Comparison of Amino Acid Sequence of the C-Terminal Domain of Insulin-Responsive Glucose Transporter (GLUT4) in Livestock Mammals

Hiroyuki ABE, Yumi KAWAKITA, Toshikazu MIYASHIGE, Masami MORIMATSU1* and Masayuki SAITO1
Department of Animal Nutrition, National Institute of Animal Industry, Tsukuba 305–0901, and 1)Laboratory of Biochemistry, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060–0818, Japan
(Received 16 December 1997/Accepted 7 February 1998)

**ABSTRACT.** The nucleotide sequence of cDNA coding the 38-amino acid of the C-terminal domain of insulin-responsive glucose transporter (GLUT4) was determined by the method of reverse transcription-polymerase chain reaction in the sheep, goat and pig, and compared with that of bovine which have been shown to have a unique amino acid conversion of Asn508 to His. The deduced amino acid sequence was completely identical in the three species, and did not have the amino acid conversion at position 508. Western blot analysis confirmed that an antiserum raised against a rat C-terminal peptide cross-reacted efficiently with GLUT4 of these livestock mammals. — KEY WORDS: glucose, GLUT4, livestock.

Facilitated diffusion of glucose across the plasma membrane is mediated by glucose transporter proteins (GLUTs). Among six isoforms of GLUT so far reported in mammals, GLUT4 is an isofrom present in skeletal muscle and adipose cells and plays a key role in cellular glucose uptake stimulated by insulin, and thereby is called insulin-responsive GLUT [6]. Molecular cloning studies have demonstrated that GLUT4 is a single polypeptide of 509–510 amino acids, the sequence of which is 90–94% homologous with human [5], rat [2, 3, 8] and mouse [9]. Particularly, the 25-amino acid sequence in the C-terminal domain (Arg486-Asp509) is identical in these species. One of the characteristics of glucose metabolism specific to ruminants is insulin-resistance [12]: that is, the ability of insulin to stimulate cellular glucose uptake is much lower than non-ruminants. Previously, we determined the complete nucleotide sequence of GLUT4 cDNA derived from skeletal muscle of Holstein cows, and found one amino acid conversion (Asn508 to His) in the C-terminal domain [1]. Since the C-terminal domain has been proposed as the targeting signal of GLUT4 relating to the post-receptor events of insulin [7], it is possible that this amino acid conversion might be a common reason for insulin resistance in ruminants including sheep and goat. To test this idea, in the present study, we examined GLUT4 cDNA structure of other ruminant species, sheep and goat, focusing on the C-terminal domain. The structure of pig and another strain of bovine (Japanese Black). The corresponding cDNA was obtained by the method of rapid amplification of the cDNA 3’-end [4] using 3’AmpliFINDER RACE kit (Clontech): that is, single strand cDNA was synthesized from total RNA using NN,-oligo(dT)CDS primer and AMV reverse transcriptase, according to the manufacturer’s instructions. The first PCR was carried out with the cDNA, primary gene-specific primer (5'-GTCCCTACGTCTTTTCTCTA-3’) and anchor primer, and the second PCR with the cDNA from the first PCR, secondary gene-specific primer (5’-CACGAATTCGAGCGAGGACGTTTGACC-3’) and anchor primer. The final PCR product was cloned into the plasmid vector (pCR II) with a TA cloning kit (Invitrogen), and amplified in E. coli (DH5α). Nucleotide sequencing was performed by the fluorescent dideoxy-terminator method[11] using an automatic DNA sequencer (Perkin Elmer - Applied Biosystems, model 373A).

The cDNA thus analyzed consisted of 1044-1061 nucleotides with 926–943 bp of 3’flanking sequence and 118 bp of the sequence coding 38 amino acids of the C-terminal domain. Figure 1 shows the deduced 38-amino acid sequences of the C-terminal domain of the livestock mammals, as well as those of rodents and human. Although the sequence identity was rather high (91–97%), there were three amino acid conversion sites at positions 482, 484 and 508. There was no difference in the sequence between the two bovine strains, Japanese Black and Holstein, suggesting the C-terminal amino acid conversion at His508 is common to bovine. The sequences of sheep, goat and pig were completely identical, but differed from that of cattle at two positions, 484 and 508. It should be noted that Asn508 is common in all species, except bovine, including other ruminants (sheep and goat). Thus, the conversion of Asn508 to His is unique to bovine but not common to ruminants, and it is not the primary reason for the insulin resistance widely seen in ruminant species. Some other differences in GLUT4 itself and insulin-related regulatory mechanisms may contribute to ruminants’ insulin resistance, although little information is available at present.
In most previous studies on GLUT4 protein, antisera raised against various oligopeptides corresponding to the C-terminal domain were widely used. We used a rabbit antiserum against a 12-amino acid peptide corresponding to the positions 498–509 for Western blot analysis of rat and mouse GLUT4s [10, 13]. Based on the sequence shown in Fig. 1, it was expected that this antiserum could also be used for the detection of GLUT4 protein of livestock mammals including bovine. To confirm this, we examined tissue extracts obtained from pigs and Holstein cows by Western blot analysis using our antiserum. Crude tissue extracts obtained from various organs were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose filters, and incubated with ×500 diluted antiserum as described previously [10, 13]. The protein blots were visualized using an ECL-detection kit (Amersham). As shown in Fig. 2, a band was found at the expected size (48 kDa) in the extracts from skeletal muscles and adipose tissue, but not liver, brain and kidney, of pig. A similar 48 kDa band was also detected in cattle skeletal...
muscles, while two slightly larger bands (53 kDa and 55 kDa) were detected in adipose tissue. Similar variations in apparent molecular size, probably because of different post-translational modifications such as glycosilation, were also reported in GLUTs 1 and 4 of the mammary glands of lactating rats and cows [14]. In addition, much larger (>80 kDa) and smaller (<30 kDa) bands were also observed, not only in muscle and adipose tissues but also in brain, liver and kidney. Since GLUT4 mRNA is expressed only in muscle and adipose tissues [1], it is more likely that these bands were derived from some non-specific reaction of the ECL-detection kit used. Collectively, the results of molecular size and tissue distribution are essentially the same as seen in rodents and humans, indicating that our antiserum cross-reacts efficiently with GLUT4 of livestock mammals including bovine. Thus the amino acid conversion at position 508 impairs only a little the cross-reaction between bovine GLUT4 and the antiserum. Extensive studies on expression and regulation of GLUT4s of bovine and pig using this antiserum and also cDNA probes are now in progress.

REFERENCES