EVIDENCE FOR A ROLE OF GLUCOSE REMOVAL FROM CORE OLIGOSACCHARIDES*

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The insulin proreceptor is a 190-kDa glycoprotein that is processed to mature α (135-kDa) and β (95-kDa) subunits. In order to determine the role of carbohydrate chain processing in insulin receptor biogenesis, we investigated the effect of inhibiting glucose removal from core oligosaccharides of the insulin proreceptor with glucosidase inhibitors, castanospermine and 1-deoxynojirimycin.

Cultured IM-9 lymphocytes treated with inhibitors had 50% reciubition in surface insulin receptors as demonstrated by ligand binding, affinity cross-linking with 125I-insulin, and lactoperoxidase/Na125I labeling studies. Degradation rates of surface labeled receptors were similar in both control and inhibitor-treated cells (t1/2 = 5 h); thus, accelerated receptor degradation could not account for this reduction. Biosynthetic labeling experiments with [3H]leucine and [3H]mannose identified an apparently higher molecular size proreceptor (≈205 kDa) that failed to show the characteristic decline with time seen in the normal 190-kDa proreceptor. Along with this finding, the biosynthetic label appearing in the mature subunits was reduced in these inhibitor-treated cells. Endoglycosidase H treatment of both precursors produced identical 170-kDa bands. Carbohydrate chains released from the 205-kDa precursor by endoglycosidase H migrated in the same position as the Glc2-Man9GlcNAc standards when separated by high performance liquid chromatography, whereas the 190-kDa proreceptor oligosaccharides migrated similar to the Man9GlcNAc chains. Although the mature subunits of control and inhibitor-treated cells demonstrated equal electrophoretic mobility, the endoglycosidase H-sensitive oligosaccharides of the mature subunits in treated cells also contained residues that migrated similar to the Glc2-Man9GlcNAc standards. Thus, glucose removal from core oligosaccharides is apparently not necessary for the cleavage of the insulin proreceptor, but does delay processing of this precursor, which probably accounts for the reduction in cell-surface receptors.

The recent identification of the cDNA of the insulin receptor has revealed 15 potential N-linked glycosylation sites in the α subunit 4 on the presumed extracellular portion of the β subunit (10, 11). However, the biological significance of N-glycosylation of the insulin receptor is unclear. Complete inhibition of glycosylation by tunicamycin, which prevents the transfer of the core oligosaccharide, blocks any further processing of the proreceptor (12). On the other hand, inhibition of Golgi mannosidase II by swainsonine does not appear to have any effect on the translocation and function of the insulin receptor (13). Thus, whereas complete lack of N-linked carbohydrate chains is incompatible with normal receptor processing, abnormalities of late carbohydrate processing may not be sufficient to alter further receptor maturation and function. Castanospermine, a plant alkaloid, and 1-deoxynojirimycin, a glucose analogue antibiotic, inhibit glucosidases I and II, respectively. The oligosaccharide chains of glycopeptides produced in the presence of either inhibitor retain 1–3 glucose residues and a variable number of mannosides (14, 15). The preservation of the glycosylated high-mannose oligosaccharide on the insulin receptor precursor allowed us to investigate the significance of oligosaccharide trimming for insulin receptor structure and function. We have found that in the presence of these inhibitors, cultured lymphocytes produce an abnormal proreceptor of approximately 205 kDa. The processing of this precursor was delayed and probably accounted for the observed 50% reduction in cell-surface insulin receptors of inhibitor-treated cells.

The insulin receptor is an integral membrane glycoprotein which is synthesized in the endoplasmic reticulum as a single-chain polypeptide precursor of 190 kDa (2, 3). This proreceptor contains oligosaccharides exclusively of the N-linked high-mannose-type. After translocation to the Golgi complex, it is proteolytically cleaved to yield pre-α (120-kDa) and pre-β (80-kDa) subunits (4). Additional post-translational events are the processing of a number of the high-mannose carbohydrate chains to complex-type chains (2) and fatty acylation (5). The mature α (135-kDa) and β (95-kDa) subunits are inserted into the plasma membrane as disulfide-linked heterodimers (6, 7).

