Restricted localization of the adipocyte/muscle glucose transporter species to a cell surface-derived vesicle fraction of 3T3-L1 adipocytes

Inhibited lateral mobility of integral plasma membrane proteins in newly inserted membrane areas of differentiated 3T3-L1 cells

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The recent demonstration of a large cell surface-derived pool of insulin-sensitive glucose transporters, presumably concentrated in the microvilli of 3T3-L1 adipocytes, induced the assumption that in differentiated adipocytes, newly inserted plasma membrane areas may display restricted lateral mobility, thereby preventing diffusion of integral membrane proteins out of these areas into the adjoining plasma membrane. In order to test this assumption, the cell surface distributions of the two glucose transporter species expressed by 3T3-L1 cells were determined using specific antisera against the HepG2/erythrocyte transporter, GluT1, which is synthesized in both fibroblasts and adipocytes, and the adipocyte/muscle-specific transporter, GluT4, expressed for the first time 3–4 days after induction of adipose conversion. GluT1 was shown to be localized in the plasma membrane of both 3T3-L1 preadipocytes and adipocytes, whereas GluT4 was almost entirely restricted to the low density surface-derived vesicle (LDSV) fraction of 3T3-L1 adipocytes most likely consisting of microvilli-derived vesicles. In contrast to the minor portion of GluT4 found in the adipocyte plasma membrane fraction, equal amounts of the GluT1 protein were detected in both the plasma membrane and the LDSV fractions of adipocytes. Both transporter species were present in the microsomal and the LDSV fractions of adipocytes. The observed distribution of the two transporter species is in accordance with the postulated restriction of the lateral mobility in plasma membrane areas formed by newly inserted transgolgi vesicles of differentiated adipocytes.

Glucose transporter cluster; Microvilli; Insulin action; Adipocyte (3T3-L1); Fibroblast

1. INTRODUCTION

Recently, experimental evidence was presented that hexose transport in C6 glioma cells [1] as well as in 3T3-L1 adipocytes occurs through a small surface located 'entrance compartment' which is separated from the cytoplasm by a diffusion barrier for low molecular weight solutes [2]. Hexose uptake experiments and morphological studies further suggested that this entrance compartment is represented by microvilli which were formed on the apical surface of 3T3-L1 cells during adipose conversion in parallel with the development of insulin responsiveness, 3-*O*-Methylglucose uptake kinetics indicated that the internal diffusional properties of this entrance compartment are subject to metabolic and hormonal control [2]. Furthermore, from 3T3-L1 adipocytes but not from preadipocytes a low density cell surface-derived vesicle (LDSV) fraction was prepared that contained the bulk of the cellular cytochalasin B binding activity including the total insulin-sensitive transporter pool of 3T3-L1 adipocytes [3]. The isolation of this vesicle fraction was achieved by a hydrodynamic low-stress shearing technique originally introduced by Carothers Carraway et al. [4] for the isolation of microvilli from ascites tumour cells. This treatment leaves the bulk of the cells intact but removes plasma membrane extensions such as microvilli from the cell surface. A two-step sedimentation protocol yielded the LDSV fraction containing mainly spherical vesicles of a size (100–200 nm in diameter) similar to that of the spherical enlarged tips of the microvilli observed on these cells [3].

The aim of the present study was to confirm the observed cell surface distribution of the glucose transporters in 3T3-L1 adipocytes and to identify the transporter species occurring in the plasma membrane and LDSV fraction by use of specific antibodies against the two types of transporters found in 3T3-L1 adipocytes, the erythrocyte/brain transporter (GluT1) [5–8] and the adipocyte/muscle transporter (GluT4) [9–11]. Furthermore, since our concept of the receptor regulated entrance compartment for glucose transport implies that glucose transporters must be concentrated within special microdomains of the plasma membrane, presumably the tips of the microvilli [3], the mechanism of transporter clustering has to be explained.

Morphological examination of differentiating 3T3-L1 cells revealed that adipose conversion is accompanied by the development of microvilli on the apical

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surface of the cells [2]. These microvilli evolved from small surface blebs (100–200 nm in diameter) which appeared soon after the induction period. Growth of the microvillar stalks then raised these blebs to the tips of the microvilli [2,12]. Based on these observations, clustering of glucose transporter proteins in microvillar membranes may be explained by the assumption, that in differentiating adipocytes, newly inserted integral membrane proteins, due to inhibition of lateral diffusion are retained within their original membrane environment. Apparently, the surface blebs observed on differentiating adipocytes represent such newly inserted membrane microdomains with restricted lateral mobility of integral proteins.

