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Electrophoretic resolution of three major insulin receptor structures with unique subunit stoichiometries

(crosslinking/plasma membranes/receptor heterogeneity/disulfide-linked subunits/insulin action)

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ABSTRACT **Plasma membrane insulin receptors, affinity labeled by covalent crosslinking to receptor-bound ^{125}I-labeled insulin, are shown to appear as a heterogeneous population of three major disulfide-linked complexes (M_r 350,000, 320,000, and 290,000) upon electrophoresis in highly porous dodecyl sulfate/polyacrylamide gels in the absence of reductant. This pattern is consistent in all rat and human tissues that were analyzed. Upon reduction of disulfide bonds, each of these receptor structures is dissociated in two successive steps. Low concentrations of dithiothreitol promote a first step of disulfide bond reduction in which the M_r 350,000 species splits into a M_r 210,000 form and the M_r 290,000 species splits into a M_r 160,000 form. In contrast, both the M_r 210,000 and M_r 160,000 receptor fragments are generated from the native M_r 320,000 species upon partial reduction, indicating an asymmetrical structure. The second step of receptor reduction occurs upon treatment of the native disulfide-linked receptor complexes with high concentrations of dithiothreitol. Under these conditions, the M_r 350,000 receptor yields a M_r 125,000 subunit, denoted as α, and a M_r 90,000 subunit, denoted as β, whereas the M_r 290,000 receptor dissociates into the α subunit and a M_r 49,000 subunit, denoted as β_1. The M_r 320,000 receptor band is found to consist of α, β, and β_1 subunits upon complete reduction. The partially reduced M_r 210,000 receptor fragment is composed of the α subunit disulfide-linked to the β subunit, whereas the M_r 160,000 species consists of the α subunit disulfide-linked to the β_1 subunit. Thus, the stoichiometry of the three ubiquitous native insulin receptor structures of M_r 350,000, 320,000, and 290,000 are $(\alpha)_2(\beta)_2$, $(\alpha)_2(\beta)(\beta_1)$, and $(\alpha)_2(\beta_1)_2$, respectively.**

Recent efforts of this (1, 2) and other (3–8) laboratory groups to elucidate the structural features of the insulin receptor in adipocyte and liver plasma membranes have shown that the receptor contains at least one subunit in the 125,000–135,000 M_r range and is disulfide-linked in the native membrane (2, 4, 5). Although the presence of additional subunits has also been reported (4–9), the data obtained in different laboratories on the molecular characteristics are variable and controversial. Thus, Jacobs *et al.* (4) have reported that insulin receptor purified from liver membranes consists of M_r 45,000 and M_r 135,000 subunits. More recently, these workers have obtained data suggesting that the insulin receptor structure consists of two M_r 135,000 and two M_r 45,000 subunits, all disulfide-linked (5). However, Yip and coworkers (7) claim that M_r 90,000 and M_r 130,000 species are the only components specifically labeled when liver plasma membrane insulin receptors are subjected to photoaffinity labeling and to electrophoresis under dissociating conditions. Lang *et al.* (9) have also visualized putative receptor subunits of M_r 90,000 and lower. Taken together, these data have presented a puzzling picture of the insulin receptor subunit composition.

We provide here direct evidence for the presence of a heterogeneous population of three major insulin receptor structures in membranes from all rat and human tissues studied. The resolution of these heterogeneous insulin receptor structures clarifies the apparent diversity of results obtained in different laboratories concerning subunit components of the receptor. Our results reveal that the three receptor structures resolved consist of the M_r 125,000–135,000, M_r 90,000, and M_r 45,000–49,000 subunits, identified in various laboratories (1–9), in unique stoichiometries.

MATERIALS AND METHODS

Preparation of Membranes. Adipocytes were isolated from the epididymal fat pads of 150- to 175-g Sprague–Dawley rats by digestion with collagenase (Worthington) (10); plasma membranes were prepared from these cells as described (2). Rat liver plasma membranes were purified by the method of Neville (11) as modified (12). To prepare crude plasma membrane fractions from rat kidney and lung and from human placenta, we minced samples of these tissues and homogenized them in 250 mM sucrose/10 mM Tris, pH 7.4/1 mM EDTA with a Potter–Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 10,000 × *g* for 10 min. The supernatants were centrifuged at 30,000 × *g* for 30 min, the pellets were resuspended in the same buffer, and the latter centrifugation step was repeated twice. Final pellets were resuspended in 10 mM Tris, pH 7.4/1 mM EDTA.