The N-glycosylation of the insulin receptor appears to follow the general pathway described for many secretory and membrane proteins in animal cells (8, 9). After the initial transfer of a high-mannose oligosaccharide (Glc3Man9GlcNAcβ) from a lipid carrier to the nascent polypeptide chain, a number of processing steps take place. Glucosidases I and II remove the 3 glucose residues and mannosidases I and II may remove all but 3 mannose residues. Following the trimming of glucoses and mannoses, complex-type chains are generated by the addition of N-acetylglucosamine, galactose, fucose, and sialic acid.

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**EXPERIMENTAL PROCEDURES**

**Materials**—Castanospermine and endoglycosidase H (endo-β-N-acetylglucosaminidase, EC 3.2.1.96) were purchased from Genzyme, Inc. (Boston, MA), and 1-deoxynojirimycin was a generous gift from Drs. E. Truscheit and D. Schmidt (Bayer AG, Wuppertal, Germany). Peroxidase (EC 1.11.1.7) was obtained from Boehringer. Biosynthetic labeling, the cells were preincubated with the appropriate with and without labeled insulin M) for 36 h at 37 °C. The effect of glucosidase inhibitors on (575) and 18F-labeled receptors were excised, washed twice with cold PBS. For the insulin receptor turnover study, 12 were analyzed. Effects of Glucosidase Inhibitors on **125I-Insulin Binding**—Insulin binding was assayed as previously described (16). Cells were incubated with [125I]insulin (~15,000 cpm (0.2 ng/ml)) and various concentrations of unlabeled insulin in lymphocyte buffer (100 mM HEPES, 120 mM NaCl, 1 mM MgSO4, 1 mM EDTA, 10 mM glucose, 15 mM sodium acetate, and 0.1% bovine serum albumin, pH 8.0) for 90 min at 15 °C. After incubation, the cells were sedimented by centrifugation, the supernatant was aspirated, and the radioactive content was measured. Labeled oligosaccharide standards were run adjacent to all samples.

**RESULTS**

**Effect of Glucosidase Inhibitors on **125I-Insulin Binding**—Insulin binding to IM-9 lymphocytes was determined after a 24-h preincubation at 37 °C in the presence or absence of inhibitors. Cells treated with 100 µg/ml castanospermine had approximately a 50% reduction in specific insulin binding (Fig. 1). Competition binding with unlabeled insulin in treated and untreated cells revealed similar one-half maximal displacement (~5–10 ng/ml insulin). Thus, reduction in binding was attributed to a decrease in receptor number, not affinity. The effect of castanospermine was dose-dependent, with maximum inhibition in binding occurring at a concentration of 100 µg/ml. The earliest detectable decrease in binding occurred at 12 h of preincubation; the effect was maximal at 18–24 h and persisted for up to 36 h at 37 °C (data not shown).

**Immunoprecipitation and SDS-Polyacrylamide Gel Electrophoresis**—After labeling, the cells were solubilized with 1% Triton X-100 (v/v) at 0.15 M NaCl, 50 mM HEPES buffer, pH 7.6, supplemented with phenylmethylsulfonyl fluoride (2 mM) and aprotinin (1.5 trypsin inhibitor units/ml). Solubilization was performed at 4 °C for 30 min, and nondissolved material was sedimented by ultracentrifugation at 200,000 × g for 1 h. The insulin receptors were immunoprecipitated with human autoantibodies (serum B-7) as previously described (19). The antiserum or the normal nonimmune sera were added directly to the protein A-Sepharose extract (1:100 dilution). In the case of biosynthetic labeling, the extracts were treated with S. aureus cell suspension or protein A-Sepharose (200 µl/1 ml sample) for 1 h prior to the addition of antibodies. After 12–14 h at 4 °C, the receptor-antibody complexes were immunoadsorbed with S. aureus cells or protein A-Sepharose (100 µl) for 2 h. The receptors were released from the immunoadsorbent by boiling in sample buffer (2% SDS, 0.1 M diithiothreitol, 0.02% bromophenol blue, 10% glycerol, and 10 mM phosphate buffer, pH 4–5) for 5 min. The receptor components were then separated by SDS-polyacrylamide gel electrophoresis (7.5%) according to the method of Laemmli (20). Gels with tritium-labeled receptors were washed with ENHANCE prior to drying. Autoradiography or fluorography was performed by exposure at -70 °C of Kodak X-Omat AR film to the dried gels (19). Quantitative measurements of radioactivity in receptor bands were obtained by excising and counting in a γ-counter for 125I label or in a scintillation counter for tritium label after eluting with 3% Protosol in Econofluor for 18 h at 37 °C.

