This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2001)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2001 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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Glucose Transporters: A GLUTton for Vitamin C

by

JENNIFER R. PFAFFLY

1178 ML
Biosciences Department
The University of Iowa
Iowa City, IA 52242

For 77:222, Spring 2001

March 8, 2001

ABBREVIATIONS: (DHA) dehydroascorbic acid, (H_2O_2) hydrogen peroxide

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ABSTRACT: Glucose is an essential molecule for most aerobic species, but too much glucose can be harmful, or even deadly, to a cell. Because of this, controlling the levels of glucose inside the cell is of monumental importance. To accomplish this, cells have glucose transporters that regulate the flux of glucose into and out of the cell. A malfunction in one or more of these glucose transporters can be disastrous to the cell. This paper will review the basics of glucose transporters, their function as dehydroascorbic acid transporters and the effect oxidative stress has on them.

INTRODUCTION

Glucose is an extremely diverse molecule. Not only is it the primary energy source for most organisms, but it can also be toxic to cells if allowed to accumulate in high concentrations. Too little glucose can also be toxic to the cells [16]. Because of this, it is important to the cell that the levels of glucose inside the cell be kept well controlled. To accomplish this, cells contain one or more glucose transporters. Glucose transporters are responsible for transporting six-carbon sugars and other carbon compounds into the cell. There are several different types of glucose transporters. Bacteria contain several unique types of glucose transporters, such as proton symporters, substrate-binding transporters and group translocation systems [9]. Mammalian cells employ at least two types of transporters, Na^+-dependent cotransporters and facilitative transporters. This paper will focus on the simple facilitative transporters, most of which are members of the GLUT protein family.

THE GLUT FAMILY

The majority of glucose transporters belong to a family of proteins, called GLUT. There are seven glucose transporters identified to date, GLUT1-5, 7 and SGLT1 (Table I).

Table I. The different types of primary glucose transporters [9,10].

Protein	Tissue Distribution	Proposed Function	K_m
SGLT1	kidney, intestine	Na+-dependent active transport; concentration across apical epithelial membranes	---
GLUT1	multiple fetal and adult tissues, most abundant in human erythrocytes, endothelia and immortalized cell lines	Basal glucose and increased supply for growing / dividing cells; transport across blood brain barrier and other barrier tissues	16.9-26.2 mM
GLUT2	hepatocytes, pancreatic β cells, intestine and kidney	high-capacity low-affinity transport; transepithelial transport (basolateral membrane)	40 mM
GLUT3	widely distributed in human tissues, found only in brain in other species	Basal transport in many human cells; uptake from cerebral fluid into brain parenchymal cells	10.6 mM
GLUT4	skeletal muscle, heart, adipocytes	Rapid increase in transport in response to elevated blood insulin; important in whole-body glucose disposal	1.8-4.8 mM
GLUT5	intestine, adipose, muscle, brain and kidney	Intestinal absorption of fructose and other hexoses	---
GLUT6	hepatocytes and other gluconeogenic tissues	Mediates flux across endoplasmic reticulum membrane	---

GLUT1 is found in most cell types, but primarily in red blood cells and the endothelial cells of blood vessels. It composes 5% of the entire erythrocye membrane. GLUT2 is distinguished from the other family proteins by its low affinity for glucose, and when it is coupled with a high K_m hexokinase IV isozyme, it can act as a glucose-sensor, allowing it to respond to subtle changes in glucose concentration. GLUT 3 is found mostly in brain tissue, primarily neurons. GLUT 4 is found in insulin-responsive tissues, such as adipose and muscle. Insulin-responsive tissues are responsible for 20% (basal) and 75-95% (hyperinsulinemic) of whole-body glucose disposal, making GLUT4 an extremely important glucose transporter. Insulin causes GLUT4, which is stored in transport vesicles during basal state, to be translocated to the plasma membrane, where it is able to function as a glucose transporter (Figure 1). Subsequent to insulin stimulation, the concentration of GLUT4 found in the plasma membrane is ten-fold that of GLUT1. GLUT5 is primarily a fructose transporter, so it will not be discussed here. GLUT7 is the most recently discovered protein in the GLUT family, at this point little is known about it [9].