In order to test this assumption, the distribution of the two types of glucose transporters between the plasma membrane and the LDSV fraction in differentiated and undifferentiated 3T3-L1 cells was examined by Western blot analysis. According to a recent publication [13], GluT1 is expressed both in 3T3-L1 fibroblasts and adipocytes. In contrast, GluT4 is first synthesized 3–4 days after differentiation of the cells has started. If the assumption of stabilized membrane domains holds true, the adipocyte plasma membrane should be devoid of the adipocyte-specific transporter GluT4 which should largely be restricted to the LDSV (and microsomal) fraction. This type of GluT4 distribution was indeed observed.

2. MATERIALS AND METHODS

2.1. *Antibodies against the glucose transporters*

Polyclonal antisera against the C-terminal peptide of the adipocyte/muscle transporter and the HepG2/erythrocyte glucose transporter were kindly provided by Dr H.G. Joost (Göttingen) and Dr M. Mueckler (St. Louis), respectively.

2.2. *Cell culture*

3T3-L1 cells were cultured and differentiated as described earlier [2].

2.3. *Preparation of subcellular membrane fractions*

Plasma membrane, microsomal and LDSV fractions were prepared as recently described [3] except that the protease inhibitors, phenylmethylsulfonyl fluoride (1 mM) and pepstatin (1 μg/ml) were present during the preparation. The LDSV fraction could only be isolated from 3T3-L1 cells after adipose conversion.

3. RESULTS

Using C peptide antisera against GluT1 and GluT4, Western blot analysis was carried out for LDSV, plasma membrane and the microsomal fractions of differentiated and undifferentiated 3T3-L1 cells. As assessed by displacement with the antigenic peptide (data not shown), a 35 kDa protein was specifically labeled by the anti-GluT1 serum in addition to the broad band of the 45 kDa transporter proteins recognized by both antisera.

As shown in Fig. 1, the GluT1 transporter was expressed in preadipocytes and adipocytes as well. Based on protein content, this transporter species was much less abundant in the undifferentiated than in the differentiated state. In addition to its occurrence in the plasma membrane and microsomal fraction of the adipocytes a high level of GluT1 was also detected in the LDSV fraction indicating that this transporter species was expressed in differentiated cells because the LDSV fraction could not be isolated from uninduced 3T3-L1 preadipocytes.

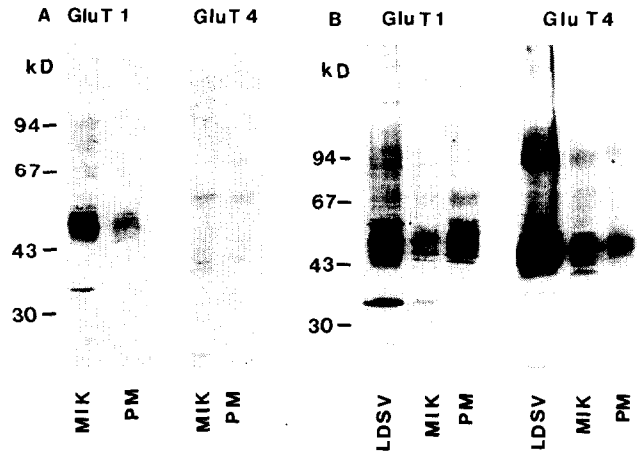

Fig. 1. Immunoblot analysis of the subcellular distribution of the HepG2/erythrocyte (GluT1) and the adipocyte/muscle (GluT4) transporter proteins (43 kDa) in 3T3-L1 preadipocytes and adipocytes. Aliquots of each of the fractions containing the same amount of protein (100 μg/lane) were electrophoresed on 8% SDS-polyacrylamide gels. The gels were blotted on nitrocellulose and probed with antibodies followed by treatment with horseradish peroxidase-labeled anti-IgG antiserum and dye development according to Leary et al. [15]. MIK, microsomal fraction; PM, plasma membrane fraction; LDSV, low density surface derived vesicles (not present in preparations from preadipocytes). Subcellular fractions were prepared according to [3].

