Production of Antisera to Synthetic Decapeptide of the Cooh-Terminus of Rat Glut2

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ABSTRACT

In the present study, the production of an anti-GLUT 2 antibody is reported. The antibody (S-5096) was raised in New Zealand rabbits immunized with an immunogen prepared by cross-linking the synthetic peptide and a carrier protein. A COOH-terminal decapeptide of rat GLUT 2 predicted sequence was synthesized and coupled to keyhole limpet hemocyanin, using the glutaraldehyde method. Subcellular membrane fractions were prepared from renal cortex (C) and medulla (M), and subjected to Western blotting analysis using the antiserum in a 1:200 dilution and, subsequently, [^{125}I]protein A. As the results showed, the S-5096 anti-serum showed clear blots in kidney samples, stronger in cortex than in medulla as expected, whereas white adipose tissue and heart samples did not show any immunoreactivity. In addition, immunoblots were detected in samples prepared from duodenum and jejunum, as well as from isolated pancreatic B cells. In conclusion, the results clearly show that an anti-Glut 2 antibody, efficient enough to detect the rat protein by Western blotting analysis, was obtained.

Key words: glucose transporter, GLUT 2, antibody, coupling peptide.

INTRODUCTION

Recent progress in molecular biology has provided much knowledge about glucose transporter proteins. The facilitated glucose transport in all mammal cells involves a family of related proteins that have been called GLUTs (Thorens et al., 1990). These transporters are membrane proteins, whose primary sequence was predicted from cDNA molecules, and, in fact, these proteins had never been characterized or purified at all (Carruthers, 1990). Tissues specificities are responsible for the expression of one or more isoforms, and the GLUT 2 protein is known to be expressed in liver, pancreatic B cells, and intestinal and renal epithelial (Fukumoto et al., 1988; Thorens et al., 1988) . GLUT 2 transporter permits high glucose flux, since this isoform presents low affinity and high Km for glucose transport (Johnson et al., 1990). The detection of a specific isoform of glucose transporter is only possible using Western blotting analysis or immunohistochemistry, and, for that, the production of a specific antibody is a key step. Since the GLUTs have never been purified, the antibodies production must be done by using synthetic peptides selected from the predicted primary protein structure. Immunization with synthetic peptides offers the advantage of using pure immunogens. However, the main problem of synthetic peptides is their low immunogenic power, and because of that they must be cross-linked to a carrier protein such as keyhole limpet hemocyanin (KLH). In this report, the production of an anti-GLUT 2 antibody, raised against a synthetic carboxy-terminal decapeptide of the rat GLUT 2 protein is described. The peptide was made according

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to the predicted amino acid sequenced of the GLUT 2 (Thorens et al., 1988). We selected a 10 amino acid length peptide considering that, comparing to the rat GLUT 1 protein which has an ubiquous tissue distribution, this sequence has 8 different residues, and that can guarantee a high immunospecificity.

MATERIAL AND METHODS

A decapeptide NH_2-Met-Glu-Phe-Leu-Gly-Ser-Ser-Glu-Thr-Val-COOH was synthetized according to the predicted sequence of rat GLUT 2 glucose transporter protein, and was kindly made available by Dr. M. Saito, Laboratory of Biochemistry, Department of Biomedical Sciences, Faculty of Veterinary Medicine, Hokkaido University, Japan. The peptide was coupled with the carrier protein KLH using the glutaraldehyde method (Coligan, 1991). By this, 10 mg of synthetic peptide and 10 mg of KLH were dissolved in 2 mL of borate buffer (pH 10). Slowly, 1 mL of freshly prepared 0.3% glutaraldehyde solution was added to the reaction mixture with continuous stirring at room temperature, and 2 hours were allowed for reaction. Finally, 0.25 mL of 1 M glycine was added and allowed to react for 30 min, blocking unreacted glutaraldehyde. After this, the peptide/carrier conjugate was dialysed against 4 L of water overnight at 4 °C, replaced with fresh water and dialysed again for 6 hours. This immunogen was used according to standard schedule for immunization of New Zealand rabbits (Coligan, 1991). Briefly, 1.0 mg of peptide/carrier conjugate in 0.5 mL PBS was mixed 1:1 with complete Freunds adjuvant. The rabbit was immunized subcutaneously at multiple sites with this mixture. The animal was boosted with 0.5 mg peptide/carrier conjugate in 0.5 mL PBS mixed 1:1 with incomplete Freunds adjuvant, after 2 weeks and again after 4 weeks. The animal was bleed after 8 weeks after the immunization to take serum, which was directly checked by Western blotting. The final bleeding was performed at 12 weeks, and the anti-serum was identified as S-5096.