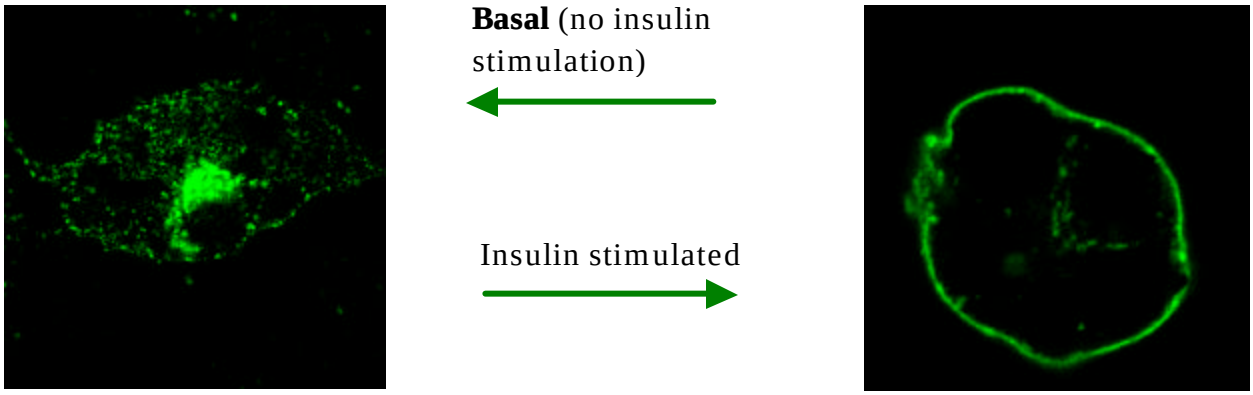


Figure 1. GLUT4 expression in NIH 3T3L1 adipocytes with and without insulin stimulation.

All of the GLUT proteins are developmentally regulated and at least three of them are known to be regulated by many factors, such as hypoglycemia, hypoxia, mitochondria inhibitors, prolonged insulin exposure, tumor necrosis factor and iron chelators [6].

PROPOSED STRUCTURE

The precise structure is not known for any of the GLUT proteins. This is primarily due to the difficulty of obtaining a good quality crystal to perform crystal diffraction studies. Membrane proteins are notoriously difficult to crystallize. Despite this complication, much has been deduced about the general structure of the GLUT proteins.

The GLUT proteins all contain approximately 500 amino acids, with GLUT1-5 exhibiting 39-65% sequence identity [9]. They all share a similar structure, but GLUT1 is the one for which the most information is known. The GLUT proteins contain 12 transmembrane helices, with the amino and carboxyl termini situated on the cytoplasmic face [10]. A large intracellular loop is found between helices 6 and 7 and a N-linked glycosylation site is found between helices 1 and 2 (Figure 2).