**Endoglycosidase H Treatment**—Enzymic digestion with endoglycosidase H was performed as previously described (2). After immunoprecipitation, labeled receptors were recovered from immunoadsorbent by boiling in 2% SDS, 0.01 M dithiothreitol, and 10 mM Tris-HCl buffer, pH 7.0, and the supernatant was diluted 1:4 with 0.5 M citrate buffer, pH 5.5, and endoglycosidase H was added to a final concentration of 0.2–0.5 unit/ml. Digestion was performed for 6 h at 37 °C in the presence of phenylmethylsulfonyl fluoride (1 mM) and pepstatin (0.5 µg/ml) and terminated with ice-cold 10% trichloroacetic acid. Precipitates were washed twice with ethyl ether/ethanol (1:1, v/v), air-dried, and analyzed by SDS-polyacrylamide gel electrophoresis under reducing conditions as described above.

**HPLC of Endoglycosidase H-sensitive Oligosaccharides**—Analysis of labeled high-mannose oligosaccharides present in insulin receptor after a 24-h preincubation at 37 °C in the presence of phenylmethylsulfonyl fluoride (1 mM) and pepstatin (0.5 µg/ml) and terminated with ice-cold 10% trichloroacetic acid. Precipitates were washed twice with ethyl ether/ethanol (1:1, v/v), air-dried, and analyzed by SDS-polyacrylamide gel electrophoresis under reducing conditions as described above.
binding sites in castanospermine-treated cells (Fig. 1). Insulin binding to IM-9 cells was performed as described under "Experimental Procedures." Bound/free $^{125}$I-insulin, normalized to $6 \times 10^6$ cells/ml, is plotted versus increasing total insulin concentrations. $\bigcirc$, control cells; $\bullet$, castanospermine-treated cells. Each point represents a mean of three experiments. The horizontal bars indicate one-half maximal displacement.

Exclusion or by protein synthesis as assessed by incorporation of $[^3]$H]leucine into trichloroacetic acid-precipitable material.

Affinity and Cell-surface Labeling of the Insulin Receptor—To investigate further the nature of cell-surface insulin receptors after treatment with castanospermine, affinity cross-linking with $^{125}$I-insulin was performed. After a 24-h preincubation in the presence or absence of the inhibitor, cells were incubated with $^{125}$I-insulin and cross-linked with disuccinimidyl suberate. The labeled receptors were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Affinity labeling confirmed the reduction of specific insulin-binding sites in castanospermine-treated cells (Fig. 3). Quantitation of the ligand-bound $\alpha$ subunit showed an approximate 50% decrease in the labeled band from treated cells. The electroforetic mobility of the 135-kDa band, however, was similar to control (Fig. 3). Because of the possibility that a population of receptors might go undetected by equilibrium binding or cross-linking studies, surface labeling experiments were undertaken. Following a 24-h preincubation in the presence or absence of castanospermine, IM-9 cells were labeled by the lactoperoxidase/Na$^{125}$I method, and the insulin receptor was immunoprecipitated with antireceptor antibodies. Analysis of SDS-polyacrylamide gel electrophoresis and autoradiography allowed the identification of both the $\alpha$ (135-kDa) and $\beta$ (95-kDa) receptor subunits in control and treated cells (Fig. 3). The labeling of both receptor subunits in treated cells was clearly reduced. Quantitation of the radioactivity present in the receptor bands showed that this reduction was approximately 50%, in agreement with the estimates obtained in the $^{125}$I-insulin binding and affinity labeling studies.