Subcellular membrane samples were taken from rat the kidneys, previously perfused with KRb buffer to avoid blood contamination. The kidneys were excised, longitudinally cut, and tissue slices were taken from the out area and from the transition area of medulla and cortex (according to the visual control) and referred to as cortex (C) and medulla (M), respectively. The tissue slices were weighed and immediately homogenized in 10 mL/g buffer (10 mM Tris-HCl, 1 mM EDTA and 250 mM sucrose, pH 7.4), using a polytron for 30 s. The homogenates were centrifuged at 3,000 x g for 15 min, and the supernatant was submitted to a second centrifugation at 12,000 x g for 15 min to sediment plasma membrane.

Membrane samples (100 μg protein) were solubilized in Laemmli's sample buffer, subjected to SDS-PAGE (10%) and electrophoretically transferred to nitrocellulose paper. After blocking with skimmed powdered milk, the sheets were incubated for 2 hours with the antiserum, and after washing, incubated for 2 hours with [^{125}I]protein-A (Amersham Life Science, UK). The autoradiograms were obtained 15 days after exposure at -70 °C. Experiments were performed with 1:200 diluted preimmunization serum (Pre) and anti-GLUT 2 antisera developed (S-5096), as well as with a 1:100 diluted commercial anti-GLUT 2 antibody (East) purchased from East-Acres Biologicals (Southbridge, MA, USA).

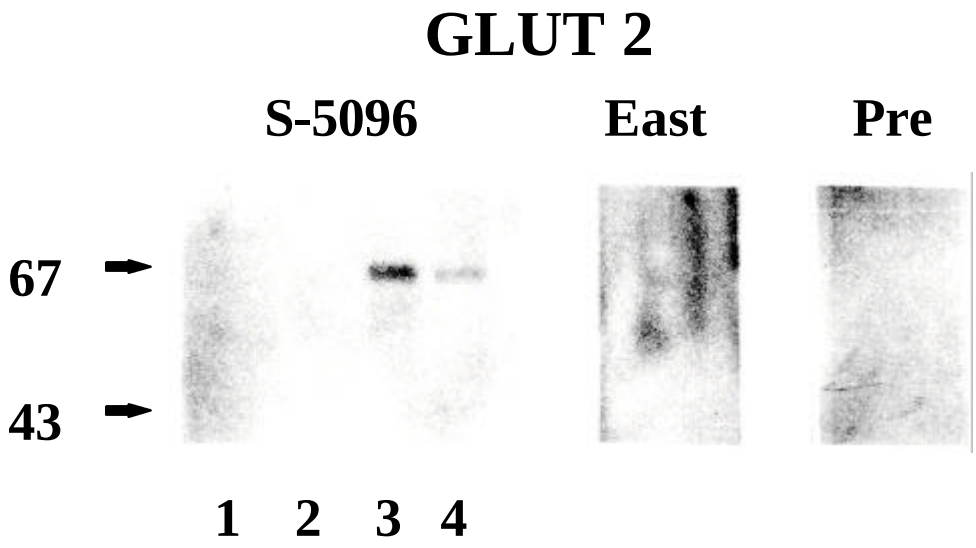

Figure 1. Western blotting analysis of GLUT 2. Membrane proteins were prepared from white adipose tissue (1), heart (2) and renal cortex (3) and medulla (4). Membrane proteins were separated by SDS-PAGE and transferred to nitrocellulose membrane. Detection of the transporter was carried out with anti-GLUT 2 antiserum developed (S-5096) in all tissues. Renal cortex samples were also immunoblotted using Pre-immunization serum (Pre) and commercial antibody (East). The position of protein markers (sizes in kD) are indicated on the left.

RESULTS AND DISCUSSION

As shown in Figure 1, the S-5096 anti-serum showed clear blots in kidney samples, stronger in cortex than in medulla as expected, whereas white adipose tissue and heart samples did not show any immunoreactivity. Figure 1 also shows that in kidney samples, the pre-immunization serum did not cause immunoblots, and the commercial anti-GLUT 2 antibody, despite the low dilution used, showed high background image, without specific GLUT 2 immunoreactivity.

