Hepatic microsomal glucose transport
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Glucose transport and phosphorylation in cells which cannot make significant amounts of glucose

Glucose is an important metabolic substrate for mammalian cells. Most tissues cannot make sufficient glucose to maintain their normal levels of metabolic function. It is therefore important that tissues receive a steady supply of glucose from the blood and the transport of blood glucose across the plasma membrane of mammalian cells is a vital event. One or more plasma-membrane glucose transport proteins are present in nearly all mammalian cells.

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Abbreviation used: ER, endoplasmic reticulum.

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Mechanisms and Regulation of Sugar Transport

pump glucose against a concentration gradient by coupling its transport to sodium which is being transported down a concentration gradient. Sodium–glucose transporters have been described in small intestine and kidney but not in liver (see [3] for a recent review). A family of plasma membrane facilitative glucose-transport proteins have been found in virtually all mammalian cells. These transporters allow the movement of glucose from blood across the plasma membrane of cells down the chemical gradient. The facilitative plasma membrane glucose-transport proteins are specific for α-glucose and do not need energy, i.e. they are not linked to H+ gradients or ATP hydrolysis [4].

To date, four different plasma membrane glucose transport proteins called GLUT 1, 2, 3 and 4, have been cloned (two further similar sequences were cloned the first was termed GLUT 5 it has been shown to be a high affinity fructose transport protein (e.g. [5,6]), the second was termed GLUT 6 and it is a pseudogene with a high level of identity to GLUT 3 [7]), each being encoded by different genes and they have different tissue distributions which reflect the different glucose requirements of the individual tissues and/or cells (see [3,7–9] for reviews).

GLUT 1 was the first plasma membrane facilitative glucose transport protein to be purified [10] and cloned [11–13]. GLUT 1 is present in many tissues and is kinetically asymmetric as the net influx \( K_{in} \) for glucose is significantly lower than the net efflux \( K_{out} \) or the equilibrium exchange \( K_{eq} \) [4,14–16]. It has therefore been proposed [4] that GLUT 1 can function as a unidirectional transporter to allow cellular uptake of glucose in conditions where there is a high demand for glucose but extracellular glucose is low [4]. GLUT 2 is the major liver plasma membrane glucose transport protein and is discussed in the section below. The major site of expression of GLUT 3 protein and mRNA is brain [8,17], where it is thought to act in tandem with GLUT 1 to meet the high glucose demands of brain cells [4]. GLUT 4 is predominately present in muscle and adipose tissue where it is regulated by insulin (e.g. [18,19]).

Cells that cannot make significant levels of glucose usually obtain glucose from the blood via GLUT 1, 3 or 4 (see Figure 1). The \( K_m \) of these three plasma-membrane transport proteins is relatively low (see [4] for a more detailed review) so the amount of glucose that they transport will not alter significantly if blood glucose levels change within the normal physiological range. When the glucose enters these cells it is converted to glucose-6-phosphate by one of the three hexokinase isoenzymes (Figure 1). The hexokinase isoenzymes have a low \( K_m \) for glucose which is well below normal physiological blood glucose concentrations. This means that once it enters the cell most of the glucose is converted via hexokinase to glucose-6-phosphate. The cells do not have an enzyme which can reconvert the glucose-6-phosphate back to glucose (Figure 1) so the glucose concentration gradient (even at very low blood glucose concentrations) is always such that significant levels of glucose are not released by cells into the blood.

