Intramolecular Subunit Interactions between Insulin and Insulin-like Growth Factor 1 $\alpha\beta$ Half-Receptors Induced by Ligand and Mn/MgATP Binding†

Judith L. Treadway,‡ Anne L. Frattali, and Jeffrey E. Pessin*,§

Department of Physiology and Biophysics, The University of Iowa, College of Medicine, Iowa City, Iowa 52242

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ABSTRACT: We have previously demonstrated that isolated insulin and IGF-1 $\alpha\beta$ half-receptors can be reconstituted into a functional $\alpha_2\beta_2$ hybrid receptor complex [Treadway et al. (1989) *J. Biol. Chem. 264,* 21450–21453]. In the present study, we have examined this assembly process by determining the effect of ligand occupancy and Mn/MgATP binding on the dimerization of mutant and wild-type insulin and IGF-1 $\alpha\beta$ half-receptors. IGF-1 or Mn/MgAMPPCP binding to wild-type IGF-1 $\alpha\beta$ half-receptors resulted in the specific assembly of the $\alpha\beta$ half-receptors into an $\alpha_2\beta_2$ heterotetrameric IGF-1 holoreceptor complex. Similarly, insulin binding to the kinase-deficient mutant (A/K_{1018}) insulin $\alpha\beta$ half-receptor also resulted in the specific assembly into an $\alpha_2\beta_2$ holoreceptor complex. In contrast, Mn/MgAMPPCP treatment of A/K_{1018} mutant insulin $\alpha\beta$ half-receptors did not induce heterotetramer assembly, consistent with the inability of this mutant receptor to bind ATP. The ability of the insulin $\alpha\beta$ receptors to assemble with the IGF-1 $\alpha\beta$ half-receptors was used to examine the intermolecular subunit interactions responsible for dimerization. In the presence of Mn/MgAMPPCP, the wild-type insulin and wild-type IGF-1 $\alpha\beta$ half-receptors were observed to assemble into an insulin/IGF-1 $\alpha_2\beta_2$ hybrid receptor complex. Similarly, a combination of insulin and IGF-1 induced hybrid receptor formation between wild-type IGF-1 and A/K_{1018} mutant insulin $\alpha\beta$ half-receptors. In contrast, mixing insulin-occupied A/K_{1018} mutant insulin $\alpha\beta$ half-receptors with Mn/MgAMPPCP-occupied wild-type IGF-1 $\alpha\beta$ half-receptors did not result in hybrid receptor formation. Thus, these data demonstrate that ligand binding to the α subunit and Mn/MgATP binding to the β subunit do not result in complementary conformational changes necessary for $\alpha\beta$ half-receptor dimerization.

Insulin and insulin-like growth factor 1 (IGF-1)[1] bind with high affinity to two distinct cell-surface growth factor receptors which share a high degree of structural and functional similarity (Ebina et al., 1985; Ullrich et al., 1985, 1986). Structural analyses of these holoreceptors have indicated a minimal subunit composition of two α subunits and two β subunits disulfide-linked in to an $\alpha_2\beta_2$ heterotetrameric complex (Rechler & Nissley, 1985; Goldfine, 1987). The α subunits are covalently linked to each other through what have been defined as class I disulfide bonds (Massague & Czech, 1982), whereas the individual α and β subunits, derived from the proteolytic cleavage of a single $\alpha\beta$ fusion precursor protein (Hedo et al., 1981; Jacobs et al., 1983a), are covalently linked to each other by class II disulfide bonds. Specific high-affinity ligand binding is encoded within the extracellular α subunits of the insulin and IGF-1 $\alpha_2\beta_2$ holoreceptors (Grunfeld et al., 1985; Pilch et al., 1986; Andersen et al., 1990; Gustafson & Rutter, 1990; Zhang & Roth, 1991). The β subunits contain a single membrane-spanning domain, a cytoplasmic portion encoding an ATP binding domain, a protein tyrosine kinase domain, and phosphotyrosine acceptor sites (Kasuga et al., 1982; Jacobs et al., 1983b; Roth & Cassel, 1983; Rubin et al., 1983; Tornqvist et al., 1988; White et al., 1988). Insulin and IGF-1 receptors share an overall 53% amino acid sequence similarity and an 84% sequence identity in the conserved tyrosine kinase domain (Ullrich et al., 1986).