Affinity Crosslinking of Insulin Receptors. The affinity crosslinking protocol was typically performed by incubating membranes (1–2 mg of protein per ml of Krebs–Ringer phosphate buffer containing 1% bovine serum albumin) with 10 nM ^{125}I-labeled insulin (^{125}I-insulin) for 30 min at 23°C. The incubation was ended by addition of excess cold Krebs–Ringer buffer and centrifugation (30,000 × *g*, 30 min). The membrane pellet was resuspended in the same buffer and incubated with 0.25 mM disuccinimidyl suberate for 15 min at 0°C. The crosslinking reaction was quenched by addition of excess ice-cold 10 mM Tris, pH 7.4/1 mM EDTA. Finally, the crosslinked material was pelleted at 30,000 × *g* for 30 min and resuspended in 10 mM Tris, pH 7.4/1 mM EDTA.

Dodecyl Sulfate/Polyacrylamide Gel Electrophoresis and Autoradiography of Gels. Crosslinked membranes in 10 mM Tris, pH 7.4/1 mM EDTA were subjected to electrophoresis according to Laemmli (13). Prior to electrophoresis, samples were boiled for 1 min in Laemmli's sample buffer in the presence of the concentration of dithiothreitol indicated in each experiment. A second electrophoresis of individual receptor bands resolved after a first electrophoresis was performed by slicing the first-dimension gel bands into 2-mm sections (Figs. 2 and 4). The radioactivity in the gel slices was determined in a gamma counter. The second electrophoresis was carried out

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Abbreviation: ^{125}I-insulin, ^{125}I-labeled insulin.

by grinding the gel slices in a stainless steel mortar that had been chilled at −30°C, soaking them in 100 mM Tris, pH 6.9/20% (vol/vol) glycerol/2% $NaDodSO_4$ and dithiothreitol as indicated for 30 min, and electrophoresing them in the presence of the same buffer. The gel slabs were stained in 0.05% Coomassie blue, dissolved in 25% 2-propanol/7% acetic acid (vol/vol) and destained in 5% 2-propanol/7% acetic acid (vol/vol). The gels were dried and subjected to autoradiography (14). To estimate M_r values, we subjected the following standards to electrophoresis in the presence of 50 mM dithiothreitol: myosin (M_r 200,000), β-galactosidase (M_r 116,000), phosphorylase *b* (M_r 94,000), bovine serum albumin (M_r 68,000), ovalbumin (M_r 45,000), and carbonic anhydrase (M_r 31,000).

All autoradiographic results depicted were verified by multiple experiments performed on different membrane preparations. At least 30, 3, 9, and 5 experiments confirmed the results of Figs. 1, 2, 3, and 4, respectively.

Reagents. Porcine insulin was a gift of R. Chance (Eli Lilly). ^{125}I-Insulin was prepared by the lactoperoxidase method (Enzymobeads, Bio-Rad). Disuccinimidyl suberate was prepared in this laboratory (1).

RESULTS

The presence of a native, unreduced, disulfide-linked complex of insulin receptor ($M_r \approx 300,000$) that minimally penetrated standard dodecyl sulfate/polyacrylamide gels has been reported (2). Fig. 1 shows that more detailed resolution of the affinity-labeled disulfide-linked complexes of insulin receptor was obtained when highly porous gels (5% acrylamide; acrylamide/bisacrylamide, 100:1) were used. In the absence of dithiothreitol, ^{125}I-insulin-linked insulin receptors appeared as three major distinct species with M_r 350,000, 320,000, and 290,000. It was striking that all three receptor species were present in membranes from all tissues studied, including rat fat, liver, lung, and kidney and human placenta. Nonradioactive insulin effectively competed with ^{125}I-insulin for the affinity-labeling of all three insulin-binding species (Fig. 1).