**Plasma membrane glucose transport and the glucose/glucose-6-phosphate cycle in liver**

The gluconeogenic organs, i.e. the liver and to a lesser extent the kidney, play a vital role in maintaining blood glucose homeostasis. Whenever blood glucose levels fall the liver releases glucose into the bloodstream for use by other tissues such
as brain which need a constant supply of glucose (see Figure 1) [20,21]. The liver therefore needs a different type of facilitative plasma membrane glucose transport protein to be able to transport relatively large amounts of glucose into and out of its cells. GLUT 2 is the major liver plasma membrane glucose transport protein (low levels of GLUT 1 [22] and GLUT 3 have also been reported in liver [4]). GLUT 2 is a high-capacity high-\(K_m\) glucose transporter which is symmetrical, i.e. it transports equally well in both directions depending only on the glucose gradient between the hepatocyte cytosol and the blood. GLUT 2 was isolated by screening liver cDNA libraries with GLUT 1 cDNA probes [23,24]. It has the highest \(K_m\) of the four plasma membrane glucose transport proteins and an equilibrium exchange \(K_m\) for 3-O-methyl D-glucose of 42 mM when the cDNA from GLUT 2 is expressed in oocytes [4] is similar to \(K_m\) data for plasma membrane glucose transport in intact hepatocytes, e.g. 66 mM [25]. The high \(K_m\) of GLUT 2, which is much higher than even reported pathophysiological blood glucose levels, means that the higher blood glucose levels rise the more glucose is transported into the liver. Immunofluorescence studies have shown that in liver, GLUT 2 is only present in the basolateral membranes of hepatocytes [26]; this is consistent with its role in the regulation of blood glucose levels.

Once glucose enters the hepatocyte via GLUT 2 it can be converted to glucose-6-phosphate. The major enzyme in liver which converts glucose to glucose-6-phosphate is glucokinase (Figure 1). Glucokinase has a much higher \(K_m\) than the hexokinases. Its \(K_m\) of \(-10\) mM means that glucokinase activity can also be affected by increasing blood glucose levels and, unlike the hexokinases, is not inhibited by physiological levels of glucose-6-phosphate. Liver glucokinase is also decreased in starvation and diabetes and increased by insulin and re-feeding [27,28]. The high \(K_m\) of both GLUT 2 and glucokinase mean that the liver can take up significant amounts of glucose whenever blood glucose levels rise.

The liver also has the capacity to produce high levels of glucose via the pathways of gluconeogenesis and glycogen breakdown and it does so at times of stress or whenever blood glucose falls. The reason the liver can produce large quantities of glucose is that it can hydrolyse glucose-6-phosphate to glucose and inorganic phosphate, catalysed by the enzyme glucose-6-phosphatase (Figure 1) [20,21]. The glucose produced by glucose-6-phosphatase can leave the liver via GLUT 2 but first it must be able to reach GLUT 2.

**Hepatic glucose-6-phosphatase and endoplasmic reticulum glucose transport**

Glucose-6-phosphatase is an endoplasmic reticulum (ER) protein and it is situated with the active site inside the lumen of the ER [21,29]. The other enzymes of gluconeogenesis and glycogen breakdown are not inside the lumen of the ER. Therefore the substrates and products of the glucose-6-phosphatase enzyme need to cross the ER membrane (Figure 1). Studies of the type 1 glycogen storage diseases (genetic deficiencies of glucose-6-phosphatase activity) have greatly helped to clarify our understanding of the essential role played by not

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**Figure 2**

A schematic representation showing the ER transport proteins required for normal glucose-6-phosphatase activity with glucose-6-phosphate as substrate.
only the glucose-6-phosphatase enzyme but also several ER transport proteins in the maintenance and regulation of normal glucose-6-phosphatase activity in vivo. The deficiency of the glucose-6-phosphatase enzyme is termed type 1a glycogen storage disease [21,29–31]. It is a severe metabolic disorder [28] and it is usually diagnosed by enzymic analysis of glucose-6-phosphatase activity in microsomes isolated from needle biopsy samples of the patient’s liver (see [32] for a recent review). In complete deficiencies of the glucose-6-phosphatase enzyme there is no glucose-6-phosphatase activity in either intact or fully disrupted microsomal vesicles [28]. In contrast, some patients were found to have no activity in intact microsomal vesicles but normal glucose-6-phosphatase activity in fully disrupted microsomal vesicles (where the need for the substrate to cross the microsomal membrane had been removed). This was called type 1b glycogen storage disease [33] and it is a defect of T1, the microsomal glucose-6-phosphatase transport protein (see Figure 2). Since then it has also been clearly shown that glucose-6-phosphatase is transported into the liver microsomes in vitro [34] although T1 has not yet been purified to homogeneity.