Selective reduction of the class I disulfide bond(s) of purified and partially purified receptor preparations, followed by the functional characterization of the resultant isolated $\alpha\beta$ half-receptors, demonstrated that the mature $\alpha_2\beta_2$ holoreceptor state of insulin and IGF-1 receptors is required for ligand-stimulated transmembrane signaling (Boni-Schnetzler et al., 1986, 1988; Sweet et al., 1987a; Tollefsen & Thompson, 1988; Swanson & Pessin, 1989; Wilden et al., 1989a,b). In these studies, both the insulin and IGF-1 $\alpha\beta$ half-receptors were found to assemble into functional $\alpha_2\beta_2$ holoreceptors in vitro upon binding ligand or Mn/MgATP (Boni-Schnetzler et al., 1987, 1988; Sweet et al., 1987b; Feltz et al., 1988; Morrison et al., 1988; Wilden et al., 1989a,b). However, the specific conformational changes resulting from ligand or Mn/MgATP binding which dictate these intramolecular subunit interactions have not yet been experimentally addressed. In the present study, we have examined the domains required for intersubunit interaction during $\alpha_2\beta_2$ heterotetramer assembly of wild-type and kinase-deficient mutant insulin and IGF-1 $\alpha\beta$ half-receptors in vitro. These data demonstrate that ligand- and Mn/MgATP-induced heterotetramer assembly are not complementary events, but rather are the result of specific intersubunit conformational changes.

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* Address correspondence to this author at the Department of Physiology and Biophysics, Room 6-532, Bowen Science Building, The University of Iowa College of Medicine, Iowa City, IA 52242.

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[1] Abbreviations: IGF-1, insulin-like growth factor 1; A/K, alanine for lysine substitution at amino acid position 1018.

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EXPERIMENTAL PROCEDURES

Materials. Protease inhibitors, dithiothreitol, wheat germ agglutinin–Sepharose, and polylysine–Sepharose were purchased from Sigma Chemical Co. Triton X-100, Affi-Gel 10, and Bio-Gel A1.5m resin were obtained from Bio-Rad. Recombinant human IGF-1 was obtained from Toyobo, and porcine insulin was obtained from Dr. Ronald Chance, Eli Lilly Co. Monoclonal antibodies αIR-3 (Kull et al., 1983) and 83-7 (Soos et al., 1989) were gifts from Dr. Steven Jacobs and Dr. Kenneth Siddle, respectively. The monoclonal antibodies as well as mono[^{125}I]iodo(A_{14})-insulin (375 Ci/g) and ^{125}I-IGF-1 (100–150 Ci/g) were prepared by the Diabetes and Endocrinology Research Center, The University of Iowa.

Membrane Preparation. Human placenta membranes were prepared from freshly obtained human placenta by the procedure of Harrison and Itin (1980). Membranes were also prepared from stable transfected tissue culture cell lines expressing either wild-type or A/K_{1018} kinase mutant human insulin receptors as previously described (Treadway & Pessin, 1990). The membranes were stored at −70 °C in 250 mM sucrose, 10 mM Tris-HCl, pH 7.4, 2 mM EDTA, 1 mM phenylmethanesulfonyl fluoride, 25 mM benzamidine hydrochloride, 10 μM leupeptin, 50 trypsin inhibitor units/mL aprotinin, 1 mM 1,10-phenanthroline, and 1 μM pepstatin A. Protein concentration was determined by a modification of the method of Lowry et al. (1951).

Isolation and Purification of αβ Half-Receptors. Human placental membranes (20 mg/mL) were treated with 2 mM dithiothreitol for 5 min at pH 8.5 to reduce and dissociate the $\alpha_2\beta_2$ heterotetrameric receptors, followed by detergent solubilization and partial purification by Bio-Gel A1.5m gel filtration or wheat germ agglutinin–Sepharose chromatography (Swanson & Pessin, 1989). The wild-type IGF-1 αβ half-receptors were isolated by immunoaffinity purification using the anti-IGF-1 receptor monoclonal antibody αIR-3 or by immunodepletion of the wild-type insulin αβ half-receptors using the anti-insulin receptor monoclonal antibody 83-7 (Kull et al., 1983; Soos et al., 1989). The wild-type insulin and A/K_{1018} mutant insulin αβ half-receptors were isolated from cDNA-transfected cell lines (Whittaker et al., 1987; McClain et al., 1987). Cell membranes (3 mg/mL) were prepared and treated with alkaline pH plus dithiothreitol, followed by partial purification on Bio-Gel A1.5m gel filtration columns or polylysine–Sepharose affinity chromatography as previously described (Treadway et al., 1989).