Because various groups of investigators have identified insulin receptor subunits of M_r 125,000–135,000 (1–8), 90,000 (7–9), and 45,000 (4, 5) in the fully reduced state, it was of particular interest to identify which subunits were associated with each of the three native receptor species identified. Fig. 2 depicts the subunit composition of the M_r 350,000, M_r 320,000, and M_r 290,000 receptors after their elution from gel bands and a

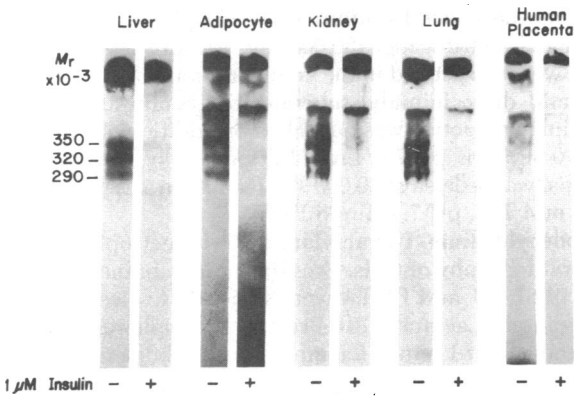

FIG. 1. Electrophoretic resolution of affinity-labeled insulin receptor complexes from several rat and human tissues. Membranes prepared and affinity labeled as described in the text were boiled in Laemmli's sample buffer for 1 min in the presence of 5 mM *N*-ethylmaleimide and in the absence of dithiothreitol. Samples were subjected to electrophoresis in 5% polyacrylamide gels with acrylamide/bisacrylamide, 100:1. Autoradiograms were obtained from dried gels.

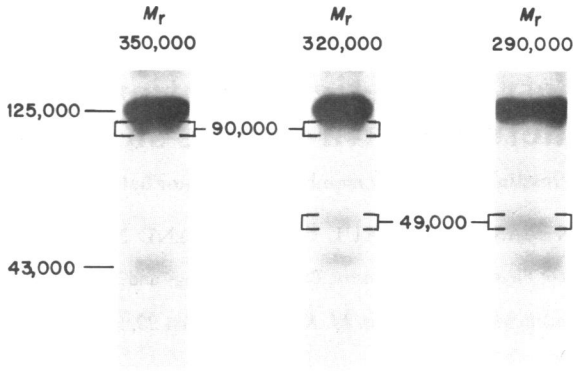

FIG. 2. Electrophoretic identification of affinity-labeled insulin receptor subunits. Affinity-labeled adipocyte membranes were subjected to dodecyl sulfate/polyacrylamide gel electrophoresis in the presence of 5 mM *N*-ethylmaleimide and in the absence of dithiothreitol. The three specifically labeled bands (M_r 350,000, M_r 320,000, and M_r 290,000) resolved in this first electrophoresis were selectively isolated and individually subjected to electrophoresis in the presence of 50 mM dithiothreitol in 8.5–13% polyacrylamide gradient gels (acrylamide/bisacrylamide, 100:2.6). The autoradiogram of a representative gel is shown.

second electrophoresis with complete reduction. All receptor forms under these conditions yielded a strongly labeled band at M_r 125,000 that represents a subunit we denote as α and a M_r 43,000 species which we conclude represents a proteolytic fragment of this α subunit. This conclusion is based on peptide mapping experiments showing that homologous peptides were generated from the M_r 125,000 and M_r 43,000 species upon proteolysis (not illustrated). The M_r 43,000 species therefore will not be further considered. A key observation was that the M_r 350,000 receptor yielded a M_r 90,000 subunit, that we denote as β, and the M_r 290,000 receptor revealed a M_r 49,000 subunit, denoted as β_1, in addition to the α subunit upon complete reduction (Fig. 2). In contrast, the M_r 320,000 receptor consisted of α, β, and β_1 subunit types, as exhibited on this reducing gel. The extent of labeling of the β and β_1 subunits by the crosslinking techniques was much lower than that of the α subunit and was not visualized unless the autoradiographs were extensively exposed. The same pattern of insulin receptor subunits was observed in all other tissues examined.