A number of cases of type 1c glycogen storage disease [29,32,35,36], which is a deficiency of microsomal pyrophosphate and/or phosphate transport, have also been reported (see Figure 2). Studies of patients with type 1c glycogen storage disease [37] together with other biochemical studies [38] have demonstrated the existence of at least two ER transport proteins (T2α and T2β), one of which, T2β, has been purified [36]. While the existence of plasma membrane glucose transport proteins has been widely accepted since the 1980s, the existence of a liver ER glucose transport protein remained very controversial until recently. The reason was that it had been suggested that glucose produced inside the lumen of the ER by glucose-6-phosphatase crossed the ER membrane via simple diffusion [39]. This suggestion was widely believed to be true for two main reasons: cytochalasin B, which was a well known inhibitor of the plasma-membrane facilitative transport proteins, does not significantly inhibit glucose-6-phosphatase activity in intact microsomal vesicles; and genetic deficiencies of microsomal glucose transport (type 1d glycogen storage disease) had not been reported.

However, it seemed logical that glucose transport across the ER membrane would also require the presence of a glucose-transport protein and we succeeded in isolating a 52 kDa glucose-transport protein from rat liver ER [40]. Antibodies raised against the protein were later used to isolate a rat liver cDNA clone [41]. The deduced amino acid sequence showed sequence similarities to GLUTs 1–6 and it was most similar to GLUT 2, the liver plasma-membrane glucose-transport protein [41]. GLUTs 1–6 were named in the order of their discovery therefore the ER glucose transport protein was called GLUT 7. The GLUT 7 sequence contained a consensus sequence motif for retention of membrane-spanning proteins in the ER [42,43]. Expression of GLUT 7 in Cos 7 cells (which do not normally contain either significant amounts of glucose-6-phosphatase activity or GLUT 7) resulted in the expression of microsomal glucose-transport capacity [41].

The liver ER glucose-transport protein and its regulation are not yet nearly as well characterized as the facilitative plasma-membrane glucose-transport proteins. The main reason is the difficulty in directly assaying glucose efflux from microsomes and/or ER. The plasma-membrane facilitative glucose-transport proteins are normally assayed by measuring uptake or efflux of radioactively labelled glucose into the cells where the transport proteins occur naturally or cells in which they have been expressed, e.g. oocytes [15,16,44]. Similar types of experiments are very difficult to do with the ER transport proteins (T1, T2 and GLUT 7) as the microsomal intraluminal space is very small and the substrate can only equilibrate with the lumenal water space. The intraluminal volume of liver microsomes varies somewhat depending on the composition of the buffer in which they are suspended but the normal intraluminal volume measured is only ~1 µl/mg of microsomal protein (e.g. [45]). This means that direct measurement of labelled glucose (or indeed phosphate, pyrophosphate or glucose-6-phosphate) uptake or efflux into microsomes is difficult because the transport is very rapid and relatively low numbers of counts are taken into or released from the microsomal lumen. However, conventional transport assays in combination with kinetic analysis of the glucose-6-phosphatase system [46] and assessment of microsomal uptake using light-scattering measurements [47] all clearly indicate that high-capacity high- Km glucose transport occurs in liver microsomes. Two obvious differences between microsomal glucose transport and the mammalian facilitative plasma-membrane glucose-transport proteins are the lack of inhibition by cytochalasin B and the fact that uptake is transported into microsomes.