Bio-Gel A1.5m Column Gel Filtration Chromatography. Isolated αβ half-receptors (2 pmol/mL) were mixed as indicated in the individual figure legends and incubated in 50 mM Hepes (pH 7.6) containing either 100 nM insulin, 100 nM IGF-1, 100 nM insulin plus 100 nM IGF-1, or Mn/MgAMPPCP (2 mM $MnCl_2$, 10 mM $MgCl_2$, and 100 μM AMPPCP) for 1 h at 23 °C. Samples were then applied to Bio-Gel A1.5 gel filtraton columns (1.6 × 50 cm) equilibrated in 50 mM Tris-HCl, pH 7.6, 0.1% Triton X-100, 150 mM NaCl, and 0.02% NaN_3 at 4 °C in the presence of ligands and Mn/MgAMPPCP as indicated in the figure legends. Fractions containing the $\alpha_2\beta_2$ heterotetrameric and αβ heterodimeric insulin and/or IGF-1 receptors were identified by ^{125}I-insulin and ^{125}I-IGF-1 binding (Sweet et al., 1987a; Feltz et al., 1988).

Immunoabsorption of Insulin and IGF-1 Receptor Complexes. Bio-Gel A1.5m gel filtration column fractions containing the $\alpha_2\beta_2$ heterotetrameric or αβ heterodimeric insulin and IGF-1 receptors were incubated (1:500 dilution) with the anti-insulin receptor monoclonal antibody 83-7 or the anti-IGF-1 receptor monoclonal antibody αIR-3 coupled to Sepharose beads for 16 h at 4 °C. The antibody/receptor complexes were collected by microcentrifugation, and the supernatants and/or the washed immunoprecipitates were measured for ^{125}I-insulin and ^{125}I-IGF-1 binding.

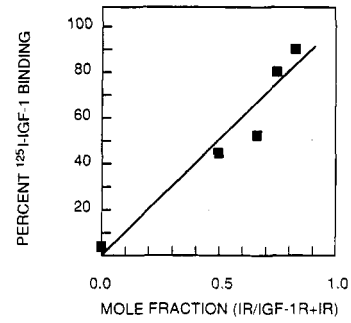

FIGURE 1: Ligand-induced insulin/IGF-1 hybrid receptor formation occurs in a concentration-dependent manner. Human placenta membranes (1 g) or transfected rat-1 fibroblast membranes (10 mg) were treated with 2 mM dithiothreitol for 5 min at pH 8.5 to generate A/K insulin and wild-type IGF-1 αβ half-receptors, respectively. Membranes were then solubilized, and the receptors were purified as described under Experimental Procedures. Immunoaffinity-purified IGF-1 αβ half-receptors (0.4 pmol/mL) were mixed with 0–2 pmol/mL A/K insulin αβ half-receptors in the presence of 100 nM insulin plus IGF-1 for 1 h at 22 °C. Samples were then incubated for 16 h at 4 °C with the anti-insulin receptor monoclonal antibody 83-7 coupled to Sepharose. The immunoprecipiates were extensively washed with 50 mM Hepes, pH 7.6, and 0.05% Triton X-100 and then measured for specific ^{125}I-IGF-1 binding. The results represent the correlation between the formation of insulin/IGF-1 hybrid receptors versus the mole fraction of the A/K insulin and wild-type IGF-1 αβ half-receptor species. ^{125}I-IGF-1 binding was determined in 83-7 Sepharose immunoprecipitates from various αβ insulin and IGF-1 half-receptor mixtures. The percent ^{125}I-IGF-1 bound for each ratio was normalized to the amount of binding in 83-7 immunoprecipitates isolated from samples containing a 10-fold excess of αβ insulin half-receptors to αβ IGF-1 half-receptors. This is a representative experiment independently performed 2 times.