Degradation Rate of Cell-surface Insulin Receptor—Having demonstrated a decrease in cell-surface insulin receptors in the presence of glucosidase inhibitors, we wished to determine whether this could be due to accelerated receptor degradation. IM-9 cells were incubated for 24 h with and without castanospermine and then labeled by the lactoperoxidase/Na$^{125}$I method. After washing, the cells were returned to control or inhibitor-supplemented culture media and incubated at 37 °C for up to 9 h. At various time points, aliquots of cells were solubilized, and the insulin receptors were analyzed by SDS electrophoresis and autoradiography. Degradation rates of the receptor subunits were estimated by quantitation of the decrease in their radioactive content. The degradation rates of both subunits from control and castanospermine-treated cells were essentially identical ($t_{\nu} \sim 5$ h (Fig. 4)). Thus, degradation of surface receptors was unaffected by the presence of inhibitor. The $t_{\nu}$ for 5 h observed differs from that reported by Kasuga et al. (22), but is similar to the $t_{\nu}$ of 6.5 h observed by McElduff et al. (23). This is consistent with the differences that have been seen over the years among IM-9 cells, other transformed lymphocytes, and even different batches of IM-9 cells.

**Biosynthetic Labeling of the Insulin Receptor with $[^3]$H Leucine and $[^3]$H Mannose—Having demonstrated that the number of cell-surface insulin receptors was reduced in the presence of glucosidase inhibitors and that this reduction could not be accounted for by accelerated receptor degradation, we next wished to investigate the effect of the inhibitors on the biosynthetic process.

Cells were preincubated with castanospermine for 4 h, pulsed with $[^3]$H]leucine, and chased with unlabeled leucine for up to 4 h. Castanospermine was present throughout the pulse and chase incubations. Control and castanospermine-treated cells were then solubilized and immunoprecipitated with antireceptor antibody, and the insulin receptor bands
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Fig. 4. Degradation rate of surface labeled receptor subunits of control and castanospermine-treated cells. Cells incubated with and without castanospermine were labeled by the lactoperoxidase/NaI method (see "Experimental Procedures"). After both proreceptor and mature components were identified on SDS-polyacrylamide gel electrophoresis (7.5%), and autoradiography. As shown in Fig. 5, several points are of note. First, the proreceptor, normally a 190-kDa band, as seen in control, had somewhat less mobility and migrated as a 205-kDa band in castanospermine-treated cells. At the 4-h chase point, the proreceptor band was more prominent in the castanospermine-treated cells than in control, and the α and β subunit bands were reciprocally less intense (Fig. 5). An essentially identical situation was found when [3H]mannose was used as the label in the same pulse-chase design (Fig. 6) except that [3H]mannose was used as label. Excess unlabeled mannose was used during the chase period, which was extended to 6 h for this study.

Fig. 5. Pulse-chase labeling with [3H]leucine of the insulin receptor in castanospermine-treated cells. IM-9 lymphocytes were preincubated in complete medium with and without inhibitor for 4 h. Cells were then pulse-labeled with [3H]leucine for 15 min in leucine-free medium and chased in complete medium with excess unlabeled leucine for 1 and 4 h. Castanospermine was maintained throughout the pulse and chase periods for treated cells. After immunoprecipitation with control or antireceptor antisera, the receptor components were identified on SDS-polyacrylamide gel electrophoresis (7.5%) and autoradiography.

were identified by electrophoresis and fluorography. By 1 h of chase, the insulin proreceptor was prominently seen in both control and castanospermine-treated cells; and by 4 h of chase, both proreceptor and mature α and β subunit bands were seen (Fig. 5). Several points are of note. First, the proreceptor, normally a 190-kDa band, as seen in control, had somewhat less mobility and migrated as a 205-kDa band in castanospermine-treated cells. At the 4-h chase point, the proreceptor band was more prominent in the castanospermine-treated cells than in control, and the α and β subunit bands were reciprocally less intense (Fig. 5). An essentially identical situation was found when [3H]mannose was used as the label in the same pulse-chase design (Fig. 6) except that the labeling of the 205-kDa proreceptor was more intense with this monosaccharide than with [3H]leucine. As in the cross-linking and surface labeling experiments, the α and β subunits from control and castanospermine-treated cells migrated to a similar position in the gel (compare Fig. 3 with Figs. 5 and 6).

