a premix of 125I-IGFBP-3 and biotinylated IGFBP-3 in the presence of a 100-fold excess of unlabeled IGFBP-3 or ~1.6 mM biotin (Fig. 6, lanes 3 and 2). These results further verify the ability of IGFBP-3 to form dimers at the cell surface.

TGF-β is known to stimulate cellular responses by inducing hetero-oligomerization of TGF-β receptors through its covalent dimeric structure (30, 31). We hypothesize that IGFBP-3 inhibits its cellular growth by a similar mechanism in which the dimeric form of IGFBP-3 is required for activation of TβR-V. To test this hypothesis, we determined the binding of the dimeric form of IGFBP-3 to TβR-V in Mv1Lu cells in the presence and absence of DSS. As shown in Fig. 7, the covalently linked dimer of 125I-IGFBP-3 was detected in the medium (lane 1) and lysates (lane 3) of cells incubated with 125I-IGFBP-3 without added cross-linking agents. It is of importance to note that the exposure times for the autoradiograms of the medium and cell lysates were 16 and 2 h, respectively, to have comparable intensities of covalently linked 125I-IGFBP-3 dimers. In the presence of the cross-linking agent DSS, most of the covalently linked dimer associated with the cell surface was cross-linked to TβR-V (lane 4 versus 3). These results suggest that the cell surface-associated dimeric form of IGFBP-3 preferentially binds to TβR-V. These results are also consistent with our previous observation that both TβR-V and 125I-IGFBP-3 dimers were immunoprecipitated by specific antiserum to TβR-V after the 125I-IGFBP-3-affinity labeling of TβR-V in Mv1Lu cells (19).

**FIG. 7.** Binding and cross-linking of the cell surface covalently linked 125I-IGFBP-3 dimer to TβR-V in Mv1Lu cells. Cells were incubated with 5 nM 125I-IGFBP-3 at 0 °C for 3 h and then treated with (+) or without (−) DSS (0.3 mM) for an additional 15 min. The medium and cell lysates were analyzed by 5% SDS-PAGE under reducing conditions and autoradiography. The exposure times for the autoradiograms of the medium and cell lysates were 16 and 2 h, respectively. Arrows indicate the locations of the 125I-IGFBP-3-TβR-V cross-linked complex and covalently linked 125I-IGFBP-3 dimers. The crescent shape of the 125I-IGFBP-3 dimer in the medium (lanes 1 and 2) is because of the influence from a large quantity of bovine serum albumin in the binding medium, which migrates closely with the 125I-IGFBP-3 dimer on the SDS-polyacrylamide gel.

**DISCUSSION**

High affinity IGFBPs are important modulators of IGF actions (1–3). Accumulated evidence suggests that IGFBPs are also able to induce cellular responses in an IGF-independent manner (5–15). The IGF-independent actions for IGFBPs are believed to be mediated by specific cell-surface receptors or membrane-binding proteins (1, 16–18). Several membrane-binding proteins for IGFBPs were identified, but none of these proteins were well characterized (6, 16–18). We have recently identified the IGFBP-3 receptor as TβR-V, which mediates the IGF-independent growth inhibitory response induced by IGFBP-3 (19). In this communication, we show that IGFBP-4 and -5 are also specific ligands for TβR-V, although their affinities for TβR-V are weaker than that of IGFBP-3. The TβR-V is likely the same receptor for IGFBP-5, which has been recently identified in mouse osteoblasts (34). The TβR-V and putative...
IGFBP-5 receptor in osteoblasts share similar properties including the following: 1) they have almost identical molecular weights (∼400,000) (19–25, 34), 2) both show ligand (TGF-β/IGFBP-3 and IGFBP-5)-stimulated serine-specific autophosphorylation and kinase activity toward caseins2 (23, 24, 34), and 3) the TβR-I is expressed in most cell types including osteoblasts (21).3

