Signal Transduction Mechanism of Insulin and Insulin-Like Growth Factor-1

TAKASHI KADOWAKI, KAZUYUKI TOBE, RITSUKO HONDA-YAMAMOTO, HIROYUKI TAMEMOTO, YASUSHI KABURAGI, KAORU MOMOMURA, KOJIRO UEKI, YOSHIHIKO TAKAHASHI, TOSHIYOSHI YAMAUCHI, YASUO AKANUMA*, AND YOSHIO YAZAKI

Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo 113, and
*Institute for Diabetes Care and Research, Asahi Life Foundation, Tokyo 100, Japan

Abstract. Insulin and insulin-like growth factor-1 (IGF-1) are two structurally related hormones which produce similar biological activities such as metabolic and growth promoting actions. Their receptors, insulin and IGF-1 receptors, also share similarities in both structure and functions such as tyrosine-specific protein kinase. We identified insulin receptor substrate-1 (IRS-1) as a common substrate for insulin and IGF-1 receptor tyrosine kinases. We generated IRS-1 knockout mice and showed that IRS-1 plays a physiological role in signal transduction and biological actions of insulin and IGF-1. We also identified pp190 (IRS-2) as an alternative substrate for IRS-1.

Key words: Insulin, Insulin-like growth factor-1, Tyrosine kinase, Insulin receptor substrate-1

(Endocrine Journal 43 (Suppl): S33-S41, 1996)
ligand-binding domains. Affinity labeling and mutational analyses have implicated the involvement of NH2-terminal domain and C-terminal domain of the insulin receptor α-subunit in ligand binding [8–10], whereas the residues defining IGF-1 binding are present predominantly in the cysteine-rich domain of the IGF-1 receptor [8]. For example, substitution of lysine for asparagine at position 15 in the α-subunit of the human insulin receptor, a mutation observed in a patient with a genetic form of insulin resistance impaired the affinity of insulin binding and the transport of receptors to the cell surface [9].

The β-subunit is a transmembrane protein possessing tyrosine-specific protein kinase activity in the intracellular domain [11] (Fig. 1). Insulin binding to the α-subunit of the insulin receptor leads to the phosphorylation of the β-subunit of the receptor on tyrosine residues [11–13]. This in turn activates the intrinsic tyrosine-specific protein kinase activity [14]. Several studies suggest that this tyrosine protein kinase activity plays an important role in insulin signal transduction. Chou et al. [15] overexpressed in CHO cells mutated human insulin receptor by replacing lysine-*1018 (ATP-binding site) with several different amino acids. They found that none of these cells exhibited insulin-stimulated kinase activity, uptake of 2-deoxyglucose, S6 kinase activation, glycogen synthesis, thymidine uptake and internalization of insulin [15, our unpublished observation (Table 1)]. Therefore, the autophosphorylation and/or tyrosine kinase activity of the insulin receptor is necessary in mediating many, if not all, of the biological effects of insulin.

*according to the numbering system of Ullrich, et al. [1].

Similar results were observed with mutant IGF-1 receptor (lysine-substituted with alanine) [16]. We identified a mutation in the insulin receptor gene impairing the tyrosine kinase activity in a patient with insulin resistance [17]. In the mutant recep-

---

Fig. 1. Schematic representation of cytoplasmic domain of the human insulin receptor and mutant insulin receptors described in the manuscript.

Table 1. Summary of signalling and biological actions of mutant insulin receptors

<table>
<thead>
<tr>
<th>Mutant insulin receptors</th>
<th>Tyrosine kinase</th>
<th>Auto-</th>
<th>pp185</th>
<th>2DG uptake</th>
<th>Glycogen synthesis</th>
<th>DNA synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly*96→Arg</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Lys*1018→Arg</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Tyr*146,150,151→Phe</td>
<td>↓ ↓ ↓</td>
<td>↓ ↓ ↓</td>
<td>↓ ↓ ↓</td>
<td>↓ ↓ ↓</td>
<td>↓ ↓ ↓</td>
<td>↓ ↓ ↓</td>
</tr>
<tr>
<td>Tyr*96→Phe/Ala</td>
<td>N N</td>
<td>N N</td>
<td>N N</td>
<td>N N</td>
<td>N N</td>
<td>N N</td>
</tr>
<tr>
<td>ΔC82</td>
<td>↓</td>
<td>↓</td>
<td>N</td>
<td>N N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Tyr*136,1322→Phe</td>
<td>N N</td>
<td>N N</td>
<td>N N</td>
<td>N N</td>
<td>N</td>
<td>↑</td>
</tr>
</tbody>
</table>