Because the above results indicate that the three native forms of insulin receptor exist as disulfide-linked complexes containing three subunit types, we attempted to generate and analyze partially reduced intermediate receptor fragments in order to deduce the subunit stoichiometries. Membranes containing affinity-labeled insulin receptors were solubilized in dodecyl sulfate and incubated with various concentrations of dithiothreitol prior to electrophoresis (Fig. 3). The three bands containing high M_r material present in the absence of dithiothreitol (lane A) progressively decreased in intensity as the concentration of dithiothreitol in the sample buffer was increased (lanes B–F). The decrease in the intensity of these bands was paralleled by the appearance of an increasing amount of M_r 210,000 and M_r 160,000 ^{125}I-insulin-labeled species in the gels (lanes D–G). The presence of these partially reduced forms of insulin receptor was transient, and concentrations of dithiothreitol higher than 2 mM led to their complete elimination. The α subunit began to appear at 0.9 mM dithiothreitol, and maximal amounts of it were observed upon total elimination of the other (M_r 350,000–160,000) insulin receptor species in the presence of 10 mM dithiothreitol (lane H). This gel was too porous to visualize the β and β_1 subunits. The mobility of the M_r 350,000 receptor species was decreased slightly in the presence of reductant. Trace amounts of another species (M_r

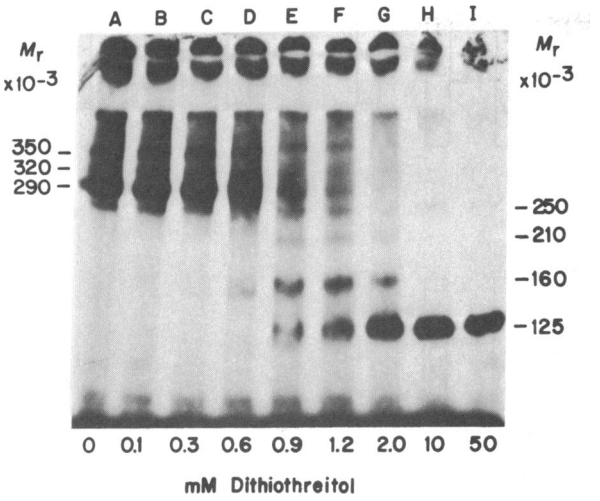

FIG. 3. Sequential dithiothreitol-induced dissociation of disulfide-linked receptor complexes. Affinity-labeled membranes from rat adipocytes were subjected to electrophoresis in the presence of the indicated concentrations of dithiothreitol. Gel composition was as for Fig. 1. The autoradiogram from a representative gel is shown.

225,000–250,000) were detectable in gels at high concentrations of dithiothreitol (lanes H and I). The amount of this species varied among experiments and its origin is unknown. However, it may be a product of further crosslinking of the M_r 125,000 form to another membrane protein (2). The experiment presented in Fig. 3 corresponds to affinity-labeled plasma membranes from rat adiopocytes, but all other tissues studied showed an identical pattern of insulin receptor dissociation upon reduction by dithiothreitol prior to electrophoresis (not illustrated). The total amount of radioactive label in lane I was less than that in lane A due to the loss of label upon dissociation of the A chain of crosslinked ^{125}I-insulin after treatment with dithiothreitol (1). Also, most of the label above the stacking gel was not inhibited by inclusion of unlabeled insulin with membranes and of ^{125}I-insulin before crosslinking, indicating that it is largely nonspecific (Fig. 1).

These results suggest that the M_r 210,000 and M_r 160,000 forms represent intermediate dissociation states of the native high M_r disulfide-linked receptor complexes. Because the results of Fig. 2 show that the M_r 350,000 receptor structure is composed of only α and β subunits, we postulated that the M_r 210,000 fragment represents one M_r 125,000 α subunit disulfide-linked to one M_r 90,000 β subunit. A similar rationale based on the composition of the M_r 290,000 subunit led us to propose that the M_r 160,000 fragment consists of one α and one β_1 subunit. These deductions lead to a definitive hypothesis concerning the minimal subunit composition of each of the three native receptor forms identified. Thus, the M_r 350,000 receptor is deduced to consist of two M_r 210,000 fragments whereas the M_r 290,000 receptor consists of two M_r 160,000 fragments. A key feature of this hypothesis is the prediction that the M_r 320,000 receptor should consist of one M_r 210,000 fragment disulfide-linked to one M_r 160,000 fragment because it contains all three (α, β, and β_1) subunit types upon full reduction (Fig. 2).

