Sortilin Is a Major Protein Component of Glut4-containing Vesicles*

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In fat and skeletal muscle cells, glucose transporter isoform 4 (Glut4) is translocated to the cell surface in response to insulin via a system of specialized recycling vesicles. Besides Glut4, these vesicles include the novel insulin-regulatable aminopeptidase, receptors for insulin-like growth factor-II/Man-6-phosphate and transferrin, and a glycoprotein with the molecular mass of 110 kDa. We report here by the criteria of the partial protein sequencing and subsequent cDNA cloning that glycoprotein 110, the last unidentified major protein component of Glut4-containing vesicles, is sortilin, a novel type I receptor-like protein recently cloned from human brain (Petersen, C. M., Nielsen, M. S., Nykjar, A., Jacobsen, L., Tommerup, N., Rasmussen, H. H., Roigaard, H., Gliemann, J., Madsen, P., and Moestrup, S. K. (1997) J. Biol. Chem. 272, 3599–3605). This protein is highly expressed in fat, brain, and lung and is dramatically up-regulated during differentiation of adipocytes in vitro.

The regulation of blood glucose levels by insulin in mammals is achieved by the hormone-dependent movement of the fat and muscle-specific glucose transporter, Glut4, from an intracellular storage vesicle to the cell surface (1–4). As an approach to understand the mechanisms underlying this process, we and others have isolated these vesicles using anti-Glut4 antibodies and have analyzed their protein content by several techniques. Thus, immunosolation of Glut4-containing vesicles following cell surface biotinylation in the presence of insulin revealed three major component proteins in these vesicles (gp230, gp160, and gp110)† that corresponded to major silver staining vesicular proteins present in the basal state (no insulin) (5). These proteins bind to wheat germ agglutinin-agarose and can be detected in an overlay assay with labeled wheat germ lectin, which are the recently described putative sorting protein/receptor, sortilin (9).

Isolation of Rat Sortilin cDNA Clone—A peptide excised from purified p110 (peptide 2 in Fig. 1) was found to be 93.7% identical to the translated peptide of Homo sapiens cDNA clone 249708 (accession number H85743) derived from a normalized human expressed sequence tag (EST) cDNA library. This clone was purchased from Genome Sequencing Center at Washington University School of Medicine, and the whole insert of the clone (1330 base pairs) was sequenced. The sequenced insert was found 99.5% identical to Human sortilin (accession number X98248). This sequence was used as a probe to screen rat skeletal muscle cDNA library. All probes were labeled by random priming using the Klenow fragment of DNA polymerase (Promega) and [α-32P]dCTP (NEN Life Science Products). The DNA insert of Homo sapiens cDNA clone 249708 was excised with XhoI/NotI digestion, labeled with [α-32P]dCTP by random priming, and used to screen 5' stretch cDNA libraries from skeletal muscle (primed with oligo(dT) + random primers, respectively) of adult male Sprague-Dawley rats (CLONTECH). Seventeen 150-mm agar plates each containing 50,000 phage plaques were transferred to nylon filter disks (NEN Life Science Products) and hybridized to probe as described in manufacturer’s instructions. Positive clones were picked and rescreened until single clones were obtained. Lambda DNA clones were purified by Nucleobond AX (The Nest Group, Inc.) and directly sequenced using gt11 primers and synthetic oligonucleotide primers corresponding to the gene-specific sequence.

Cell Culture—3T3-L1 preadipocytes were maintained in growth medium consisting of Dulbecco’s modified Eagle’s medium with 10% normal calf serum (Intergen, Co.) and were induced to differentiation as described previously (12). Adipogenesis was induced by feeding with fresh medium containing 10% fetal bovine serum (FBS), 0.5 mM methylisobutylxanthine, 1 μM dexamethasone, and 5 μg of insulin/ml for 48 h. The cells were subsequently maintained in medium containing 10% FBS and 2.5 μg of insulin/ml for an additional 48 h and were refed every 2 days with 10% FBS medium.

RNA Isolation and Analysis—Total RNA was isolated from rat tissues or 3T3-L1 cells as described by Chomczynski and Sacchi (13). Tissues were homogenized in solution D (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% Sarkosyl, 0.1 M 2-mercaptoethanol). Cells were washed twice with ice-cold phosphate-buffered saline and lysed with solution D. The lysate was extracted with acidic phenol/chloroform and then subjected to an isopropanol precipitation at −20 °C. Poly(A)+ RNA was selected using a oligo(dT) cellulose (Type 3, Collaborative Biomedical Products) according to the manufacturer’s instructions. For Northern blot analysis, 20 μg of total RNA or 5 μg of poly(A)+ were separated on formaldehyde agarose gels and transferred to Gene Screen Plus by capillary transfer (NEN Life Science Products).

MATERIALS AND METHODS

Adipocyte Fractionation and Isolation of Glut4-containing Vesicles—Adipocytes were isolated from the epididymal fat pads of male Sprague-Dawley rats (150–175 g) by collagenase digestion and fractionated into subcellular fractions by differential centrifugation according to Simpson et al. (10). Light microsomes were immunoadsorbed on monoclonal anti-Glut4 antibody, 1F8 (11), and covalently immobilized on Reacti Gel GF 2000 (Pierce), and the bound material was eluted with 1% Triton X-100 in phosphate-buffered saline. Nonadsorptive control was controlled by passage of microsomes over total mouse IgG (Sigma) immobi- lized on the same beads in the same protocol.