Among high affinity IGFBPs, four (IGFBP-3 to -6), which possess the putative TGF-β active site motif WCVD near their C termini, were initially predicted to bind to TβR-V. However, although IGFBP-3, -4, and -5 were found to interact with TβR-V in Mv1Lu cells, IGFBP-6 did not. The inability of IGFBP-6 to interact with TβR-V may be because of its unique structure. IGFBP-6 contains 10 of 12 N-terminal cysteine residues conserved in other high affinity IGFBPs and possesses additional O-linked carbohydrate moieties in the central domain and possibly near the C-terminal end (1–3, 35, 36). These distinct structural features may yield a conformation that does not allow the WCVD motif in IGFBP-6 to interact with TβR-V. It is also possible that the WCVD motif is not the only determinant required for the interactions of IGFBPs with TβR-V. The WCVD motif is contained within the thyroglobulin type-1 repeat of IGFBP-3 (37). Thyroglobulin, which contains multiple WCVD motifs per monomer, has recently been shown to exhibit an authentic TGF-β antagonist/agonist activity after activation by acidic pH/denaturing agent treatments and chemical modifications (38). This implies that certain structural configurations of the WCVD motif are required for optimal interaction with TβR-V.

Several polypeptide growth factors are known to stimulate the cytoplasmic kinase activities of their respective receptors by inducing receptor dimerization through their dimeric structures (39–41). The covalent dimeric structure is also known to be required for TGF-β activities (42). Most 125I-labeled IGFBPs form covalently linked dimers at the cell surface. Approximately 20% of cell surface-associated 125I-IGFBP-3 is estimated to be in the form of covalently linked dimers, whereas less than 2% exists as the covalently linked dimer in binding medium. This suggests that the cell surface association enhances the dimer formation of IGFBP-3. Assuming that the efficiency of the spontaneous covalent linking of 125I-IGFBP-3 is ∼20%, it is estimated that almost 100% of the cell surface-associated 125I-IGFBP-3 are dimers. The cell-surface dimeric form of IGFBP-3 appears to be the active form of IGFBP-3 for binding to TβR-V.

The major cell-surface binding sites for IGFBP-3 dimers appear to be membrane proteins other than TβR-V because cells lacking TβR-V (human colorectal carcinoma cells) express these binding sites (19). Interestingly, the binding of 125I-IGFBP-3 and 125I-IGFBP-5 dimers to their major cell-surface binding sites is blocked by β1-(41–65), a specific TGF-β peptide antagonist, whereas the binding of 125I-IGFBP-1 and -4 dimers to their major binding sites is resistant to the blocking by the TGF-β peptide antagonist (Fig. 1). This suggests that the major cell-surface binding sites for IGFBP-3 and IGFBP-5 dimers are distinct from those for other IGFBPs dimers. This suggestion has been supported by the observation that heparin inhibits the binding of 125I-IGFBP-3 and -5 dimers but not 125I-IGFBP-1 and -4 dimers to cell-surface binding sites (18, 19). The functions of these major cell-surface binding sites are unknown. However, one function may involve presentation of IGFBPs to their respective cell-surface receptors. In the case of IGFBP-3, these binding sites may present IGFBP-3 to TβR-V as demonstrated in Fig. 7. This would explain the observation that heparin inhibits the binding of 125I-IGFBP-3 to both the major cell-surface binding sites and to the TβR-V (18, 19).

The signaling mediated by TβR-V has been difficult to define because of the co-expression of TβR-I, TβR-II, TβR-III, and TβR-V in the same cells. The identification of IGFBP-3 as well as IGFBP-4 and -5 as specific ligands for TβR-V has enabled us to investigate the signaling mediated by TβR-V in cells containing other TGF-β receptors. In this communication, we show that IGFBP-3 does not stimulate the cellular phosphorylation of Smad2 and Smad3, both of which play key roles in the signaling mediated by the TβR-I and TβR-II heterocomplex (31, 32). This result is consistent with the observation that IGFBP-3 induces growth inhibition but not transcriptional activation of plasminogen activator inhibitor-1 in Mv1Lu cells (19). The TGF-β-induced expression of plasminogen activator inhibitor-1 is mainly mediated by the TβR-I/TβR-II complex (24). Furthermore, IGFBP-3 has been shown to inhibit the growth of mutant mink lung epithelial cells (DR26 and R-1B cells), which express TβR-V but lack the expression of the functional TβR-II or TβR-I (24).

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