A continuous labeling study with [3H]mannose was performed in 1-deoxynojirimycin-treated cells (7.5 mM) for up to 18 h. In the treated cells, a larger molecular size precursor was also observed, similar to that seen with castanospermine, i.e. ~205 kDa. The intensity of the label in this band was greater than the control proreceptor, and the label in the α and β subunit bands was reciprocally decreased (data not shown).

Endoglycosidase H Treatment and HPLC Analysis of Oligosaccharide Chains—To demonstrate the role of the oligosaccharide chains in the generation of the abnormal 205-kDa insulin proreceptor, endoglycosidase H digestion was performed on the precursors of control and castanospermine-treated cells. This enzyme cleaves specifically high-mannose-type oligosaccharides, which account for all the carbohydrate chains of the insulin proreceptor. After [3H]leucine pulse labeling and a 1-h chase, cells were solubilized; the immunoprecipitated receptors were then treated with endoglycosidase H, and receptor subunits were separated by SDS electrophoresis. As shown in Fig. 7 the abnormal 205-kDa precursor was endoglycosidase H-sensitive, similar to the control 190-kDa proreceptor. A 170-kDa band, representing the nascent polypeptide (24), resulted from digestion of both precursors. Thus, the difference in the molecular size of the abnormal prorecep-
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In order to verify the effects of glucosidase inhibition induced by castanospermine as well as to characterize the structure of the oligosaccharides produced under these conditions, the proreceptor carbohydrate chains were isolated and subjected to HPLC analysis. IM-9 lymphocytes were pulse-labeled with $^3$H]mannose, and at 1 h of chase, the labeled receptors were isolated. After SDS-polyacrylamide gel separation, the proreceptor bands were excised and treated with endoglycosidase H; the released oligosaccharides were separated by HPLC on an NH$_2$-derivatized column. The major components of the proreceptor oligosaccharides from control cells eluted in the same position as the Man$_9$GlcNAc standards (Fig. 8, upper), as previously reported (21). It should be noted that endoglycosidase H cleaves high-mannose chains between the two inner, $\beta 1-4$-linked N-acetylglucosamine residues; and therefore, the released oligosaccharides contain only 1 N-acetylglucosamine residue. A very different elution pattern was observed with the proreceptor oligosaccharides from castanospermine-treated cells (Fig. 8, lower); in this case, the major components migrated in the same position as the Glc$_3$Man$_n$GlcNAc standards.

The HPLC procedure we have employed separates high-mannose chains essentially by the number of hexoses. Thus, the species which migrated with the Glc$_n$Man$_n$GlcNAc standard has very likely this hexose composition since this is the longest oligosaccharide (12 hexoses) known to be transferred in animal cells (8). On the other hand, the peak eluting with the Glc$_3$Man$_n$GlcNAc marker corresponds to a Hex$_1$GlcNAc chain which could be composed of either Glc$_3$Man$_n$GlcNAc or Glc$_2$Man$_n$GlcNAc. The latter possibility is quite likely since removal of 1 or 2 mannose residues by mannosidase I can occur on fully glycosylated chains as reported for other glycoproteins synthesized under the influence of castanospermine (25). In either case, it is clear that castanospermine treatment had a dramatic effect on the structure of the proreceptor oligosaccharides.