It is known that white adipose tissue and heart do not express GLUT 2 protein whereas they express GLUT 1 and GLUT 4 isoforms, the latter in high concentration. The results showed that the S-5096 antibody did not react with any other protein in these tissues, confirming its specificity for GLUT 2 protein.

The GLUT 2 transporter is involved not only in the influx but also in the efflux of glucose. The inside or outside cell fluxes of glucose will obviously depend on its gradient. The GLUT 2 guarantees the glucose efflux in epithelial cells, as part of the glucose absorption process; and the glucose influx in the pancreatic B cells, as main signal in the insulin secretion. In the hepatocytes both efflux or influx may be mediated by the GLUT 2 transporter, according to the glucose gradient (Thorens, 1993).

The immunoreactivity of S-5096 antibody was also checked in gut and pancreatic islet samples, which are known to express the GLUT 2 isoform. As we can see in Figure 2, there was a specific immunoreactivity against the GLUT 2 in subcellular membrane samples from both duodenum and jejunum, as well as from pancreatic islets. These experiments were performed by loading 150 μg and 10 μg of total protein from gut and

pancreatic islet plasma membrane samples, respectively. The stronger image obtained with islet samples, despite the lower protein amount loaded, evidenced how rich this tissue is in GLUT 2 protein, and/or how this preparation became purified. In fact, the subcellular fractionation of pancreatic islets was performed using 1,500 pancreatic islets, previously isolated by the collagenase method, and that might be the reason for plasma membrane samples being so highly enriched.

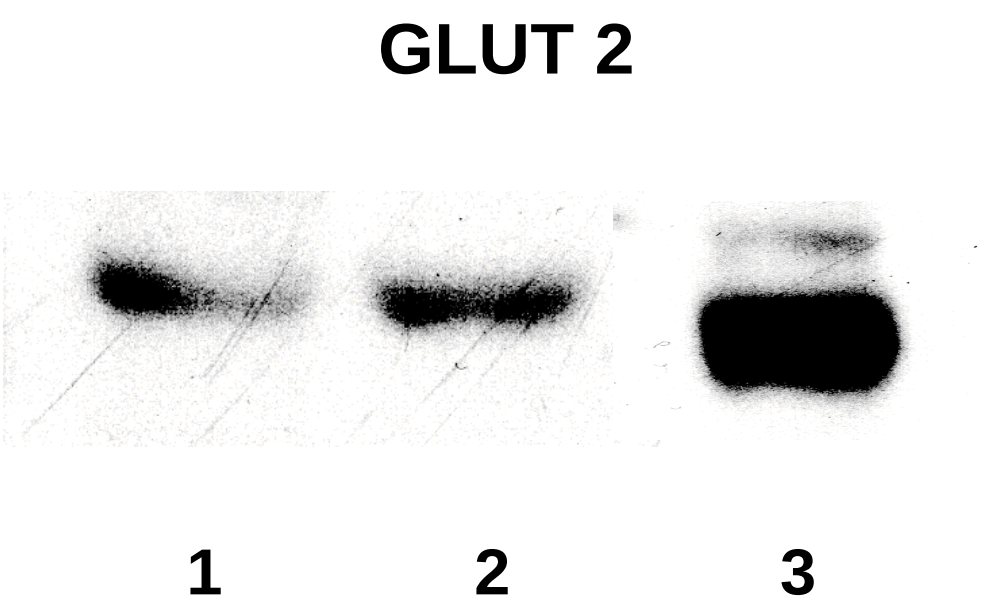

Figure 2. Western blotting analysis of GLUT 2 in intestinal and pancreatic islet samples. Membrane proteins from duodenum and jejunum (150 μg), as well as from pancreatic islets (10 μg) were separated by SDS-PAGE and transferred to nitrocellulose membrane. Detection of the transporter was carried out using the anti-GLUT 2 antiserum developed (S-5096) 1:200 diluted. The position of protein markers (sizes in kD) are indicated on the left.

Immunization with synthetic peptides has the advantage that pure immunogens are used providing monospecific antisera, which are, in fact, "site-directed" antibodies. The main problem of synthetic peptide immunogens is that, although one almost always can make anti-peptide antibodies, perhaps on average only one in three or four of these sequences generates antibodies that react with this structure in the context of the whole molecule (Gullick, 1988).

In conclusion, the results clearly showed that an efficient anti-Glut 2 antibod, was obtained, permitting to detect the protein by Western blotting analysis in kidney, gut and pancreatic islets.

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