This hypothesis was addressed by performing experiments in which each of the three native receptor bands (M_r 350,000, 320,000, and 290,000) excised from the gels was independently subjected to second-dimension electrophoresis in the presence of increasing concentrations of dithiothreitol. Fig. 4 depicts the results from one such experiment with adipocyte plasma membranes. In the presence of low concentrations of dithiothreitol, the M_r 350,000 affinity-labeled receptor (Fig. 4*a*) gave rise almost exclusively to the M_r 210,000 band. In contrast, the receptor species with the lowest M_r (290,000) yielded only the M_r 160,000 dissociation product upon partial reduction (Fig. 4*c*). Most important was the finding that both partially reduced forms of the receptor (M_r 210,000 and 160,000) were visualized when the intermediate insulin receptor population (M_r 320,000) was treated with low levels of reductant (Fig. 4*b*). Membrane insulin receptors from other tissues studied behaved like adipocyte membrane receptors when subjected to this experimental protocol (not shown). As expected, electrophoresis of the M_r 210,000 and 160,000 receptor fragments in the presence of 10 mM dithiothreitol in a second dimension yielded the α plus β and α plus β_1 subunits, respectively (not shown).

DISCUSSION

A key outcome of the present experiments is the demonstration that high-affinity insulin receptors exist in multiple forms in a variety of tissues. Previous studies in our laboratory (2) and elsewhere (4, 5) have shown that the native insulin receptor migrated in the M_r 300,000 region in dodecyl sulfate gels under conditions where the complex minimally penetrated the polyacrylamide matrix. In the more porous gel system used in the present experiments, resolution of the complex into three distinct receptor species, M_r 350,000, 320,000, and 290,000, was achieved. These values must still be regarded as estimates,

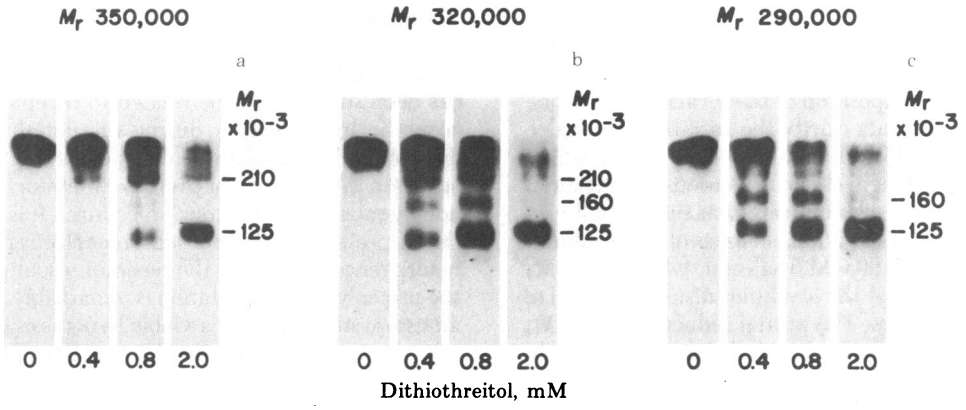

FIG. 4. Sequential dissociation of individual high M_r insulin receptor complexes by increasing dithiothreitol concentrations. Affinity-labeled adipocyte membranes were subjected to dodecyl sulfate/polyacrylamide gel electrophoresis in the presence of 5 mM *N*-ethylmaleimide and in the absence of dithiothreitol. The three high M_r radioactive bands (M_r 350,000, M_r 320,000, and M_r 290,000) resolved in this first electrophoresis were selectively isolated, processed, and individually subjected to electrophoresis in the presence of various concentrations of dithiothreitol. Gel composition was 6% polyacrylamide with acrylamide/bisacrylamide, 100:2.6. Shown is the autoradiogram from a dried gel.

however, because many possible errors are incumbent in determining the molecular weight of large glycoprotein complexes with dodecyl sulfate gels. It is noteworthy that the M_r 350,000 receptor form slightly decreases in mobility upon partial reduction (Fig. 3). This result may indicate the existence of additional disulfide bonds within receptor subunits. Serum albumin also exhibits decreased mobility in gels upon reduction and is known to contain multiple internal disulfide bonds (15).

The appearance of these different receptor complexes does not seem to result from artifacts during the preparation of membrane: (*i*) alkylation of tissue with 1 mM *N*-ethylmaleimide during homogenization, which should prevent auto-oxidation of sulfhydryl groups, did not alter the receptor heterogeneity visualized (data not shown); (*ii*) inclusion of proteolysis inhibitors (0.2 mg of ovomucoid per ml, 0.2 mM phenylmethylsulfonylfluoride, 20 kallikrein inhibitor units of aprotinin per ml, and 1 μg of antipain per ml) during homogenization did not alter receptor patterns (data not shown); and (*iii*) all tissues studied exhibited the exact same mobilities of the receptor complexes on dodecyl sulfate gels (Fig. 1). The collagenase treatment of adipose tissue cannot be invoked as a cause of proteolytic fragmentation of the receptor because the other tissues used were not dissociated into cells by enzymatic digestion prior to preparation of membranes (Fig. 1).

