An extracellular domain of the β subunit is essential for processing, transport and kinase activity of insulin receptor

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The extracellular portion of the insulin receptor (IR) β -subunit has four cysteine and four asparagine residues which are potentially involved in disulphide bond formation between the α - and β -subunits and N-linked glycosylation respectively. However, the function of this portion is not fully understood. In order to investigate the role of the extracellular domain of β -subunit, we created a deletion mutant of IR cDNA which lacked 47 amino acid residues encoded by 141 bp corresponding to exon 13 of the IR gene. Insulin binding and surface labelling of COS 7 cells transiently expressing the mutant insulin receptors (IR Δ Ex13) showed that the mutated receptors were not expressed on the cell surface. However, immunoblot analysis showed that uncleaved form (190 kDa) of the mutant receptors were intracellularly expressed. Deglycosylation with endoglycosidase H

showed that the mutant receptors had mainly high-mannose oligosaccharide chains. The mutant IRs bound with high affinity to lentil lectin but with low affinity to wheat germ agglutinin. Therefore, it is suggested that misfolding of the mutant receptors inhibits transport to the Golgi apparatus where processing of oligosaccharide chains, as well as proteolytic cleavage into subunits, takes place. The binding affinity of the mutant receptors for insulin was 50 % of normal. Furthermore, insulin-stimulated autophosphorylation of IR Δ Ex13 was markedly impaired. These data provide the evidence for a critical role of the extracellular domain of IR β -subunit for processing and transport as well as the intramolecular signal transduction to activate IR tyrosine kinase.

INTRODUCTION

The insulin receptor (IR) is a heterotetrameric transmembrane protein composed of two α - and two β -subunits ($\alpha\beta$)₂ linked by disulphide bonds (Kahn and White, 1988). The α -subunits are totally extracellular and the β -subunits are anchored in the plasma membrane. When insulin binds to the α -subunits, this activates the intrinsic tyrosine-specific kinase of the intracellular β -subunits to phosphorylate tyrosine residues of the β -subunits and also to phosphorylate intracellular substrates. These events are necessary for the signal transduction to elicit various insulin actions. Both subunits are derived from a single-chain precursor molecule, which undergoes glycosylation with high-mannose oligosaccharide chains, disulphide isomerization and dimerization at the endoplasmic reticulum (Olson et al., 1988). Proteolytic cleavage of the precursor into the α - and β -subunits as well as the maturation of carbohydrate chains occurs at the Golgi apparatus. Then, the mature ($\alpha\beta$)₂ heterotetrameric receptor is transported to the plasma membrane (Olsen et al., 1988). Since the extracellular portion of the β -subunit is linked with the α -subunits by disulphide bonds, this region should be involved in the intramolecular signal transduction between the α - and β -subunits. This portion has four cysteine residues which are potentially involved in disulphide bonds between the α - and β -subunits and also has four asparagine residues potentially involved in N-linked glycosylation (Ebina et al., 1985; Ullrich et al., 1985). Modifications of N-glycosylation sites of the extracellular β -subunit resulted in impairment of transmembrane signalling (Leconte et al., 1992). However, other mutations in the extracellular β -subunit have not been reported thus far. Thus the function of this portion has not been fully clarified.

In order to evaluate the role of the extracellular β -subunit, we created a deletion of 47 amino acid residues encoded by exon 13

of the IR gene. The deleted region contained no asparagine or cysteine residues which were potentially involved in N-linked glycosylation or disulphide linkage. In this report we have characterized the mutant insulin receptors (IR Δ Ex13) and found the importance of the deleted region for post-translational processing, transport and intramolecular signal transduction of IR.

MATERIALS AND METHODS

Materials

Transient expression vector of the wild-type IR, pGEM3SVHIR was previously constructed (Kobayashi et al., 1990). pBluescriptIIISK+ was purchased from Stratagene (La Jolla, CA, U.S.A.). Universal primers for M13 were purchased from Takara (Japan). Na¹²⁵I was purchased from New England Nuclear (Boston, MA, U.S.A.). [³⁵S]Methionine/cysteine (> 37 TBq/mmol) (Tran³⁵S-label) was purchased from ICN Biomedical (Irvine, CA, U.S.A.). [¹²⁵I]Insulin, mono-iodinated at the A-14 position, [³²P]ATP, horseradish peroxidase-conjugated Protein A and enhanced chemiluminescence (ECL) reagents were purchased from Amersham (Amersham, Bucks., U.K.). Anti-IR serum (α IR) was obtained from a type-B insulin-resistant patient with Sjögren's syndrome (Kobayashi et al., 1988a) and α CT antibody directed against the C-terminus of IR β -subunits was kindly supplied by J. M. Olefsky (University of California, San Diego, CA, U.S.A.). Monoclonal anti-IR antibody (29B4) directed against intracellular β -subunit was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Protein A-Sepharose was purchased from Pharmacia (Uppsala, Sweden). Endoglycosidase H (EC 3.2.1.96) was pur-