The mature $\alpha$ and $\beta$ subunits of the insulin receptor are known to contain a mixture of high-mannose and complex-type oligosaccharides (18). Although the electrophoretic mobility of the receptor subunits synthesized in the presence of castanospermine was not altered, endoglycosidase H-sensitive oligosaccharides were subjected to a similar HPLC analysis as that performed with the proreceptor chains. The chromatographic pattern of the oligosaccharides of the $\alpha$ and $\beta$ subunits from control cells was similar to that reported previously (21) and showed the presence of chains with a generally lower number of mannose residues (Man$_9$GlcNAc) than those of the proreceptor (Fig. 9, A and C). In contrast, analysis of the oligosaccharides from castanospermine-treated cells demonstrated the presence of two larger components which migrated with the Glc$_3$Man$_n$GlcNAc and Glc$_2$Man$_n$GlcNAc markers (Fig. 9, B and D) in a similar fashion to that observed in the proreceptor chains. The presence of these glucosylated chains was observed in both the $\alpha$ and $\beta$ receptor subunits. Thus, the abnormal glycosylation of the proreceptor oligosaccharides induced by castanospermine persists in some high-mannose chains of the mature $\alpha$ and $\beta$ subunits.

**DISCUSSION**

Our data show that glucosidase inhibition in IM-9 lymphocytes with two chemically dissimilar compounds, castanospermine and 1-deoxynojirimycin, induces a reduction (a 50%) in cell-surface insulin receptors. This reduction was demonstrated with $^{125}$I-insulin binding, affinity labeling of the $\alpha$ subunit, and cell-surface labeling of the $\alpha$ and $\beta$ receptor subunits. The usefulness of inhibitors is frequently limited by their toxicity and adverse effects on cell metabolism. In this regard, both castanospermine and 1-deoxynojirimycin were excellent tools to probe the role of glucosidases since their use was not accompanied by any alteration of cell viability or protein synthesis.

Given that the insulin receptor is subject to constant turnover and its concentration at the cell surface is the result of a dynamic equilibrium (22), a reduction in the number of receptors could be due to either an enhanced degradation rate or a decreased synthesis rate. The fact that the degradation rate of the receptor subunits was not modified in the presence of the glucosidase inhibitors suggested an effect of these compounds on the biosynthetic pathway. Indeed, biosynthetic
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Fig. 9. HPLC profile of high-mannose oligosaccharides from the mature subunits of the insulin receptor. Mature insulin receptor subunits were isolated from cells incubated with and without castanospermine, labeled with \(^{(3H)}\)mannose, and chased for 6 h as shown in Fig. 6. The high-mannose oligosaccharides were released and analyzed as described under "Experimental Procedures." The elution profiles of the 135-kDa subunit oligosaccharides from control and castanospermine-treated cells are shown in A and C, respectively; the profiles of the 95-kDa subunit oligosaccharides from control and castanospermine-treated cells are shown in B and D, respectively. The axis and abbreviations are as described for Fig. 8.

labeling studies with \(^{(3H)}\)leucine and \(^{(3H)}\)mannose demonstrated the production of an abnormal, higher molecular size insulin proreceptor (205 versus 190 kDa). This large proreceptor failed to decline with time at the same rate as the proreceptor from control cells, and the generation of the mature \(\alpha\) and \(\beta\) subunits was proportionally reduced. Therefore, a delayed processing of the abnormal proreceptor appeared to account for the reduction of insulin receptors on the plasma membrane in inhibitor-treated cells.