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† The abbreviations used are: gp, glycoprotein; IRAP, insulin-regulat-­ed aminopeptidase; Man-6-P, mannose 6-phosphate; IGF, insulin- like growth factor; EST, expressed sequence tag; FBS, fetal bovine serum; RAP, receptor-associated protein.

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FIG. 1. Identification of gp110 as rat sortilin. The figure shows the alignment of the amino acid sequences of human sortilin (top) and rat gp110. Three fragments obtained by protein sequencing of purified gp110 are in bold type. Peptide 2, used as an antigen for immunization, is underlined. Hyphens indicate the amino acid residues that are identical to those found in rat gene, and gaps are added for optimal alignment.

After ultraviolet+ cross-linking, filters were prehybridized, hybridized, and subjected to analysis as described previously (14). For rehybridization, the probe was stripped from membrane by washing the membrane in boiled 0.1 M NaOH and resubmitted to hybridization with 1% SDS twice, each for 10 min. The CDNA utilized in these studies were: sortilin (rat) and Glut4.

Gel Electrophoresis and Immunoblotting—Protein samples were separated in SDS-polyacrylamide gels according to Laemmli (15) and transferred to Immobilon-P membrane (Millipore) in 25 mM Tris, 192 mM glycine. Following transfer, the membrane was blocked with 10% nonfat dry milk, and proteins were visualized with the help of a chemiluminescent substrate kit (Amersham Corp.). An antiserum to sortilin from Glut4-containing vesicles to the plasma membrane obtained earlier data showing insulin-dependent translocation of gp110/sortilin translocation by blotting plasma membrane and light microsome fractions from insulin-treated and untreated adipocytes; lanes 2 and 3 correspond to 2 and 7 days after induction of differentiation, respectively. Total RNA was isolated from the 3T3-L1 cells (bottom panel), and 20 μg of total RNA was analyzed by Northern blot with specific probes (top panels).

RESULTS

As discussed in the Introduction, the polypeptide composition of Glut4-containing vesicles as revealed by the silver staining and/or biotinylation of the material specifically bound to 1F8-beads consists of three major glycoproteins that we have called gp230, gp160, and gp110 (5, 6, 16). The first two are the IGF-II/Man-6-phosphate receptor (8) and IRAP, respectively (6, 7, 17). To identify gp110, we isolated adipocytes from 60 rats, lung, fat, and brain and to a lesser extent in muscle and heart and is practically absent from liver. During 3T3-L1 adipocyte differentiation, sortilin is not expressed in the preadipocytes but is dramatically induced during differentiation of these cells, along with Glut4 (Fig. 2A). Similar results were obtained with several lines of differentiating myoblasts (not shown). One of the sequenced peptides, namely the 16-mer NECSL-HIHASISIQK (peptide 2 in Fig. 1), was synthesized in vitro, coupled to keyhole limpet hemocyanin, and used as an antigen for immunization of two rabbits. As shown in Fig. 3, these antisera recognize a protein in Glut4-immunoadsorbed vesicles of M, 110,000. As a function of time of insulin exposure, this protein is depleted from Glut4-containing vesicles to the same extent as Glut4 (Fig. 3). This result is consistent with our earlier data showing insulin-dependent translocation of gp110/sortilin from Glut4 vesicles to the plasma membrane obtained using the independent technique of cell surface biotinylation (5). On the other hand, we were unable to directly confirm sortilin translocation by blotting plasma membrane and light microsome fractions from insulin-treated and untreated adipocytes.
cells may indeed be phosphorylated by an unidentified vesicle-associated protein kinase in an insulin-dependent manner. Although the biological effect of this phosphorylation is not yet known, it may mediate interaction of sortilin with adaptor complex, as is the case with Man-6-P receptors (19).

A third possibility is that sortilin may be involved in the biogenesis of vesicles in which it resides. Sortilin was purified from brain via its interaction with receptor-associated protein (RAP). RAP is a luminal protein of the endoplasmic reticulum and Golgi apparatus with chaperone-like functions, and it interacts with a variety of receptors (20, 21). The ability of sortilin to interact with RAP (9) suggests that RAP may play a role in the formation and functioning of Glut4-containing vesicles in insulin-sensitive fat and skeletal muscle cells. This remains to be determined, and RAP has never been studied in these cell types or with this perspective. If RAP has some particular role in fat cells, it is more likely in biogenesis of Glut4-containing vesicles rather than in regulation of their translocation.

Obviously, a more defined role in biology for sortilin awaits its more detailed study, particularly with regard to the identification of a putative ligand. Because it is very abundant in the brain, it will be intriguing to explore localization of this protein in this tissue and, more specifically, its potential presence in synaptic vesicles. Our preliminary data (not shown) suggest that this indeed may be the case. If so, this will provide another interesting parallel between the molecular composition and functioning of Glut4-containing and synaptic vesicles and may shed light on the biological role of sortilin.

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