RESULTS

We have previously demonstrated that a wild-type IGF-1 αβ half-receptor and a kinase-defective A/K_{1018} insulin αβ half-receptor could in vitro assemble into an insulin/IGF-1 $\alpha_2\beta_2$ hybrid receptor complex when simultaneously incubated with both insulin and IGF-1 (Treadway et al., 1991). Since approximately 50% of the reassociated $\alpha_2\beta_2$ heterotetramers were insulin/IGF-1 hybrid receptors under these conditions, these data suggested that ligand-induced hybrid receptor formation occurred in direct proportion to the ratio of αβ half-receptors present. To test this hypothesis, the effect of relative concentrations of the two αβ half-receptor species on in vitro hybrid receptor assembly was determined (Figure 1). A fixed amount of wild-type IGF-1 αβ half-receptor was mixed with variable amounts of A/K_{1018} mutant insulin αβ half-receptor, and insulin and IGF-1 were added. The samples were then subjected to specific immunoprecipitation with anti-insulin receptor antibody 83-7 coupled to Sepharose. The immunoprecipitate was washed extensively to remove the ligands required for heterotetramer assembly, and the amount of ^{125}I-IGF-1 binding in the immunoprecipitates was determined as a measure of the insulin/IGF-1 hybrid receptors present. In the absence of the A/K_{1018} insulin αβ half-receptor, IGF-1 binding was not detected in the 83-7 antibody precipitates as would be expected given the specificity of the antibody for the insulin receptor subtype. However, as the concentration of the A/K insulin αβ half-receptor was increased, the proportion of IGF-1 binding precipitated by

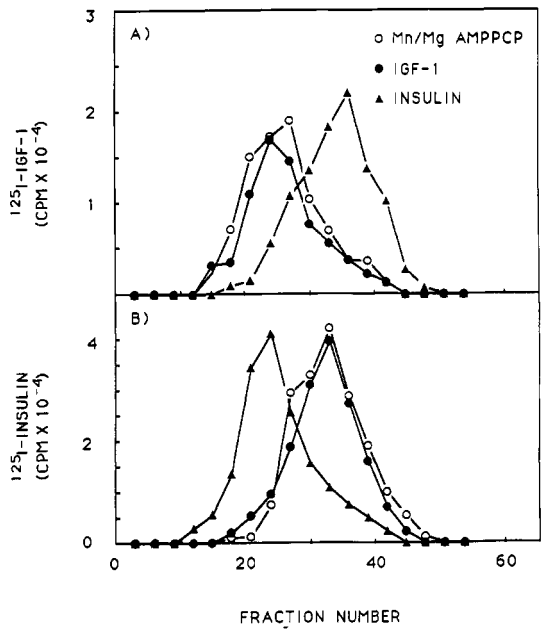

FIGURE 2: Bio-Gel A1.5m gel filtration column profile of insulin and IGF-1 binding to purified $\alpha\beta$ heterodimeric wild-type IGF-1 and A/K insulin receptors incubated in the presence of insulin, IGF-1, and Mn/MgAMPPCP. Human placenta wild-type IGF-1 $\alpha\beta$ half-receptors and transfected rat-1 fibroblast A/K insulin $\alpha\beta$ half-receptors were prepared as described in Figure 1. (A) The IGF-1 $\alpha\beta$ half-receptors and (B) A/K insulin $\alpha\beta$ half-receptors (0.8 pmol/mL) were incubated with 100 nM insulin (▲), with 100 nM IGF-1 (●), or with Mn/MgAMPPCP (○) for 1 h at 23 °C. The ligand-treated samples were then directly applied to Bio-Gel A1.5m gel filtration columns equilibrated in Bio-Gel buffer as described under Experimental Procedures. The Mn/MgAMPPCP-treated samples were applied to Bio-Gel A1.5m columns equilibrated in Bio-Gel buffer containing 2 mM $MnCl_2$ and 10 mM $MgCl_2$ plus 100 μM NaAMPPCP (○). Fractions (0.45 mL) were collected afer voiding 20 mL and assayed for ^{125}I-IGF-1 (A) or ^{125}I-insulin (B) binding as described under Experimental Procedures. This is a representative experiment independently performed 2 times.