Recently, significant new information has been obtained about the subunit composition of insulin receptors by using affinity purification techniques. Jacobs *et al.* (4, 5) showed that in addition to the M_r 135,000 subunit of the insulin receptor, which we refer to as α, a lower molecular weight (M_r 45,000) species was purified with the receptor. Antibodies against the former subunit (M_r 135,000) were shown to precipitate the native insulin receptor complex. The M_r 45,000 subunit was shown to be a peptide distinct from the α subunit by peptide mapping on dodecyl sulfate gels (5) and appears to correspond to the M_r 49,000 species we denote as β_1. These data and the observation that both the α subunit and the M_r 45,000 subunit are derived from the affinity-purified disulfide-linked receptor complex (4, 5) suggest that the native receptor species under study in those experiments consisted of two α and two β_1 subunits, all disulfide-linked. Although this hypothesis is consistent with the data available, it has been puzzling that several groups (7, 8) have visualized a M_r 90,000 protein species which also appeared to be associated with the insulin receptor. Until now, the relationship between the M_r 90,000 subunit and the other subunit species consistently identified in several laboratories has eluded clarification.

The observation that multiple receptor complexes exist in rat and human tissues and the ability to split these receptor complexes by partial reduction in dodecyl sulfate allow the deduction of the subunit composition of these various receptors. More importantly, these data clarify the variable occurrence of the M_r 90,000 and M_r 45,000–49,000 subunit species (which we denote as β and β_1, respectively) in the various investigations performed to date. Our results indicate that the partially reduced receptor M_r 210,000 fragment consists of the α subunit disulfide-linked to the M_r 90,000 β subunit, whereas the M_r 160,000 fragment consists of the α subunit disulfide-linked to the M_r 49,000 or β_1 species. The partial reduction of the M_r 350,000 receptor species to only the M_r 210,000 species indicates that the former consists of two M_r 210,000 fragments linked by disulfide bonds. Similarly, the data indicate that the M_r 290,000 receptor species consists of two M_r 160,000 fragments. The fact that both a M_r 210,000 and M_r 160,000 species are dissociated from the M_r 320,000 receptor complex strongly suggests that this receptor structure is composed of an α-S-S-β

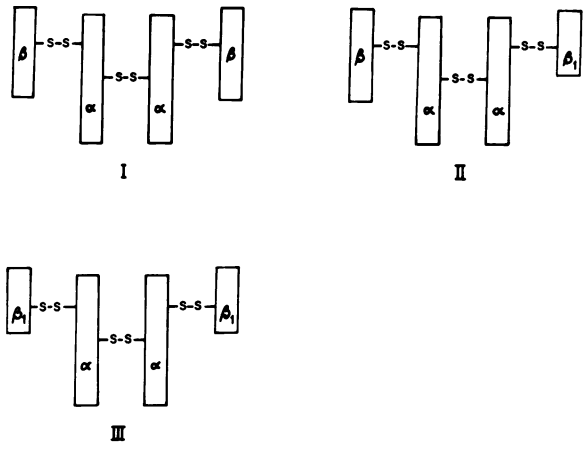

FIG. 5. Proposed insulin receptor structures ubiquitous to all tissues studied. The three high M_r insulin receptor complexes affinity labeled in the membranes of all rat and human tissues studied here are termed I, II, and III and correspond to M_r 350,000, M_r 320,000, and M_r 290,000 species, respectively. We propose that they consist of $(\alpha)_2(\beta)_2$, $(\alpha)_2(\beta)(\beta_1)$, and $(\alpha)_2(\beta_1)_2$ subunit stoichiometries, respectively. Thus, partial reduction splits the M_r 350,000 receptor $(\alpha)_2(\beta)_2$ into M_r 210,000 receptor "halves" $(\alpha)(\beta)$ due to reduction of a sensitive disulfide linkage which may link the α subunits as depicted. Our data do not establish that this link between the two $(\alpha)(\beta)$ M_r 210,000 fragments involves the α subunit as depicted in this scheme. However, the hypothetical subunit stoichiometries presented here for all three receptor complexes are consistent with the fragments obtained by partial reduction (Fig. 4).