In contrast to GluT1, the adipose/muscle transporter GluT4 was almost absent from the plasma membrane but occurred at a high level within the LDSV fraction of the 3T3-L1 adipocytes. In accordance with previous publications [9–11], this transporter species was totally absent from subcellular membrane fractions of 3T3-L1 preadipocytes.

The presented results are highly reproducible. Preparation of subcellular fractions and subsequent immunoblotting analysis were carried out three times always yielding the same results.

4. DISCUSSION

By use of specific antibodies against GluT1 and GluT4 the distribution of the two transporter proteins between subcellular membrane fractions of differentiated 3T3-L1 adipocytes was determined. The bulk of the transporter protein was shown to be localized within the LDSV fraction which was isolated by gentle shearing from the cell surface. The data confirmed the results of a preceding study [3], in which the distribution of the

glucose transporters in the plasma membrane, the LDS fraction, and the microsomal fraction was quantified by cytochalasin B binding.

The present results further show that in contrast to the continually expressed GluT1 protein which was localized in the plasma membranes of both undifferentiated and differentiated 3T3-L1 cells, the adipocyte/muscle-specific transporter protein GluT4 almost exclusively occurred in the LDSV (and the microsomal) fraction of the adipocytes; roughly estimated, less than 10% of the total GluT4 was found in the plasma membrane fraction of adipocytes, and even this amount may well be due to contamination of the plasma membrane fraction with surface-derived vesicles caused by incomplete removal of the microvilli from the adipocyte surface.

The observed subcellular GluT4 distribution strongly supports the assumption of stabilized microdomains on the surface of differentiated cells formed by newly inserted membrane areas. Apparently, membrane proteins, inserted into the plasma membrane after the initiation of adipose conversion, are firmly anchored within their original lipid bilayer microenvironment. The existence of stabilized microdomains was suggested by recent morphological studies [2]. In proliferating cells, newly inserted membrane areas transiently form surface blebs which are rapidly integrated into the plasma membrane soon after their formation. The integral membrane proteins of these blebs migrate out of their original lipid environment into the surrounding plasma membrane by lateral diffusion. In contrast, in GD-arrested induced 3T3-L1 cells [12], trans-Golgi vesicle insertion results in the formation of small spherical surface protrusions which subsequently form the tips of the microvilli.

The existence of both transporter species within the LDSV fraction is in accordance with previously published data on the insulin-induced redistribution of both GluT1 and GluT4 from the inactive storage pool to the plasma membrane in 3T3-L1 adipocytes [16,17]. According to the concept of the receptor-regulated microvillar entrance compartment [2], microvilli-located transporters, regardless of the type, should be integrated into the plasma membrane by the action of insulin.

As assessed by antibody displacement with the C-terminal GluT1 peptide antigen, a small (about 35 kDa) presumably proteolytic fragment of the GluT1 is observed in the LDSV and microsomal fraction of the adipocytes and the microsomal fraction of the preadipocytes. The formation of this protein did not result from proteolytic cleavage of GluT1 during the preparation because the amount of this component did not depend on the presence or absence of protease inhibitors. Omission of protease inhibitors, however, resulted in the in a general loss of immunoreactivity of both transporter types indicating proteolytic attack at the endofacial antigenic C-terminal part of the transporter molecule. In contrast, the natural 35 kDa peptide still contains the intact C-terminal region suggesting that this cleavage product was produced by exofacial hydrolytic attack probably due to the action of an ectoenzyme on the fibroblast cell surface. Such exofacial hydrolytic attack could not be observed for the GluT4 protein expressed after adipose conversion. A quite similar finding was recently published for isolated rat adipocytes [13]. A 24-kDa anti-GluT1 antiserum-reactive fragment occurred in the low density microsomal fraction of isolated adipocytes from rats which were fasted for 2 days and subsequently refed for 6 days. There was also no similar degradation of the GluT4 protein in 3T3-L1 adipocytes.

The present data confirm the previously reported transporter distribution within the LDSV and the plasma membrane fraction of 3T3-L1 adipocytes based on cytochalasin B-binding and strongly support one of the essential conclusions drawn from the notion of a regulated entrance compartment for hexose transport, the restriction of lateral mobility in certain plasma membrane domains of differentiated adipocytes.

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