The larger molecular mass of the proreceptor in inhibitor-treated cells was due solely to changes in the carbohydrate chains. Endoglycosidase H digestion of the \(^{(3H)}\)leucine-labeled proreceptors from control and treated cells generated polypeptides which migrated electrophoretically in identical position (170 kDa). Furthermore, molecular sizing by HPLC of the carbohydrate chains of the insulin proreceptor demonstrated the presence of Hex\(_{10-12}\)GlcNAc\(_2\) chains in treated cells, whereas the largest chain found in control cells was Hex\(_6\)GlcNAc\(_2\). Although monosaccharide analysis was not performed, the largest oligosaccharide detected in treated cells, Hex\(_3\)GlcNAc\(_2\), must correspond to Glc\(_3\)Man\(_9\)GlcNAc\(_2\); the identification of this chain confirms that this is the initial form of high-mannose oligosaccharide in the insulin proreceptor, and the rapid removal of this oligosaccharide in the absence of glucosidase inhibitors accounts for the failure of its detection in previous studies (21). The other major oligosaccharide found in the proreceptor of treated cells was a Hex\(_1\)GlcNAc\(_2\) species; it is likely that its composition is Glc\(_3\)Man\(_9\)GlcNAc\(_2\) rather than Glc\(_2\)Man\(_9\)GlcNAc, since removal of 1 or 2 mannose residues has been reported on fully glucosylated chains in other glycoproteins synthesized in the presence of castanospermine (25).

Previous work has shown the presence of a mixture of complex-type and high-mannose chains in the mature \(\alpha\) and \(\beta\) subunits of the insulin receptor (19). Furthermore, the recent identification of the nucleotide sequence of a cDNA clone of the insulin receptor has predicted 15 potential N-linked sites in the \(\alpha\) subunit and 4 on the extracellular portion of the \(\beta\) subunit (10, 11), although the number of actual glycosylation sites and the type of chains attached to each site are still unknown. Our data show that the mature \(\alpha\) and \(\beta\) subunits generated in the presence of glucosidase inhibitor had identical electrophoretic mobility as the mature subunits in control cells. However, the subunits in treated cells showed a somewhat greater sensitivity to endoglycosidase H (data not shown). Nevertheless, HPLC analysis of high-mannose oligosaccharides from treated cells also demonstrated the presence of glycosylated chains (Hex\(_{10-12}\)GlcNAc\(_2\)) in the \(\alpha\) and \(\beta\)
subunits. Therefore, it appears that at least some glucosylated high-mannose chains persist in the α and β subunits after processing. The normal electrophoretic mobility of the intact subunits may be explained if the high mannose chains make a smaller contribution to the electrophoretic behavior as compared to the complex-type chains.

Our results are consistent with the conclusion that prevention of glucose removal from core oligosaccharides retards processing of the insulin receptor and produces a marked decrease in cell-surface receptors. However, proteolytic cleavage of the proreceptor is not blocked, although it takes place at a slower rate; and further processing of some of the carbohydrate chains is not completely inhibited. Furthermore, the processed receptors are inserted in the plasma membrane, and their insulin binding affinity is normal despite the presence of an undetermined number of glucosylated chains. The occurrence of complex-type oligosaccharide chains suggests that some glucosidase activity is resistant to the inhibitors as previously noted by other authors (14).

The present data agree with those reported for certain membrane and secretory glycoproteins, although other glycoproteins appear to be unaffected by glucosidase inhibitors (26). Secretion of IgD (27), α-antitrypsin, and α-antichymotrypsin (26) as well as the intracellular transport of the glycoprotein E of mouse hepatitis virus (28) are greatly reduced in the presence of glucosidase inhibitors. Furthermore, the epidermal growth factor receptor of 1-deoxyribofuranosyl-butyric acid-treated A431 cells show delayed acquisition of both ligand binding and endoglycosidase H resistance (29). In addition, cell-surface acetylcholine receptors of BC3H1 cells are reduced in the presence of 1-deoxyribofuranosyl-butyric acid because of an increase in receptor degradation rate (30).

The mechanism by which prevention of glucose removal retards the processing of the insulin proreceptor is not fully understood. The presence of glucose residues may reduce the affinity of the substrate for the subsequent enzyme(s) involved in the processing pathway. The presence of the glucose residues might impair binding to a putative transport receptor, retarding the movement from the endoplasmic reticulum to the Golgi complex. Alternatively, it could be speculated that the glucosylated chains may interfere with oligomerization or with the tertiary configuration of the polypeptide chain which may be required for its appropriate vesicular transfer (31).

In conclusion, we found that glucose removal from core oligosaccharides represents an important signal in the translocation and rate of processing of the insulin receptor to the plasma membrane.

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