Abbreviations used: ECL, enhanced chemiluminescence; DMEM, Dulbecco's modified Eagle's medium; IR, insulin receptor; IR Δ Ex13, mutant IR with exon 13 deleted; PMSF, phenylmethanesulphonyl fluoride; WGA, wheat germ agglutinin.

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chased from Boehringer–Mannheim (Germany). All other reagents were of analytical grade.

Construction and expression of deletion mutant IR cDNA

A 2.5 kb *Bst*EII–*Bst*EII fragment of pCVSVHIRc (McClain et al., 1987) was used to replace the corresponding fragment pGEM3SVHIR giving rise to an isoform of wild-type IR cDNA lacking exon 11 (Ullrich et al., 1985). We constructed a mutant cDNA with deletion of 141 nucleotides corresponding to exon 13 of the IR gene encoding 47 amino acid residues of the extracellular β -subunit, i.e. residues 821–867 according to the numbering system of Ebina et al. (1985). A 266 bp *Pst*I–*Pst*I fragment cloned into pBluescriptII SK+ was used as a template for PCR-based mutagenesis (Higuchi et al., 1988) with the universal primers for M13 (M4; 5'-GTTTTCCCAGTCACGAC-3', RV; 5'-CAGGA-AACAGCTATGAC-2') and synthetic oligonucleotide primers (5'-ATGCCTGAAGAGCTGCATCTCTGCGTCTC-3', 5'-GATGCAGCTCTTCAGGCATGGTCCTCGCA-3'). The deletion and the entire nucleotide sequence produced by PCR was verified by dideoxy sequencing and the *Pst*I–*Pst*I fragment was finally reinserted into the original expression vector.

COS7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal-calf serum and transfection was performed by the DEAE-dextran method as previously described (Haruta et al., 1993).

Insulin-binding assay and surface labelling

Insulin binding to COS7 cells and surface labelling of the cells with Na^{125}I were performed as previously described (Haruta et al., 1993). Insulin binding to reconstituted IRs from poly(ethylene glycol)-precipitated cell lysate was performed according to the method described by Marshall (1985). Insulin binding to solubilized receptors was measured as described previously (Takata et al., 1986; Sasaoka et al., 1989b).

Immunoblot analysis

Proteins were separated by SDS/PAGE on 7.5% gels under reducing conditions and transferred to poly(vinylidene difluoride) membranes. The membranes were blocked for 30 min in phosphate-buffered saline (PBS) containing 0.1% Tween 20 and 5% (w/v) dried milk and incubated for 1 h with αCT (1:500) in PBS/Tween 20. After washing in PBS/Tween 20, the membranes were incubated for 30 min with horseradish peroxidase-conjugated Protein A(1:1000) in PBS/Tween 20/2% BSA. Membranes were then washed extensively in PBS/Tween 20 and chemiluminescence was detected by the ECL system according to the manufacturer's instructions (Amersham).

Pulse-chase labelling

At 60 h after transfection of 1×10^6 cells with mutant or wild-type cDNA, COS7 cells in 100-mm-diam. plates were incubated in methionine-free DMEM with 10% (v/v) dialysed fetal-calf serum for 1 h. The cells were then pulsed in 3 ml of medium containing 1 mCi of Tran^{35}S -label for 30 min. After washing, incorporation of [^{35}S]methionine/cysteine into the receptor protein in the cells incubated in normal medium was chased for 0–2 h. The cells were solubilized in 1% (v/v) Triton X-100, 120 mM NaCl, 5 mM KCl, 1 mM MgSO_4 , 1 mM CaCl_2 , 20 mM Hepes, pH 7.4, containing 0.2 mM phenylmethanesulphonyl fluoride (PMSF), 0.5 mg/ml bacitracin, 800 k.i.u./ml aprotinin, 8 mM EDTA, 2 mM dichloroacetic acid and 15 mM benzamidine. After ultracentrifugation, soluble materials were preadsorbed with Protein A–Sepharose for 1 h. Then, the IRs were

immunoprecipitated overnight at 4 °C with 29B4 (1:100 dilution) followed by incubation with Protein A–Sepharose for 1 h at 4 °C. The samples were eluted in sample buffer (Laemmli, 1970) with boiling and separated by SDS/PAGE.