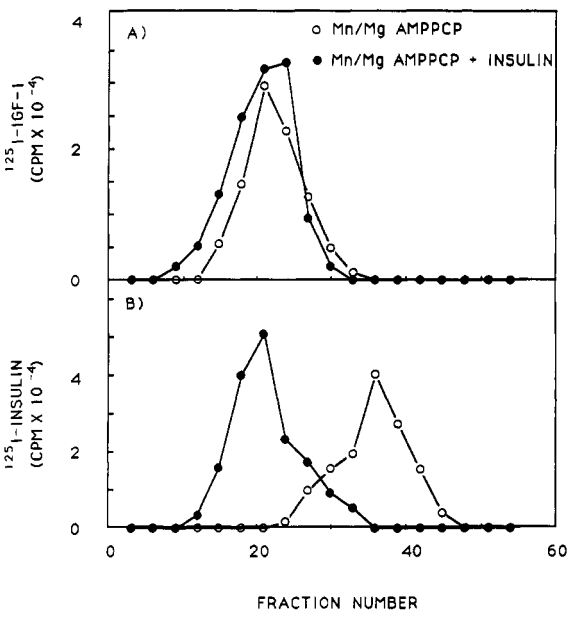

FIGURE 3: Bio-Gel A1.5m gel filtration column profile of insulin and IGF-1 binding to mixed $\alpha\beta$ heterodimeric wild-type IGF-1 plus A/K insulin half-receptors incubated in the presence of Mn/MgAMPPCP or Mn/MgAMPPCP plus insulin. Human placenta wild-type IGF-1 $\alpha\beta$ half-receptors and transfected rat-1 fibroblast A/K insulin $\alpha\beta$ half-receptors were prepared as described in Figure 1. Equal amounts of the wild-type IGF-1 and A/K insulin $\alpha\beta$ half-receptors (0.8 pmol/mL) were mixed and incubated with Mn/MgAMPPCP (○) or with Mn/MgAMPPCP plus 100 nM insulin (●) for 1 h at 23 °C and then applied to Bio-Gel A1.5m gel filtration columns equilibrated in Bio-Gel buffer containing Mn/MgAMPPCP. Fractions (0.45 mL) were collected as described in Figure 2 and assayed for ^{125}I-IGF-1 (A) or ^{125}I-insulin (B) binding. This is a representative experiment independently performed 3 times.

83-7 also increased. At a 1:1 ratio (0.5 mol fraction), approximately 44% of the IGF-1 binding was immunoprecipitated by 83-7, and at a ratio of 5:1 (0.83 mol fraction), essentially 90% was immunoprecipitated by 83-7. These data directly demonstrate that the in vitro ligand-dependent formation of insulin/IGF-1 hybrid receptors occurs in a concentration-dependent manner which can be driven to completion by an excess of one of the $\alpha\beta$ half-receptor species.

The effect of ligand and Mn/MgAMPPCP treatment on subunit association state was next determined for wild-type IGF-1 and mutant A/K_{1018} insulin $\alpha\beta$ half-receptors (Figure 2). In these experiments, Mn/MgAMPPCP was used instead of Mn/MgATP to assess the effect of β subunit ATP binding in the absence of autophosphorylation of the resultant receptor species (Morrison et al., 1988). Purified wild-type IGF-1 $\alpha\beta$ half-receptors were incubated in the absence or presence of 100 nM IGF-1 or 100 μM Mn/MgAMPPCP and subjected to Bio-Gel A1.5m gel filtration chromatography (Figure 2A). The treated $\alpha\beta$ half-receptors displayed a shift in gel filtration mobility consistent with the formation of an $\alpha_2\beta_2$ heterotetrameric complex. Ligand-induced heterotetramer assembly of the wild-type IGF-1 $\alpha\beta$ half-receptors was specific for IGF-1, as incubation with 100 nM insulin did not affect the column profile of ^{125}I-IGF-1 binding activity. Similarly, the A/K_{1018} insulin $\alpha\beta$ half-receptor also displayed ligand-specific $\alpha_2\beta_2$ heterotetramer formation in response to insulin but not IGF-1 (Figure 2B). Incubation of A/K_{1018} insulin $\alpha\beta$ half-receptors with Mn/MgAMPPCP did not alter the column profile of ^{125}I-insulin binding activity, consistent with the disruption of the Mn/MgATP (hence Mn/MgAMPPCP) binding site by the A/K_{1018} mutation. These data demonstrate that the β subunit of the A/K_{1018} insulin $\alpha\beta$ half-receptor does not undergo the necessary conformational change in response to Mn/MgAMPPCP to induce half-receptor dimerization. In contrast, insulin binding to the α subunit results in efficient A/K $\alpha\beta$ half-receptor dimerization.

