The Insulin Receptor Activation Process Involves Localized Conformational Changes*

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Insulin regulates cellular metabolism and growth through activation of its specific receptor. This is a heterotetrameric glycoprotein consisting of two extracellular α-subunits con-...
peptides were eluted using 1.5 M MgCl₂, 120 mM sodium borate, pH 7.5. Elution was immediately followed by a 7-fold dilution in 30 mM Hepes, 150 mM NaCl, 0.1% bovine serum albumin, 0.1% Triton X-100, pH 7.5 (final volume, 60 μl). A second chromatography on wheat germ agglutinin was performed to eliminate the elution buffer and to concentrate the purified receptors.

Preparation of Different ³⁵S-Labeled Phosphoreceptor Immunoprecipitation by Unlabeled Native or Phosphorylated Receptors

Increasing amounts (ranging from 0.1 to 6 pmol as determined by Scatchard analysis) of unlabeled insulin receptors, phosphorylated or not, were incubated for 3 h at 15 °C with the antipeptide to sequence 962-972 for 2 h at 37 °C. These receptors were then added at increasing concentrations for 2 more hours. Protein A-Sepharose was used to immunoprecipitate the complex, and pellets were counted in a γ-counter.

Immunoprecipitation of Receptors Activated in Intact Cells

Cells were labeled with [³⁵S]methionine (0.5 mCi/15-cm dish) and then cultured with medium containing 2.0% bovine serum albumin for 3 h at 15 °C. Insulin (10⁻⁸ M) or buffer alone were added to the cells for 5 min at 37 °C. Stimulation was stopped by three washes with 50 mM Hepes, 150 mM NaCl, 10 mM EDTA, 10 mM Na₂PO₄, 2 mM sodium orthovanadate, 100 mM NaF, 10% glycerol, pH 7.5. Cells were scraped using the same buffer plus protease inhibitors, and homogenization was performed by 15 passages through a 26-gauge syringe. After centrifugation at 15,000 × g during 30 min at 4 °C, pellets containing the membrane fraction were solubilized in 1% Triton X-100 for 30 min at 4 °C. After another centrifugation step, supernatants were immunoprecipitated with different antibodies previously adsorbed on protein A-Sepharose. The antibodies were used at optimal concentrations, i.e. concentrations giving the maximal difference between the receptor forms in vitro. Pellets were washed five times, and proteins were analyzed by SDS-polyacrylamide gel electrophoresis. A fraction of each supernatant was kept to determine by ³⁵S-insulin binding the receptor number in the stimulated and unstimulated conditions.

RESULTS

To determine whether β-subunit domains other than the C terminus are implied in the hormone-induced conformational change (16), we used polyclonal antipeptides against (i) the juxtamembrane domain corresponding to amino acids 962–972 and (ii) the intermediate region between the kinase and C terminus domains, i.e. the sequence corresponding to amino acids 1247–1251. We measured the ability of these antipeptides to distinguish between native and insulin-bound receptors. To do this, we used [³⁵S]methionine-labeled receptors in immunoprecipitation experiments with increasing antibody concentrations. Results obtained with the antipeptide to sequence 962–972 are shown in Fig. 1. No striking difference was observed with the two receptor forms, indicating that these antibodies did not discriminate between occupied and nonoccupied receptors. We also found that the antipeptide to receptor sequence 1247–1251 did not immunoprecipitate the native receptors nor the insulin-bound receptors (data not shown). Thus, the insulin-induced change could be limited to the C terminus, or at least was not detected in other domains. A possible role of this change could be to render the receptor

![Fig. 1. Immunoprecipitation of native and insulin-occupied αS-labeled receptors.](image-url)
capable of binding ATP. Therefore, it would take place before nucleotide binding in the chronology of events involved in the molecular activation process. We hypothesized that a kinase-deficient receptor, mutated at the ATP-binding site (replacement of lysine 1018 by alanine), should undergo the ligand-induced modification. To examine this possibility, we tested the two anti-C terminus antipeptides to sequences 1294–1317 and 1309–1326 in experiments with a kinase-deficient receptor, in the presence or absence of insulin. As shown in Fig. 2, the antibodies immunoprecipitated hormone-occupied receptors to a lesser extent as compared with native receptors, and at optimal antibody concentrations, the difference reached 40–50% of maximal immunoprecipitation. Thus, the kinase-deficient receptor remains capable of sensing insulin-induced conformational changes. Further, this result reinforces the notion that the observed structural change is independent of receptor autophosphorylation or ATP binding per se.