fragment (M_r 210,000) disulfide-linked to an α-S-S-β_1 fragment (M_r 160,000). Hence, our data are consistent with the interpretation made by Jacobs *et al.* (5) concerning the subunit composition of the receptor species most prominent in their studies, which must have been the equivalent of our M_r 290,000 species. It is not clear why the lower molecular weight receptor apparently predominated in their membrane preparations. In any case, the foregoing analysis allows the following conclusion about the minimal subunit composition of each of the receptor species visualized on our gels (we use the nomenclature that the M_r 125,000 subunit is α, the M_r 90,000 subunit is β, and the M_r 49,000 subunit is β_1). As schematically illustrated in Fig. 5, the M_r 350,000 receptor species is assigned a subunit composition denoted as $(\alpha)_2(\beta)_2$, the M_r 320,000 species is $(\alpha)_2(\beta)(\beta_1)$, and the M_r 290,000 species is $(\alpha)_2(\beta_1)_2$. The interpretation described here and depicted in Fig. 5 is consistent with all the data presented in this paper as well as that provided by four other laboratories (3–9) by both affinity purification and affinity labeling techniques.

The variability in detection of the α, β, and β_1 subunits in the various laboratories in which receptor subunit composition has been studied may be related to receptor proteolysis that occurs in intact cells or during the initial phases of cellular disruption. We cannot rule out the possibility that proteolysis occurs in the presence of protease inhibitors added during the homogenization step of our procedures. It is important to note that if proteolysis of the insulin receptor is responsible for the heterogeneous forms of the receptor visualized in our studies, the proteolytic fragmentation is remarkably ubiquitous among all tissues studied. Thus, a viable hypothesis is that specific and selective receptor processing by a variety of tissues occurs with uniformity and precision.

The documentation of the existence of heterogeneous insulin receptor complexes in all tissues studied raises a number of interesting physiological questions. For example, Olefsky *et al.* (16) have presented data indicating that insulin–receptor dissociation rates varied with the time of incubation of insulin with

adipocytes or plasma membranes. These results suggested that two or more receptor populations may be present in these cells and may contain different affinities for the hormone. It would thus be of interest to know whether the distinct receptor structures identified here might exhibit different kinetics in respect to ^{125}I-insulin binding.

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1. Pilch, P. F. & Czech, M. P. (1979) *J. Biol. Chem.* **254,** 3375–3380.
2. Pilch, P. F. & Czech, M. P. (1980) *J. Biol. Chem.* **255,** 1722–1731.
3. Jacobs, S., Shechter, Y., Bissel, K. & Cuatrecasas, P. (1977) *Biochem. Biophys. Res. Commun.* **77,** 981–988.
4. Jacobs, S., Hazum, E., Shechter, Y. & Cuatrecasas, P. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4918–4921.
5. Jacobs, S., Hazum, E. & Cuatrecasas, P. (1980) *J. Biol. Chem.* **255,** 6937–6940.
6. Yip, C. C., Yeung, C. W. T. & Moule, M. L. (1978) *J. Biol. Chem.* **253,** 1743–1745.
7. Yip, C. C., Yeung, C. W. T. & Moule, M. L. (1980) *Biochemistry* **19,** 70–76.
8. Wisher, M. H., Baron, M. D., Jones, R. H., Sönksen, P. H., Saunders, D. J., Thamm, P. & Brandenburg, D. (1980) *Biochem. Biophys. Res. Commun.* **92,** 492–498.
9. Lang, U., Kahn, C. R. & Harrison, L. C. (1980) *Biochemistry* **19,** 64–70.
10. Rodbell, M. (1964) *J. Biol. Chem.* **239,** 375–380.
11. Neville, D. M., Jr. (1968) *Biochim. Biophys. Acta* **154,** 540–552.
12. Rodbell, M., Kraus, H. M. J., Pohl, S. L. & Birnbaumer, L. (1971) *J. Biol. Chem.* **246,** 1861–1871.
13. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
14. Swanstron, R. & Shank, P. R. (1978) *Anal. Biochem.* **86,** 184–192.
15. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10,** 2606–2617.
16. Olefsky, J. M., Kobayashi, M. & Chang, H. (1979) *Diabetes* **28,** 460–471.