On the basis of these results, we next addressed whether insulin binding to the α subunit of an A/K_{1018} insulin $\alpha\beta$ half-receptor resulted in a conformational change complementary to that induced by Mn/MgAMPPCP binding to the β subunit of a wild-type IGF-1 $\alpha\beta$ half-receptor. Equal amounts of A/K_{1018} insulin and wild-type IGF-1 $\alpha\beta$ half-receptors were mixed and incubated with either Mn/MgAMPPCP alone or the combination of Mn/MgAMPPCP plus insulin (Figure 3). Samples were then applied to the Bio-Gel A1.5m gel filtration columns to determine the effect of this treatment on subunit association state. ^{125}I-IGF-1 binding of the mixed receptor samples incubated with Mn/MgAMPPCP alone demonstrated the expected increase in mobility of the IGF-1 receptor indicative of $\alpha\beta$ half-receptor dimerization into an $\alpha_2\beta_2$ heterotetrameric state. Since the in vitro assembly of the IGF-1 $\alpha\beta$ half-receptor is specific for IGF-1 (Figure 2A), the inclusion of insulin with Mn/MgAMPPCP did not have any additional effect (Figure 3A). Thus, under the condition of combined Mn/MgAMPPCP plus insulin incubation, the induction of $\alpha_2\beta_2$ heterotetramer assembly of ^{125}I-IGF-1 binding activity was due to the specific binding of the ATP analogue to the IGF-1 $\alpha\beta$ half-receptor. In contrast, insulin specifically shifted ^{125}I-insulin binding activity of the A/K_{1018} insulin receptor from an $\alpha\beta$ to an $\alpha_2\beta_2$ state (Figures 2B and 3B). Further, Mn/MgAMPPCP treatment alone or

Table I: Immunoabsorption of Insulin and IGF-1 Binding from Bio-Gel A1.5m Column Fractions of Mixed A/K Insulin $\alpha\beta$ Half-Receptors and Wild-Type IGF-1 $\alpha\beta$ Half-Receptors ($\alpha\beta_{INS\text{-}A/K}$ + $\alpha\beta_{IGF\text{-}WT}$) either Untreated or Incubated with Mn/MgAMPPCP, Mn/MgAMPPCP plus 100 nM Insulin, or 100 nM Insulin plus IGF-1[a]

	$\alpha\beta_{INS\text{-}A/K}$ + $\alpha\beta_{IGF\text{-}WT}$			
	^{125}I-insulin, % precipitated		^{125}I-IGF-1, % precipitated	
treatment	83-7	αIR-3	83-7	αIR-3
untreated (n = 2)	99 ± 1	0	0	100
Mn/MgAMPPCP (n = 2)	96 ± 4	2 ± 2	8 ± 2	100
Mn/MgAMPPCP + insulin (n = 3)	99 ± 1	6 ± 5	0	99 ± 1
insulin + IGF-1 (n = 1)	100	54	40	100

[a] The $\alpha_2\beta_2$ heterotetrameric and $\alpha\beta$ heterodimeric receptors were immunoprecipitated with 83-7 or αIR-3 followed by the determination of ^{125}I-insulin and ^{125}I-IGF-1 binding in the resultant supernatants. The results are presented as the percentage of ligand binding precipitated ± the standard deviation of the mean from n independent experiments each performed in duplicate.

in combination with IGF-1 had no effect on A/K$_{1018}$ insulin $\alpha\beta$ half-receptor gel filtration mobility (Figure 3B and data not shown, respectively).