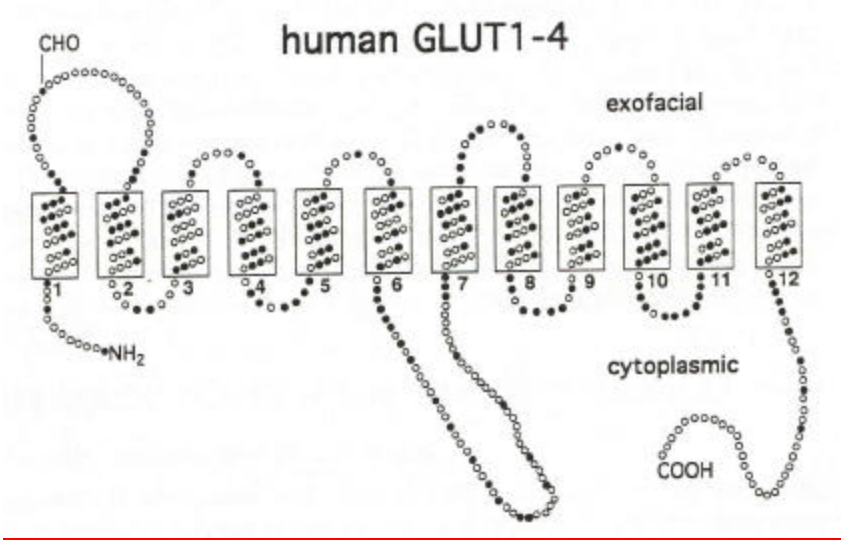


Figure 2. The predicted two-dimensional structure of the GLUT family proteins. This sketch shows the transmembrane helices and intra and extra-cellular loops, it does not depict the protein's tertiary structure within the membrane. The molecule contains 12 transmembrane helices, the three-dimensional structure has not been determined to date, but it is thought that the helices arrange the molecule into a pore-like structure. CHO denotes the N-linked glycosylation site [10].

The proposed three dimensional model of GLUT proteins suggests the clustering of amphipathic residues together to form an aqueous pore or barrel-like structure through which the glucose can traverse (Figure 3). The interior surface of the aqueous

pore would be made up of polar side chains, and it is projected that these polar groups provide hydrogen-binding sites for glucose and other similar molecules.

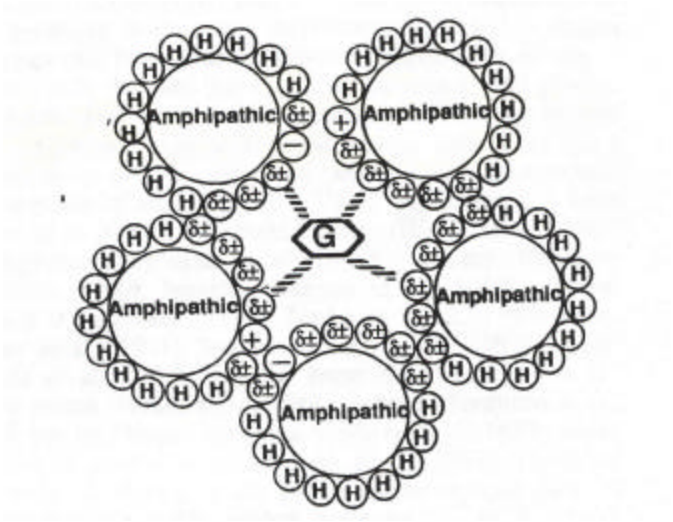
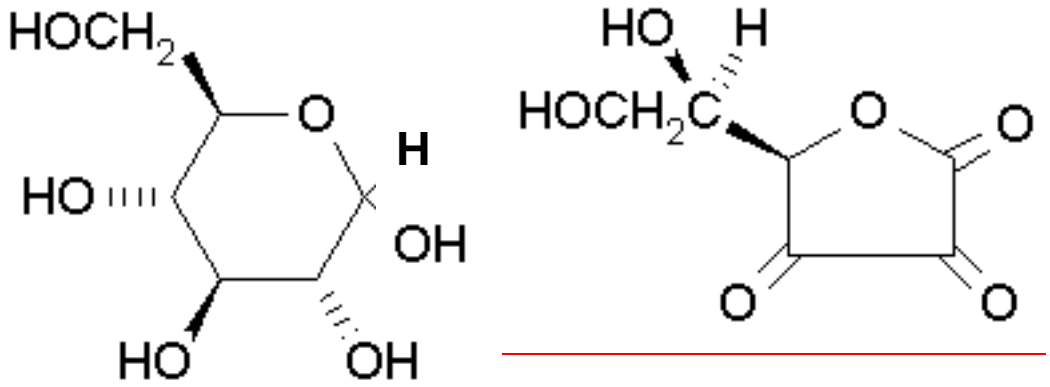


Figure 3. Proposed three-dimensional structure of GLUT family proteins. This theoretical model depicts five of the twelve membrane helices clustering together to form a aqueous pore. The hydrophobic side chains are denoted with a H and the polar side chains are depicted by a δ±, + or -. [9].