Enzymic deglycosylation

COS7 cells were lysed and soluble materials were immunoprecipitated with αIR followed by incubation with Protein A–Sepharose for 1 h at 4 °C. The resin was washed and incubated for 2 h at 37 °C in 50 μl of buffer containing 20 mM sodium acetate, pH 5.5, 0.1 mM PMSF, 0.01% SDS, 100 mM 2-mercaptoethanol and 5 units of endoglycosidase H. Sample buffer was added and boiled for 3 min followed by SDS/PAGE and immunoblotting as described above.

Lectin chromatography

Lectin chromatography with wheat germ agglutinin (WGA)–agarose was described previously (Haruta et al., 1993). For purification of high-mannose-type IR precursor, *lens culinaris* (lentil) agglutinin coupled to agarose beads was used (Hedo et al., 1981). Cell lysate prepared from 3×10^7 cells in 3 ml of solubilizing buffer was recycled three times through a 2 ml column of lentil–agarose. Final flow-through was saved and the column was washed with 0.15 M NaCl, 1 mM CaCl_2 , 1 mM MnCl_2 , 50 mM Hepes, 0.1% Triton X-100, pH 7.6, at 4 °C. Receptors were eluted with the same buffer containing 0.3 M methyl- α -D-mannopyranoside.

Autophosphorylation of lectin-purified IRs

The same amounts of lectin-purified IRs (200 fmol) as determined by Scatchard analysis were used for autophosphorylation experiments. After insulin stimulation for 16 h at 4 °C, IRs were autophosphorylated in a reaction mixture containing 5 mM MnCl_2 , 1 mM CTP, 50 μM ATP and 50 μCi of [γ - ^{32}P]ATP for 15 min at 4 °C. Phosphorylated receptors were immunoprecipitated and resolved by 7.5% SDS/PAGE under reducing condition as previously described (Haruta et al., 1993).

RESULTS

Insulin-binding and surface-labelling studies

At 72 h after transfection, insulin binding to 1×10^6 COS7 cells transfected with wild-type cDNA was increased to 11.7% i.e. a 16-fold increase, compared with that to mock-transfected cells (0.74%). However, transfection with IR ΔEx13 cDNA failed to increase insulin binding (0.86%). To clarify the mechanism of decreased insulin binding to the IR ΔEx13 cells, surface labelling of the transfected cells with ^{125}I was performed. The cells expressing wild-type IR clearly showed the increased amount of 135 kDa α -subunit, 95 kDa β -subunit and 210 kDa proreceptor (Figure 1, lane 1). In contrast, only a slightly increased amount of IR was found on the cell surface of cells expressing mutant IR (Figure 1, lane 2). Therefore, both insulin-binding and surface-labelling studies revealed that IR ΔEx13 did not exist on the cell surface of COS7 cells after transfection.

Immunoblot analysis of intracellular IRs

We then performed immunoblot analysis of total cell lysates to identify intracellularly expressed IRs. Solubilized proteins were electrophoresed, transferred to membranes and probed with anti-IR antibodies directed against an epitope of C-terminal β -subunit (αCT). The cells expressing wild-type IR had 190–210 kDa proreceptors and 95 kDa β -subunits of IR (Figure 2,

Figure 1 Surface labelling of COS7 cells with Na^{125}I

COS7 cells expressing either wild-type IR (lane 1), IR ΔEx13 (lane 2) or mock-transfected cells (lane 3) were iodinated, solubilized, immunoprecipitated with anti-IR (αIR) antibodies and analysed by SDS/PAGE and autoradiography.