To determine if any of these treatments resulted in the formation of insulin/IGF-1 hybrid receptors, the peak fractions containing the insulin and IGF-1 binding activities were immunoprecipitated with anti-receptor-specific monoclonal antibodies (Table I). Immunoabsorption of the premixed insulin and IGF-1 $\alpha\beta$ half-receptors was highly specific (<99%) for their respective monoclonal antibodies. Since Mn/MgAMPPCP treatment of the mixed $\alpha\beta$ half-receptor samples affected only the IGF-1 receptor association state (Figure 3), formation of $\alpha_2\beta_2$ heterologous insulin/IGF-1 hybrid receptor was neither expected nor observed in the immunoabsorption assay (Table I). Treatment of the mixed $\alpha\beta$ half-receptors with insulin plus Mn/MgAMPPCP induced a mobility shift of both insulin and IGF-1 binding activity upon gel filtration (Figure 3); however, the receptor-specific monoclonal antibodies 83-7 and αIR-3 still displayed absolute specificity in the precipitation of ligand binding activity. These data demonstrate a complete lack of hybrid receptor formation under these conditions. Thus, ligand occupancy of the α subunit of one insulin $\alpha\beta$ half-receptor and Mn/MgAMPPCP binding to the β subunit of another IGF-1 $\alpha\beta$ half-receptor did not result in complementary conformational changes sufficient for hybrid heterotetramer assembly.

To directly demonstrate that the A/K$_{1018}$ insulin $\alpha\beta$ half-receptor is capable of heterologous assembly with the wild-type $\alpha\beta$ IGF-1 half-receptor, these receptor species were incubated in the presence of both insulin and IGF-1 (Figure 4). As a result of this treatment, the gel filtration column profiles for both ^{125}I-insulin and ^{125}I-IGF-1 binding activities displayed the appropriate shift in gel filtration mobility. The formation of insulin/IGF-1 hybrid receptors was confirmed by cross-immunoabsorption of ligand binding activity demonstrating the presence of approximately 50% $\alpha_2\beta_2$ hybrid receptors (Table I).

DISCUSSION

To investigate the role of subunit interactions in the ligand-stimulated tyrosine kinase activity of the insulin and IGF-1 receptors, several groups have developed methods to isolate and characterize the functional properties of $\alpha\beta$ half-receptors. Initial studies have documented that curvilinear high-affinity

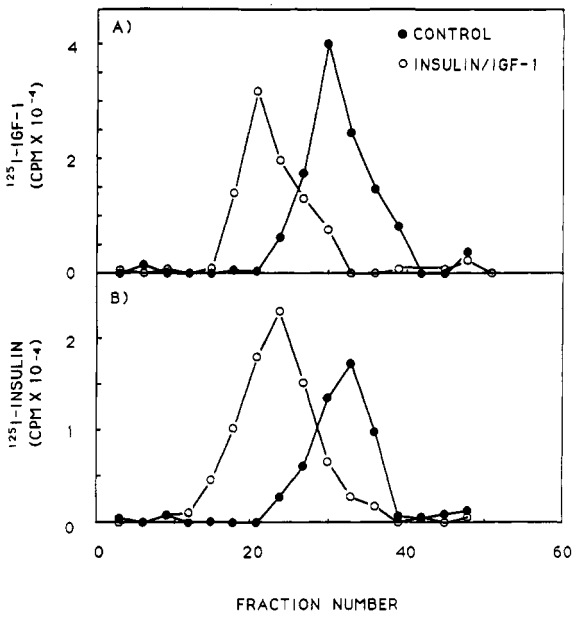

FIGURE 4: Bio-Gel A1.5m gel filtration column profile of insulin and IGF-1 binding to mixed $\alpha\beta$ heterodimeric wild-type IGF-1 plus wild-type insulin half-receptors incubated in the absence or presence of insulin plus IGF-1. Human placenta wild-type IGF-1 $\alpha\beta$ half-receptors and transfected rat-1 fibroblast mutant A/K insulin $\alpha\beta$ half-receptors were prepared as described in Figure 1. Equal amounts of the wild-type IGF-1 and A/K insulin $\alpha\beta$ half-receptors (0.8 pmol/mL) were mixed and incubated with no additions (●) or with 100 nM insulin plus IGF-1 (○) for 1 h at 23 °C and then applied to Bio-Gel A1.5m gel filtration columns equilibrated in Bio-Gel buffer. Fractions (0.45 mL) were collected as described in Figure 2 and assayed for ^{125}I-IGF-1 (A) or ^{125}I-insulin (B) binding.