It is suspected that the GLUT proteins act through conformational changes that expose a six-sugar binding site alternatively to the external and internal surfaces of the transporter. The transporter has two ligand binding sites, one on the inside and one on the outside of the transporter. The exofacial binding site is responsible for glucose entry into the transporter while the endofacial binding site is required for the efflux of glucose. This allows the transporters to control the flow of glucose through the pore, depending on the intra- and extracellular concentrations.

DHA TRANSPORT

Ascorbate is transported into cells *via* two mechanisms. Ascorbate can be brought into the cell directly through an undetermined Na^+-dependent transporter. Ascorbate can also be oxidized outside the cell, allowing it to be transported by several of the glucose transporters into the cell, where it is reduced to ascorbate again. DHA has a structure very similar to that of glucose (Figure 4), this allows several of the GLUT family to act as DHA transporters.



Glucose DHA

Figure 4. The structure of glucose and DHA [18].

GLUT1, 3 and 4 are able to move DHA into the cell (Table II). GLUT4 is only able to transport appreciable amounts of DHA after insulin stimulation, which stimulates the GLUT4-containing vesicles to transport GLUT4 to the membrane.

Table II. K_m for DHA transport by GLUT family proteins [14,15].

Protein	K_m
GLUT1	1.1 mM
GLUT3	1.7 mM
GLUT4 (+ insulin)	0.98 mM

DHA uptake and its subsequent reduction can account for an increase in ascorbate accumulation of 5-20 fold within of minutes of insulin stimulation [14]. This process, called ascorbate recycling, allows the cell to respond quickly to oxidative stress.

Diabetic patients tend to have a condition that has been termed “micro-scurvy”, meaning that the cells have a vitamin C deficiency. The amount of vitamin C entering the cells of diabetic patients through the GLUT proteins can be reduced in two different ways. If there is decreased GLUT4 translocation to the membrane, less DHA will be able to enter the cell *via* the transporter. Vitamin C must also compete with glucose for entrance into the cell via the GLUT family transporters. If the flux of glucose into the cells is increased, as it is in diabetes, then less vitamin C will be able to enter the cells. It has not been conclusively proven whether the ascorbate deficiency found in diabetic

patients is the result of reduced concentrations in the cell or an increased requirement for ascorbate.

OXIDATIVE STRESS AND GLUCOSE TRANSPORTERS

Prolonged oxidative stress has a signifcant effect on GLUT1 and GLUT4. GLUT1 expression is increased when the cell is exposed to low-grade oxidative stress for an extended period of time, while GLUT4 is reduced. An 18 hour exposure of 3T3L1 cells to H_2O_2 (produced by glucose oxidase) resulted in an approximate 3.5-fold increase in GLUT1 protein and mRNA and a simultaneous 45% reduction of GLUT4. This experiment showed a reduction in insulin-stimulated glucose uptake, glycogenesis and lipogenesis. Oxidative stress also impairs GLUT4 translocation, but has no effect on GLUT1 translocation.

GLUCOSE: THE MOTHER OF ELECTRONS

Glucose, once phosphorylated to glucose-6-phospate, is metabolized through two main processes in the cell, glycolysis and the pentose phosphate pathway. Glycolysis results in the production of pyruvate, which then goes on to react in the tricarboxylic acid cycle, among others and is also known to scavenge H_2O_2 and other hydroperoxides. The pentose phospate pathway produces NADPH, which is the primary source of reducing equivalents for the glutathione reductase system, among many other oxidizing species. So not only is glucose a primary energy source, it is also a means of removing toxic H_2O_2 and hydroperoxides from cells.

CONCLUSION

Glucose transporters are an integral part of every cell and when their function is inhibited or augmented, it can prove disastrous for the cell. Diabetic patients (Type II) are characterized by insensitivity to insulin, a decrease in the amount of GLUT4 translocation, decreased glucose transport, increased glucose in the plasma and either an increased ascorbate requirement or a decreased amount available inside the cell. These complications are all connected to each other to some extent, the majority of these conditions may be the total or partial result of the reduction of GLUT4. This underlines the importance of GLUT4 function for both normal and diabetic patients.

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