Figure 2 Immunoblot analysis of cellular proteins

Transfected COS7 cells expressing either wild-type IR (lane 1), IR ΔEx13 (lane 2) or mock-transfected cells (lane 3) were lysed in Laemmli sample buffer, electrophoresed and transferred to a membrane, followed by immunoblotting with anti-(C-terminal IR) (αCT) antibodies.

lane 1), whereas the cells expressing IR ΔEx13 showed only 190 kDa proreceptors (Figure 2, lane 2). Therefore, IR ΔEx13 was intracellularly expressed as an uncleaved proreceptor form

Figure 3 Pulse-chase labelling of IR

Cells transfected with either wild-type IR cDNA (lanes 1–5) or deletion mutant cDNA (lanes 6–10) were labelled with [^{35}S]methionine/cysteine for 30 min and then chased with unlabelled medium for the indicated number of hours. The cells were then solubilized, and IRs were immunoprecipitated with anti-IR (29B4) antibodies followed by SDS/PAGE and fluorography.

Figure 4 Enzymic deglycosylation with endoglycosidase H

COS7 cells expressing either wild-type IR (lanes 1 and 2) or IR ΔEx13 (lanes 3 and 4) were solubilized, immunoprecipitated and incubated in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of endoglycosidase H (5 m-units/50 μl) for 2 h at 37 $^{\circ}\text{C}$ followed by SDS/PAGE and immunoblotting with anti-(C-terminal IR) (αCT) antibodies.

and remained inside the cell. Apparently, IR ΔEx13 could not be transported to the plasma membrane since the surface-labelling study failed to detect the mutant receptor.

Pulse-chase labelling

In order to examine the time course of biosynthesis of the mutant IRs, transfected cells were labelled for 30 min with [^{35}S]methionine/cysteine and synthesis of IRs was chased for 21 h. Wild-type proreceptors were converted into α - and β -subunits at 3–7 h (Figure 3, lanes 1–5). However, the mutated proreceptor forms failed to be processed to subunits and remained as 190 kDa precursors up to 21 h (Figure 3, lanes 6–10). SDS/PAGE under non-reducing conditions revealed that most of the mutant receptors were normally dimerized (results not shown).

Enzymic deglycosylation

The IR is synthesized as a 190 kDa primary product containing a high-mannose-oligosaccharide core; this 190 kDa protein is then converted into 210 kDa with a low-mannose, complex-type oligosaccharide chain. Glycosylation state of the mutated receptors was assessed by endoglycosidase H which deglycosylates high-mannose oligosaccharide chains. Cellular proteins were immunoprecipitated with αIR and treated with endoglycosidase H. Before endoglycosidase H treatment, two distinct forms of precursor (210 kDa and 190 kDa) were observed in cells expressing wild-type IR (Figure 4, lane 1). After wild-type receptors

Figure 5 Lectin chromatography of IRΔEx13

COS7 cells expressing IRΔEx13 were solubilized, chromatographed through either WGA-agarose (lanes 1 and 2) or lentil lectin-agarose (lanes 3 and 4) columns. The eluate (lanes 1 and 3) or the flow-through (lanes 2 and 4) were subjected to immunoblot analysis with anti-(C-terminal IR) (α CT) antibodies.

were treated with endoglycosidase H, the 190 kDa species was deglycosylated to 170 kDa but the 210 kDa species was only slightly deglycosylated. This indicated that the 190 kDa species was a premature high-mannose-type precursor and that the 210 kDa species had fully processed low-mannose complex-type oligosaccharide chains (Figure 4, lane 2). In contrast, the molecular mass of the mutant receptors was reduced from 190 kDa to 170 kDa after treatment with endoglycosidase H (Figure 4, lanes 3 and 4). Therefore, the mutant IRs did not undergo appropriate glycosylation, probably due to abnormal tertiary structure, and had mainly unprocessed high-mannose oligosaccharide chains.

Lectin chromatography

Since IRΔEx13 appeared to have high-mannose oligosaccharide chains (Figure 4), we attempted to partially purify the mutant receptors using lentil lectin-agarose that had high affinity for mannose (Hedo et al., 1981). Bound receptors were eluted with a high concentration (0.3 M) of methyl- α -D-mannopyranoside. The eluate and flow-through were subjected to immunoblot analysis. As expected, IRΔEx13 did not bind to WGA-agarose (Figure 5, lanes 1 and 2) but had relatively high affinity for lentil lectin-agarose and could be partially purified with this method (Figure 5, lanes 3 and 4).