In previous studies, however, phosphorylation-induced changes have been described for the insulin receptor (11, 20). Therefore, we were interested in seeing whether the phosphorylation- and insulin-dependent events we observed were the same or different ones. To answer this question, 35S-labeled receptors in their native or autophosphorylated form were immunoprecipitated by increasing concentrations of antipeptide antibodies. Fig. 3 shows the results obtained with antibodies to receptor sequence 1247–1261 (panel A) and 962–972 (panel B). At every concentration tested, both antibodies immunoprecipitated the phosphoreceptor better than the native one. This indicates that the receptor epitopes recognized by these antibodies are uncovered upon autophosphorylation. This is particularly striking for the antipeptide to sequence 1247–1261, since this antipeptide fails to interact with native receptors. Hence, the β-subunit is likely to participate not only in an insulin-induced conformational change but also in a phosphorylation-dependent one.

In our previous study (16), we were not able to demonstrate in the C terminus domain any conformational change due to receptor autophosphorylation. Here, we used phosphorylated 35S-labeled receptors in competition experiments with unlabeled receptors phosphorylated or not. Using this approach, we found now that the antipeptide to sequence
the antibody. Thus, the C-terminal domain appears to be
whole cytoplasmic part of the β-subunit.

When the receptor is phosphorylated. In summary, during its
autophosphorylation, the insulin receptor appears to undergo
an important conformational change that might span the
whole cytoplasmic part of the β-subunit.

Finally, to ensure that our in vitro observation may be
physiologically relevant, we performed immunoprecipitation
experiments using receptors activated in intact cells. In these
experiments, cells were labeled with [35S]methionine and then
stimulated or not by insulin for 5 min. Receptors from solu-
ibilized cells were precipitated by different antipeptide anti-
odies and a monoclonal antibody specific for the human
insulin receptor. Fig. 5 shows an autoradiogram of such a
typical experiment. We observed that each antipeptide rec-
ognized the activated receptor better than the nonactivated
one, whereas immunoprecipitation with the monoclonal an-
tibody confirmed that we had similar amounts of the two
receptors. As the stimulation took place in intact cells, the
activated receptors correspond for the greatest part to phos-
phorylated receptors, indicating that ligand-induced in vitro
autophosphorylation is accompanied by a conformational
change.

DISCUSSION

There is an increasing body of evidence that the molecular
mechanism leading to receptor kinase activation upon insulin
binding implies conformational changes in the receptor β-
subunit. From the published data, three series of facts emerge
indicting that the insulin receptor undergoes structural
changes upon the following distinct events: (i) insulin binding
(16); (ii) ATP binding in the presence of ions, an effect
potentiated by the hormone (15); and (iii) receptor autophos-
phorylation (11, 20). Whereas the insulin-induced confor-
mational change was demonstrated in the C-terminal part of the
β-subunit, phosphorylation and ATP-induced changes have
been localized in the kinase domain, near, or including the
major autophosphorylation sites of tyrosine residues 1146, 1150, and 1151.