The main reason no cases of type 1d glycogen storage disease have been reported is that cytochalasin B is a weak inhibitor of plasma-membrane glucose transport proteins. However, the presence of a glucose-transport protein in the microsomal membrane is not required for normal intracellular metabolism. The glucose-6-phosphatase activity there is no glucose-6-phosphate activity in either intact or fully disrupted microsomal vesicles. The glucose-6-phosphatase activity in intact or fully disrupted microsomal vesicles is normally assayed by measuring uptake or efflux of radioactively labelled glucose into the cells where the transport proteins occur naturally or cells in which they have been expressed, e.g. oocytes [15,16,44]. Similar types of experiments are very difficult to do with the ER transport proteins (T1, T2 and GLUT 7) as the microsomal intraluminal space is very small and the substrate can only equilibrate with the lumenal water space. The intraluminal volume of liver microsomes varies somewhat depending on the composition of the buffer in which they are suspended but the normal intraluminal volume measured is only ~1 µl/mg of microsomal protein (e.g. [45]). This means that direct measurement of labelled glucose (or indeed phosphate, pyrophosphate or glucose-6-phosphate) uptake or efflux into microsomes is difficult because the transport is very rapid and relatively low numbers of counts are taken into or released from the microsomal lumen. However, conventional transport assays in combination with kinetic analysis of the glucose-6-phosphatase system [46] and assessment of microsomal uptake using light-scattering measurements [47] all clearly indicate that high-capacity high- Km glucose transport occurs in liver microsomes. Two obvious differences between microsomal glucose transport and the mammalian facilitative plasma-membrane glucose-transport proteins are the lack of inhibition by cytochalasin B and the fact that uptake is transported into microsomes.
storage disease (GLUT 7 deficiency) had been diagnosed is that it is technically very difficult to directly assay the microsomal glucose-transport protein in small biopsy samples. Despite the obvious potential difficulty in diagnosing type 1d glycogen storage disease we decided to develop a protocol which would allow us to do so in microsomes isolated from patients' liver needle biopsy samples. Recently, we diagnosed the first case of type 1d glycogen storage disease (A. Burchell, Scott, H. M., Waddell, I. D. and Leonard, J. V., unpublished work). The child had a severe metabolic disorder which was clinically very similar to type 1a glycogen storage disease. However, glucose-6-phosphatase activity was normal in fully disrupted microsomes, indicating that the glucose-6-phosphatase enzyme protein was normal. In intact microsomes the glucose-6-phosphatase activity had an extremely low V_max when glucose-6-phosphate was the substrate, indicating a defect in one of the transport proteins. Glucose was not taken up into or released from the microsomes, indicating a defect of microsomal glucose transport. This was confirmed by the lack of immunodetectable GLUT 7 protein in the patient's microsomes (A. Burchell, Scott, H. M., Waddell, I. D. and Leonard, J. V., unpublished work). This patient clearly illustrates the metabolic importance of the liver ER glucose-transport protein as the deficiency is very similar clinically to the complete deficiency of the glucose-6-phosphatase enzyme [28].

The role of ER transport proteins in tissues other than liver

The tissue distribution of GLUT 2 and glucose-6-phosphatase including GLUT 7 is remarkably similar although glucose-6-phosphatase has been found in some additional minor cell types which have not yet been tested for the presence of GLUT 2. So far, in every cell type in which the glucose-6-phosphatase enzyme has been found all of the components of the glucose-6-phosphatase system (see Figure 2) have also been found. High levels of glucose-6-phosphatase have been found in liver, kidney and pancreatic islets, e.g. [48,49]. Liver and kidney are both gluconeogenic organs, so logically the glucose-6-phosphatase system proteins should be in both tissues. It is not so obvious what the role(s) of glucose-6-phosphatase (including GLUT 7) are in pancreatic islets; clearly, it is not to release glucose into the blood for use by other tissues. GLUT 2 and glucokinase are also expressed in pancreatic islets [50–54] and it has been suggested that they both play a role in glucose sensing and that glucokinase is the rate-limiting step in glucose utilization by islets, (for example, see [53]). Recently, it has been shown that the rate of glucose utilization by freshly isolated islets is 100-fold lower than GLUT 2-mediated glucose transport [52] and this does not support the hypothesis that GLUT 2 expression is the major modulator of glucose-induced insulin release from islets. Most papers suggesting that glucokinase is rate-limiting in glucose utilization in islets completely ignore the fact that high levels of glucose-6-phosphatase are also expressed in islets. This is not logical as it has been shown that cycling occurs between glucose and glucose-6-phosphate (i.e. between glucose-6-phosphatase and glucokinase) in islets (see [55] for a recent review) and when substrate cycling occurs it is the combined rates of both the enzymes that determine flux through the pathway not the activity of one of the enzymes alone.

Low levels of glucose-6-phosphatase have also been found in a variety of tissues including intestine and gallbladder mucosa, some but not all brain astrocytes, and fetal adrenal glands [54–57]. It is not clear what role glucose-6-phosphatase plays in these cell types. A simple explanation would be that the cells containing glucose-6-phosphatase in these tissues supply glucose to neighbouring cells, but this has not yet been demonstrated.

The liver glucose-6-phosphatase system including GLUT 7 plays a major role in the regulation of blood glucose homeostasis but their role(s) in other tissues are still not clear. In the future, our understanding of the role and regulation of glucose-6-phosphatase and GLUT 7 in all the tissues in which they are expressed will be greatly facilitated by studies of their expression in a variety of normal and diseased states.

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Mechanisms and Regulation of Sugar Transport

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