Insulin binding to the cell lysates and lectin-purified IRs

Because IRΔEx13 is not transported to the cell surface, insulin does not bind to the cells expressing mutant receptors. However, the mutant receptor itself may have binding capability. To test this possibility, we examined insulin binding to the mutant receptor purified with lentil lectin. First, cell lysates prepared from the same number of cells were precipitated with poly(ethylene glycol) and reconstituted in a buffer, then insulin binding was examined. Insulin binding to receptors from cells expressing IRΔEx13 at a tracer insulin concentration was significantly increased ($10.0 \pm 0.8\%$ per 1×10^6 cells) (mean $\pm$ S.E.M. of three experiments) compared with receptors from mock-transfected cells ($1.4 \pm 0.4\%$ per 1×10^6 cells) and was approx. 55% of that of wild-type IRs ($17.9 \pm 1.2\%$ per 1×10^6 cells). Next, we used either WGA-purified wild-type IRs or lentil lectin-purified mutant receptors for an insulin-binding study (Figure 6). When we used the same amount of wild-type or mutant receptors (by calculating from Scatchard analysis) at a tracer concentration,

Figure 6 Scatchard plot for insulin binding to solubilized IRs

Either WGA-purified wild-type IR ($\circ$) or lentil lectin-purified IRΔEx13 ($\bullet$) were incubated with 125 I-labelled insulin and various concentrations of unlabelled insulin for 16 h at 4 °C. Specific binding was determined by the poly(ethylene glycol) precipitation method. Each point was expressed by dividing values by receptor numbers (R_o) for each receptor preparation. Error bars represent S.E.M. for three determinations.

insulin binding to the deletion mutant receptor was decreased to 50% of that to the wild-type receptor. Therefore, insulin-binding affinity of the mutant IR was decreased to half of normal.

Autophosphorylation of lectin-purified IRs

To determine whether binding of insulin to the mutated receptors can activate IR tyrosine kinase, either WGA-purified wild-type IRs or lentil lectin-purified IRΔEx13 were incubated with insulin and the autophosphorylation was examined. The same amounts of each receptors, as judged by Scatchard analysis, were used for the study. The β -subunit of wild-type IR (95 kDa) was auto-

Figure 7 Autophosphorylation of lectin-purified IRs

Samples (200 fmol) of either WGA-purified wild-type IR (lanes 1–3) or lentil lectin-purified IRΔEx13 (lanes 4–6) were incubated with various concentrations (0, 1 and 20 nM) of insulin for 16 h at 4 °C and autophosphorylated as described in the Materials and methods section.

phosphorylated in a dose-dependent manner (Figure 7, lanes 1–3). However, the autophosphorylation of the mutant receptors (190 kDa) was decreased in the basal state and was not significantly stimulated with the highest concentration of insulin, i.e. 20 nM (Figure 7, lanes 4–6). Since the precursors (190–210 kDa) of the wild type were rapidly converted into the subunits, these precursors did not exist and thus no autophosphorylation of the wild-type precursors could be detected.

DISCUSSION

In the present study, we characterized a deletion mutant of IR lacking 47 amino acid residues of the extracellular β -subunit and found that the immature precursor form of the mutated receptor with high-mannose-type oligosaccharide chains was not processed into the mature α - and β -subunits and that the mutated receptor was not transported to the cell surface but remained inside the cell. Half-life of the mutant proreceptors was longer than that of wild-type proreceptors, indicating that degradation of the mutant receptors was not accelerated by the presence of the deletion. These findings suggest that the mutated receptors are retained in the endoplasmic reticulum and can not be transported to the Golgi apparatus where proteolytic cleavage, as well as further processing of oligosaccharide side chains, takes place (Olson et al., 1988). Naturally occurring mutations in the α -subunit (Kadowaki et al., 1990, 1991; Maassen et al., 1991; Van der Vorm et al., 1992) and mutations at N-glycosylation sites in the α -subunit (Collier et al., 1993; Caro et al., 1994) also showed the similar phenomenon. On the other hand, modification of all four potential N-glycosylation sites of the β -subunit has been reported to impair transmembrane signalling but not processing or transport of IR (Leconte et al., 1992). Therefore, impaired processing and transport of IR Δ Ex13 may not be caused by altered N-glycosylation of the extracellular β -subunit of the mutant receptors. With regards to missense mutations in the α -subunit, binding of immunoglobulin heavy chain-binding (BiP) to misfolded mutant IRs has been suggested to impair transport of the mutant receptors (Accili et al., 1992). It is likely that proper folding of the extracellular domain of β -subunit is also essential for intracellular transport of precursor molecules.