(-), abolished; ↓, slightly decreased; ↓↓, moderately decreased; ↓↓↓, severely decreased; N, normal. (references 18, 20, 21, 23, 25, Momomura K and Kadowaki T, unpublished observations).
tor, valine-996 is substituted for glycine, the third
glycine in the highly conserved Gly-X-Gly-X-Gly
sequence in the receptor’s ATP-binding site. By
transfecting the mutant receptor cDNA into CHO
cells, we found that the receptor’s tyrosine kinase
activity was impaired by the Val-996 mutation.
Val-996 mutation also inhibited insulin-stimulated
uptake of 2-deoxyglucose and glycogen synthesis
[18] (Table 1). This observation suggested that a
defect in the insulin receptor tyrosine kinase could
interfere with insulin action in vivo, and that mu-
tations in the insulin receptor gene can cause
insulin resistance and diabetes.

The tyrosine kinase domain of the insulin re-
ceptor and the IGF-1 receptor contains the ATP
binding domain and 3 tyrosine residues in the
Tyr-X-X-X-Tyr-Tyr motif (Fig. 1). Three tyrosine
autophosphorylation sites in the kinase domain
(Tyr-1146, Tyr-1150, and Tyr-1151 of the insulin
receptor and Tyr-1146, Tyr-1150, and Tyr-1151 in
the IGF-1 receptor) seem to be essential for recep-
tor kinase activity and biological effects [19, our
unpublished observation] (Table 1).

Several functional domains other than tyrosine
kinase domain have been defined in the β-subunit
of the insulin receptor and the IGF-1 receptor (Fig.
1). The intracellular juxtamembrane region of the
insulin receptor β-subunit plays an important role
in signal transduction. Tyr-960 of the insulin re-
ceptor resides in an NPXY motif which also exists
in other tyrosine kinase receptors and oncogene
products including the IGF-1 receptors (Tyr-950)
(Fig. 1). Replacement of Tyr-960 with phenylala-
nine or alanine impairs phosphorylation of insulin
receptor substrate-1 (IRS-1) and Shc without dis-
rupting autophosphorylation of the β-subunit and
the tyrosine kinase activity, raising the possibility
that Tyr-960 and NPXY motif play pivotal roles in
the recognition of these substrates [20–22] (Table
1). In addition to signal transduction, the jux-
tamembrane region of the insulin receptor and
IGF-1 receptor is required for coated pit-mediated
internalization of insulin and IGF-1, respectively
[20–22].

Protein tyrosine kinases often contain a COOH-
terminal tail that extends beyond the end of the
tyrosine kinase homology region. The COOH tail
of the insulin receptor contains two autophospho-
rylation sites at tyrosine-1316 and tyrosine-1322
(Fig. 1). The COOH tail of IGF-1 receptor contains
3 possible autophosphorylation sites at tyrosine-
1250, tyrosine-1251, and tyrosine-1316. Autophosphorylation sites in the COOH tail of the
insulin receptor may behave inhibitory to mitoge-
nic signaling, while those of the IGF-1 receptor may
behave stimulatory to tumorigenic signaling. Cells
overexpressing mutant insulin receptor replacing
tyrosine-1316 and tyrosine-1322 with phenylalanine
exhibited enhanced activation of mitogen-activat-
ed protein (MAP) kinase and S6 kinase, and
increased sensitivity of thymidine uptake in re-
response to insulin, while insulin-stimulated
cytoskeleton reorganization and kinase activity were
normal [23] (Table 1). Cells overexpressing mutant
IGF-1 receptor replacing tyrosine-1250, 1251 or ty-
rosine-1316 with phenylalanine exhibiting reduced
tumorigenic potentials without significantly inhib-
iting receptor autophosphorylation and kinase
activity [24]. Cells overexpressing mutant insulin
receptor lacking 82 amino acids (ΔC82) from the
COOH terminus exhibited impaired autophospho-
ylation of the insulin receptor while tyrosine
phosphorylation of IRS-1 were comparable to cells
overexpressing [25]. Thus, COOH tail of the insu-
lin receptor seems to be involved in the interaction
of insulin receptor with IRS-1.