In an attempt to integrate these findings into a comprehen-
sive molecular model and to delineate more precisely the β-
subunit parts involved in this process, we used a series of antipeptide antibodies to detect insulin- and phosphorylation-
dependent changes. In our previous work (16), two antipep-
tides directed to the receptor C-terminal sequences 1309–1326
and 1294–1317 were shown to identify an insulin-induced conformational change. Our results did not allow us to con-
clude anything about the existence of additional changes due
to receptor phosphorylation. To address this possibility, we
took advantage of receptors biosynthetically labeled with [35S]
methionine and of antipeptide antibodies to several receptor
domains. As a whole, our data now provide direct evidence
that the insulin receptor may exist in distinct conformations
following hormone binding and autophosphorylation. We
would like to draw the following key conclusions. First, in
contrast to the phosphorylation-induced change that can be
detected at least in three regions (the juxtamembrane domain,
the kinase domain, and the C terminus), the insulin-depend-
ent process may be limited to the C terminus. This may
explain results previously published by Maddux and Goldfine
(15), showing that insulin-binding alone did not modify the
interaction between the receptor and their antibody directed
against a domain near the tyrosines 1150 and 1151. Second,
the two events are clearly distinct for the following reasons:
(i) they were not detected in the same domains, (ii) antipep-
tides to domain 1247–1261 did not interact with insulin-
occupied receptors, but did immunoprecipitate the phos-
phorylated receptors, and (iii) antipeptide to domain 1294–
1317 recognized the phosphoreceptor better than the native
one (Fig. 4) and also recognized the native receptor better
than the insulin-occupied one (16).

Phosphorylation-induced changes have been demonstrated
in several receptors with tyrosine kinase activity, for example
in the platelet-derived growth factor receptor C-terminal do-
main (12, 13), in the colony-stimulating factor-1 receptor
juxtamembrane domain (14), and in the insulin receptor ki-

nase domain (11, 15, 20). However, it is difficult to explain
why despite the close structural similarity between the plate-
let-derived growth factor receptor and the colony-stimulating
factor-1 receptor, the first one undergoes a conformational
change at its C terminus, whereas such an alteration was
detected in the juxtamembrane domain of the colony-stimu-
lating factor-1 receptor. Considering our results, it could be
possible that all tyrosine kinase receptors are subjected to a
widespread process covering the entire cytoplasmic domain.
The observation of a ligand-induced conformational change,
which is independent of autophosphorylation, leads us to
suggest that an intermediate receptor form may exist during
the activation process. This preactivated state may be eva-

nent in cells due to the presence of ATP and ions that lead
to immediate phosphorylation. We have shown that kinase-
deficient mutated receptors retain the capacity to undergo
insulin-induced changes and to shift to the intermediate,
preactivated but nonactive conformation. Our results add
further weight to the concept that before ATP binding or
autophosphorylation, an event involving a conformational
change takes place. In addition, this result indicates that the
kinase-deficient insulin receptor is not totally inert, but is
capable of transmitting ligand-induced information across the

cell membrane. This adds even more decisive evidence to the
idea that the inability of this kinase-deficient receptor to
generate a biological response is due to its enzymic deficiency
and not to a globally altered conformation as compared with
the kinase active receptor nor to a defect in the transmem-
brane activation mechanism.

Based on our observations and on previously published
data, we would like to propose the following schematic model
for the insulin receptor activation. Insulin binding to the
receptor α-subunit induces a conformational change in the
extracellular domain (9, 10), and a modification in the inter-

FIG. 5. Immunoprecipitation of insulin receptors stimulated
in intact cells. Cells were labeled with [35S]methionine and stimu-
lated or not by insulin as described under "Materials and Methods."
Solubilized fractions were immunoprecipitated by optimal amounts
of antipeptides previously adsorbed on protein A-Sepharose. Proteins
were analyzed by SDS-polyacrylamide gel electrophoresis. The auto-
radiogram represents a typical experiment.
action between the two receptor halves (21, 22). These changes are transmitted to the β-subunit down to its C terminus, leading to a short-lived, preactivated receptor that becomes competent to bind ATP. The ensuing receptor autophosphorylation induces a second conformational change, distinct from the first one, which affects the major part of the cytoplasmic domain. Although the precise role of these phenomena remains to be determined, we would like to suggest that they could lead to unmasking of the receptor catalytic domain and/or of binding sites for cellular proteins, allowing enzyme-substrate interactions.

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