Insulin-binding affinity of the deletion mutants was only decreased to 50% of normal. Therefore, proteolytic cleavage, maturation of carbohydrate chains, or deletion of 47 amino acid residues in the extracellular β -subunit was not essential for insulin-binding activity. In fact, it has been reported that newly translated proreceptor requires insulin-binding activity prior to maturation of N-linked oligosaccharide side chains (Olson and Lane 1987; Olson et al., 1988). Previously, substitutions of Leu²³³ to Pro and Gly³¹ to Arg have been reported to impair cleavage and transport of mutant proreceptors (Maassen et al., 1991; Van der Vorm et al., 1992). In contrast with our results, insulin could not bind to Pro²³³ or Arg³¹ mutant proreceptors. As WGA–Sephadex chromatography, instead of lentil lectin chromatography, was employed to purify these mutants, insulin-binding activity of the mutant precursors might be underestimated. Alternatively, Pro²³³ or Arg³¹ mutations in the α -subunit may cause marked structural change in insulin-binding sites.

As we (Kobayashi et al., 1988a,b; Sasaoka et al., 1989a,b) and others (Yoshimasa et al., 1988) previously reported, proreceptors produced by a substitution of Ser for Arg⁷³⁵ at the proteolytic cleavage site identified in patients with extreme insulin resistance showed normal processing of carbohydrate chains and normal transport to the cell surface but had a markedly decreased affinity for insulin. Another mutant, lacking all of the four basic

amino acid residues (Arg-Lys-Arg-Arg) at the proteolytic cleavage site, has also been reported to exhibit a markedly decreased affinity for insulin (Williams et al., 1990). Although proteolytic cleavage of IR Δ Ex13 was also inhibited, the mutant receptor had a relatively high insulin-binding affinity compared with Ser⁷³⁵ proreceptors. Possibly, the deletion of 47 amino acid residues corresponding to exon 13 may partially remove steric hindrance around the insulin-binding pocket produced by linkage between the α - and β -subunits. After mild trypsin digestion, which increased insulin-binding capability of Ser⁷³⁵ mutants, insulin-binding activity of deletion mutant was not increased but rapidly decreased (results not shown), suggesting that conformational change of the mutant receptors prevented the enzyme from attacking the cleavage sites.

Despite the fact that the mutated receptor had a substantially high insulin-binding affinity, insulin-stimulated autophosphorylation of the solubilized mutant receptors was markedly impaired. It has been shown that uncleaved high-mannose-type IR precursors are able to autophosphorylate following insulin stimulation (Rees-Jones et al., 1983; Arakaki et al., 1989). Furthermore, previous reports using glucosidase or mannosidase blockers indicated that the incomplete maturation of the oligosaccharide side chains did not affect the insulin-stimulated phosphorylation of these receptors (Duronio et al., 1988). Therefore, it seems unlikely that the kinase defect of IR Δ Ex13 is due to impaired post-translational processing such as proteolytic cleavage or maturation of carbohydrate chains. An anti-IR monoclonal antibody directed against the extracellular β -subunit inhibits insulin-stimulated receptor autophosphorylation and biological responses without affecting IR binding (Gherzi et al., 1989). Our findings also support the notion that the extracellular β -subunit has a regulatory role for transmembrane signalling. Mutations at N-glycosylation sites in the extracellular β -subunit have been reported to cause decreased kinase activity of the mutated receptors and impaired insulin-stimulated biological response in transfected cells (Leconte et al., 1992). Thus, N-linked oligosaccharide chains of the extracellular β -subunit are also important for transmembrane signalling. However, since all four asparagines potentially involved in N-glycosylation are preserved in IR Δ Ex13, structural change produced by the deletion of 47 residues in the extracellular β -subunit appears to be sufficient to inhibit the insulin-induced conformational change required for the activation of IR tyrosine kinase.

In conclusion, the extracellular domain of the β -subunit encoded by exon 13 is necessary for processing and transport as well as for intramolecular signal transduction of IR.

This study was supported in part by a grant-in-aid from the Ministry of Education, Science and Culture, and a research grant for intractable disease from the Ministry of Health and Welfare, and a grant from Otsuka Pharmaceutical Co., Japan.

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