Substrates for Insulin Receptor and IGF-1
Receptor Kinase

Using anti-phosphotyrosine antibodies, White et
al. [26] reported a phosphotyrosine-containing pro-
tein of relative Mr 185,000 (pp185) on SDS-PAGE,
which appeared during the initial responses of
hepatoma cells to insulin binding. Tyrosine phos-
phorylation of pp185, later named IRS-1, reaches
maximum within 30 sec after exposure of the cells
to insulin and exhibits a dose-response curve simi-
lar to that of receptor autophosphorylation [27–30].
pp185 (IRS-1) is tyrosine phosphorylated in physi-
ological target tissues of insulin such as liver,
muscle, and adipocytes [29, 30]. We showed that
pp185 (IRS-1) is also tyrosine phosphorylated with
IGF-1 [28, 31, 32]. We demonstrated that tyrosine
phosphorylation of pp185 (IRS-1) in response to
insulin occurred via the insulin receptor and that
of pp185 (IRS-1) in response to IGF-1 occurred via
the IGF-1 receptor using specific antisera to these
receptors [28]. Thus, pp185 (IRS-1) is a common
substrate for the insulin and IGF-1 receptor tyrosine kinases [28]. In contrast, pp185/IRS-1 does not serve as a substrate for EGF receptor [28] or PDGF receptor. Recently, tyrosine phosphorylation of IRS-1 is reported in response to several interleukins, interferons, angiotensin II, and growth hormone (Yamauchi T, Tsushima T, Kadowaki T, unpublished observation).

IRS-1 contains 21 potential tyrosine phosphorylation sites and over 30 potential serine/threonine phosphorylation sites. In its amino terminus, IRS-1 contains a pleckstrin-homology (PH) domain and a phosphotyrosine-binding (PTB) domain. PH domain and PTB domain of IRS-1 are thought to be important for receptors coupling between insulin and IGF-1 receptors and IRS-1.

The role of IRS-1 in signal transduction of insulin has been suggested by several lines of evidence. In CHO cells overexpressing mutant insulin receptors (Tyr-960→Phe/Ala, OC82), insulin actions appears to be parallel with the extent of tyrosine phosphorylation of IRS-1 rather than that of the receptor itself [20–22, 25]. For example, replacement of Tyr-960 with phenylalanine caused an impairment of IRS-1 tyrosine phosphorylation and a concomitant decrease in insulin's biological actions despite normal insulin receptor autophosphorylation [20–22].

**Molecules that Bound to IRS-1**

Tyrosine phosphorylation of IRS-1 provides binding sites for several distinct Src homology 2 (SH2) proteins and may mediate multiple signaling pathways. Indeed, IRS-1 binds the 85-kDa subunit of phosphatidylinositol 3-kinase (PI 3-kinase p85) through interaction with Tyr-X-X-Met motifs, thereby activating PI 3-kinase [33]. This pathway may be implicated in the translocation of glucose transporters and also activation of glycogen synthase [34]. IRS-1 also binds Ash/Grb2 (abundant src homology/growth factor receptor bound protein 2), a 23 kDa protein with one SH2 domain and two SH3 domains [35]. Ash/Grb2 associates with IRS-1 or Shc by its SH2 domain and associates with Sos with its SH3 domain. These signaling pathways may activate p21ras and the MAP kinase cascade [36–38]. We have recently shown Csk tyrosine kinase which is a negative regulator src-family tyrosine kinases can form a complex with IRS-1 in response to insulin, which appears to be implicated in insulin-induced tyrosine dephosphorylation of proteins at the focal adhesion such as focal adhesion kinase (FAK) [39]. Moreover, IRS-1 has binding sites for Syp and Nck and other SH2 signaling molecules.