insulin binding requires direct interaction between two $\alpha\beta$ half-receptors (Boni-Schnetzler et al., 1986; Sweet et al., 1987a; Swanson & Pessin, 1989). Moreover, these $\alpha\beta$ half-receptors are kinase-inactive species that regain ligand-stimulated tyrosine kinase activity upon dimerization into an $\alpha_2\beta_2$ state (Boni-Schnetzler et al., 1987, 1988; Sweet et al., 1987b; Morrison et al., 1988). Similarly, the IGF-1 $\alpha\beta$ half-receptors display reduced kinase activity which is restored upon ligand-induced $\alpha\beta$ half-receptor dimerization (Wilden et al., 1989a,b).

During these studies, we observed that the in vitro assembly of $\alpha\beta$ half-receptors could be induced by either ligand or Mn/MgATP occupancy (Morrison et al., 1988; Wilden, 1989a,b). Since the effect of Mn/MgATP was reversible and could also be mimicked by nonhydrolyzable ATP analogs, the assembly of $\alpha_2\beta_2$ heterotetrameric complexes from $\alpha\beta$ half-receptors was demonstrated to be independent of β subunit phosphorylation. Similarly, ATP and nonhydrolyzable ATP analogs have also been reported to alter the immunogenic properties of the insulin receptor β subunit toward conformation-specific monoclonal antibodies (Maddox & Goldfine, 1991). Taken together, these data strongly suggest that specific conformational changes in the three-dimensional structure of insulin and IGF-1 receptors are induced by either ligand occupancy or Mn/MgATP binding.

To examine the nature of the conformational changes induced by ligand occupancy of the α subunit and Mn/MgATP binding to the β subunit, we have taken advantage of two previous observations. First, $\alpha\beta$ insulin half-receptors can be assembled with highly related but immunologically distinct $\alpha\beta$ IGF-1 half-receptors to form $\alpha_2\beta_2$ insulin/IGF-1 hybrid receptor complexes (Treadway et al., 1989). The in vitro assembly of this hybrid receptor required the simultaneous presence of both insulin and IGF-1, suggesting that each $\alpha\beta$

half-receptor must be ligand-occupied in order to induce the appropriate conformational changes required for appropriate intermolecular subunit interactions. Second, although Mn/MgATP effectively induces the dimerization of wild-type $\alpha\beta$ half-receptors, the kinase-defective A/K mutant insulin $\alpha\beta$ half-receptor (McClain et al., 1987) is completely refractory to Mn/MgATP treatment (Figure 2B).

To determine whether α subunit ligand occupancy and β subunit Mn/MgATP binding induce a complementary conformational change, the dimerization of the A/K_{1018} insulin $\alpha\beta$ half-receptor with the wild-type IGF-1 $\alpha\beta$ half-receptor was determined in the presence of insulin, which binds exclusively to the insulin $\alpha\beta$ half-receptor, and Mn/MgATP, which binds exclusively to the IGF-1 $\alpha\beta$ half-receptor. Under these conditions, there was no detectable formation of insulin/IGF-1 hybrid receptor complexes. These data therefore demonstrate that ligand occupancy induces distinct α subunit conformational changes which do not result in equivalent β subunit conformational changes as Mn/MgATP binding. Similarly, Mn/MgATP binding to the β subunit does not result in complementary alterations in the conformational state of the α subunit to which it is covalently linked. These observations suggest that conformational changes induced by ligand and Mn/MgATP binding are unlikely to be directly transmitted between the disulfide-linked α and β subunits. This model is consistent with studies demonstrating that most mutations in the transmembrane domain do not appreciably affect the kinase activity of the insulin receptor (Frattali et al., 1991; Yamada et al., 1992). Thus, ligand- and Mn/MgATP-induced intersubunit signaling must be communicated via a complex pathway of distinct intramolecular interactions within the native $\alpha_2\beta_2$ holoreceptor structure.

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