**Growth Retardation and Insulin Resistance in IRS-1 Deficient Mice**

To understand the roles of IRS-1 in normal physiology, we and others made mice with a targeted disruption of the IRS-1 gene locus [40, 41]. Growth of the homozygous IRS-1 knockout mice was retarded. At the age of 3, 8, and 15 weeks, the weight was about 30% less than that of normal littermates (Fig. 2A); the serum IGF-1, IGF-2 and growth hormone levels were normal in the mutant mice (Hizuka N, personal communication). The growth retardation of the embryos was apparent at 15.5 embryonic day and the weight of homozygous IRS-1 knockout embryos was about 80% of that of normal and heterozygous embryos at 18.5 embryonic day.

The drop in the blood glucose levels after injection of human insulin was significantly smaller in homozygous mutant mice than those of heterozygous mutant and wild-type mice (Fig. 2B). Similar results were observed with IGF-1 injection (Fig. 2C) and also with IGF-2 (data not shown). Thus, homozygous mutant mice have resistance to IGF-1 and IGF-2 in addition to insulin. Stimulation of glucose transport activity in the isolated adipocytes with insulin [40] and that in soleus muscle with insulin and IGF-1 [42] were significantly impaired in homozygous mutant mice compared with that of the wild-type mice. In contrast, insulin-stimulated biological activities in the liver such as glycogen synthesis were normal (see below). There was no significant difference in blood glucose levels among the three genotypes during an oral glucose load (Fig. 2D). However, the serum insulin levels before and after glucose load were significantly higher in the homozygous mutant mice (Fig. 2D). Thus, IRS-1 knockout mice show mild to moderate post-receptor insulin resistance in the muscle yet normal glucose tolerance by compensatory hyperinsulinemia from pancreatic β-cells,
which resembles the phenotype of NIDDM at the pre-diabetic stage [40, 42].

This study has shown that IRS-1 is required at least in part for the pre- and postnatal growth-promoting effect and glucose-lowering effect of insulin, IGF-1, and IGF-2. The growth retardation of the IRS-1 knockout mice was milder than those of insulin receptor knockout mice [43] and leprechaunism, human insulin receptor deficient state [44], and also milder than that of IGF-1 receptor knockout mice [45], suggesting the existence of both IRS-1 dependent and IRS-1 independent pathways in signal transduction through insulin and IGF-1 receptor tyrosine kinases (Fig. 3). We studied insulin-stimulated signalling pathways and biological actions in IRS-1 deficient mice [42] (Table 2). Insulin-stimulated PI3 kinase activity in anti-phosphotyrosine antibody immunoprecipitates was reduced in the muscle from IRS-1 deficient mice, whereas it was normal in the liver. Moreover, insulin-stimulated MAP kinase activity was also reduced in the muscle, whereas it was again normal in the liver. Consistent with PI3 kinase and MAP kinase activities, insulin-stimulated glucose transport, glycogen synthesis and protein synthesis was reduced in the muscle, whereas insulin-stimulated glycogen synthesis was normal in the liver. Thus, contribution of IRS-1 independent pathway seemed to be more significant in the liver than in the muscle [42].

![Growth curve](image)

**Fig. 2.** (A) Growth curve. (B) Insulin tolerance test. (C) IGF-1 tolerance test. Homozygous (unfilled circles), heterozygous (triangles) and wild-type (filled circles) mice were injected either with human insulin or IGF-1 into the peritoneal cavity and the blood glucose levels were determined at the indicated times. ** indicates \( P<0.01 \) and * indicates \( P<0.05 \). (D) Blood glucose and serum insulin during an oral glucose load. The wild-type (filled bars), heterozygous (hatched bars), and the homozygous mutant mice (unfilled bars) were given glucose, and the blood glucose (left panel) and serum insulin (right panel) levels determined at the indicated time points. The bars show the SEM. The serum insulin levels of the homozygous mice before and after glucose load (0.41 ± 0.04 mg ml\(^{-1}\) to 0.46 ± 0.04 mg ml\(^{-1}\)) were significantly higher than those of the wild type (0.24 ± 0.02 mg ml\(^{-1}\) to 0.311 ± 0.03 mg ml\(^{-1}\)) and heterozygous mice (0.24 ± 0.02 mg ml\(^{-1}\) to 0.37 ± 0.04 mg ml\(^{-1}\)) (\( P<0.01 \) and \( P<0.05 \), respectively) [40].
In order to identify IRS-1-independent pathways, we studied insulin-stimulated tyrosine phosphorylated proteins in the liver of wild-type or homozygous mutant mice by injecting insulin via portal veins [46]. We observed a 160-kDa tyrosine phosphorylated protein (IRS-1) in liver of insulin-injected wild-type mice (Fig. 4). In addition to IRS-1, we also observed another weakly tyrosine phosphorylated protein of Mr 160-kDa as shown in lane a was detected in some, but not all, of the experiments. However, since this band was not immunoprecipitated with α-IRS-1 (1-6) as shown in lane f, it is not IRS-1 [46].

**Table 2.** Insulin-stimulated signalling pathways and biological actions in IRS-1 deficient mice

<table>
<thead>
<tr>
<th></th>
<th>Muscle</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>P13 kinase</td>
<td>↓↓</td>
<td>normal</td>
</tr>
<tr>
<td>MAP kinase</td>
<td>↓↓</td>
<td>normal</td>
</tr>
<tr>
<td>IRS-1</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>Shc</td>
<td>→or ↑</td>
<td>→or ↑</td>
</tr>
<tr>
<td>IRS-2</td>
<td>=20%</td>
<td>100%</td>
</tr>
<tr>
<td>Glucose transport</td>
<td>↓↓</td>
<td>N.A.</td>
</tr>
<tr>
<td>Glycogen synthesis</td>
<td>↓↓</td>
<td>normal</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>↓↓</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

→, unaltered; ↑, increased; ↓↓, markedly decreased; (-), absent; N.A., not applicable; N.D., not determined.

**Identification of pp190/IRS-2 in IRS-1 Deficient Mice**

In order to identify IRS-1-independent pathways, we studied insulin-stimulated tyrosine phosphorylated proteins in the liver of wild-type or homozygous mutant mice by injecting insulin via portal veins [46]. We observed a 160-kDa tyrosine phosphorylated protein (IRS-1) in liver of insulin-injected wild-type mice (Fig. 4). In addition to IRS-1, we also observed another weakly tyrosine phosphorylated protein of Mr 160-kDa as shown in lane a was detected in some, but not all, of the experiments. However, since this band was not immunoprecipitated with α-IRS-1 (1-6) as shown in lane f, it is not IRS-1 [46].
phosphorylated protein of an Mr 190-kDa (pp190) which was not recognized by anti IRS-1 antibody (Fig. 4). In homozygous IRS-1 deficient mice, we observed pp190 in response to insulin. Tyrosine phosphorylation of pp190 was significantly increased in insulin-injected IRS-1 deficient mice compared with that in insulin-injected wild-type mice (Fig. 4). Tyrosine phosphorylation of Shc was not significantly increased. We also showed that tyrosine phosphorylated pp190/IRS-2 is able to bind both p85 of PI 3-kinase and Ash/Grb2 [46]. These data suggested that insulin-stimulated tyrosine phosphorylation of pp190, which was immunologically distinct from IRS-1 yet functionally similar to IRS-1, was increased in IRS-1 deficient mice compared with that in wild-type mice, thus compensating the effects of IRS-1 deficiency.

In fact, pp190, cloned by Sun et al. and named IRS-2 [47], has many structural homologies with IRS-1 including P85 PI3-kinase and Ash/Grb2 binding sites. We found that the amount of tyrosine-phosphorylated IRS-2 (in IRS-1 deficient mice) was roughly equal to that of IRS-1 (in wild-type mice) in the liver, whereas it was only 20 to 30% of that of IRS-1 in muscles, suggesting biological roles of IRS-2 as well as IRS-1 in insulin actions. Generation of IRS-2 knockout mice as well as IRS-1/IRS-2 double knockout mice should define the roles of IRS-1/IRS-2 system in biological actions of insulin and IGF-1.

Acknowledgment

This work was supported by grants from the Juvenile Diabetes Foundation International to T. K., by a grant for diabetes research from Ohtsuka Pharmaceutical Co., Ltd., to T.K., and by a grant from Taisho Pharmaceutical Co., Ltd., to T.K. Dr. Ritsuko Honda-Yamamoto played a major role in writing this review.

References


