Functional Expression of the Human HepG2 and Rat Adipocyte Glucose Transporters in Xenopus Oocytes

**COMPARISON OF KINETIC PARAMETERS**

(Received for publication, May 22, 1989)

Konrad Keller‡, Marilyn Strube, and Mike Mueckler§

From the Department of Cell Biology and Physiology, Washington University Medical School, St. Louis, Missouri 63110

Facilitated glucose transport is a ubiquitous characteristic of animal cells carried out by a family of membrane glycoproteins. Two members of this gene family are the well characterized human erythrocyte protein that has been cloned from the HepG2 cell line and the insulin-sensitive transporter that has been cloned from adipocytes and muscle tissue. In the present study the HepG2 and adipocyte glucose transporters were functionally expressed in Xenopus oocytes after injection of synthetic mRNAs produced by transcription in vitro from cloned cDNAs. Both 2-deoxyglucose uptake and 3-O-methylglucose transport were increased several-fold over basal levels in mRNA-injected oocytes. Increased uptake of 2-deoxyglucose was inhibited completely in the presence of cytochalasin B, and 3-O-methylglucose transport was blocked by D-glucose but not by L-glucose. The half-saturation constant and turnover number for 3-O-methylglucose transport at 22 °C via the HepG2 transporter were estimated to be 21 mM and 2.2 × 10⁶ s⁻¹ under equilibrium exchange conditions. The half-saturation constant for 3-O-methylglucose transport via the adipocyte transporter under the same conditions was estimated to be 1.8 mM. These data prove the functional identity of the cloned HepG2 and adipocyte cDNAs and indicate that the HepG2 and adipocyte transporters display similar kinetic behavior when expressed in the frog oocyte membrane as compared with their native membrane environments. Thus, the difference in the equilibrium exchange kinetic parameters for glucose transport in the erythrocyte and the adipocyte is a result of the expression of two distinct glucose transporter proteins.

Transport of glucose across the plasma membrane of animal cells is carried out by members of at least two distinct protein families, the secondary active systems that cotransport glucose and sodium (1) and the facilitated diffusion transporters (2). The former proteins are restricted to a few cell types, i.e. intestinal and renal epithelial cells that concentrate glucose from the intestinal contents and the forming urine. The facilitated diffusion systems, on the other hand, are ubiquitously expressed in animal cells (3). These proteins are responsible for supplying cellular glucose for energy metabolism and for the biosynthesis of sugar-containing macromolecules, and in some tissues are involved in regulating blood glucose levels (4).

Four members of the facilitated diffusion glucose transporter gene family have been described thus far (5–13). Characterization of the properties and physiological roles of the facilitated diffusion glucose transporters is complicated by the fact that more than one species may be expressed in a single cell type (7–10, 14). The most extensively characterized of these transporters is the species present in the human erythrocyte membrane (15). The erythrocyte transporter is a ~55-kDa glycoprotein that has been purified and functionally reconstituted into liposomes (2, 16). There is strong evidence that the erythrocyte polypeptide is identical to the glucose transporter cloned from the human HepG2 cell line (5, 17). A second transporter of interest is the major species expressed in insulin-sensitive tissues such as fat and muscle (18). This transporter has not yet been purified, but the cDNA has been cloned from rat (10–12) and human (13) tissues. The adipocyte protein is 65% identical in sequence to the HepG2 transporter and is predicted to possess a very similar two-dimensional membrane topology. These two glucose transporter proteins thus exhibit considerable structural homology and almost certainly share a common transport mechanism. Interestingly, however, the kinetics of glucose transport in the human erythrocyte and the rat adipocyte are decidedly different. Several studies indicate that glucose transport in the rat adipocyte can be described by a simple symmetrical carrier model without accelerated exchange (19–21). Transport in the red cell, however, is asymmetric and exhibits accelerated exchange, and controversy exists as to whether some kinetic observations can be explained in terms of any carrier-type model (2, 22, 23).

Are the kinetic differences observed between the erythrocyte and adipocyte transporters intrinsic properties of these two related proteins, or are they due to the expression of the proteins in two distinct environments? This question can be addressed by an analysis of the functional expression of the different transporter genes in the same cell type. Xenopus oocytes exhibit extremely low basal levels of glucose transport and are thus ideally suited for the expression of heterologous glucose transporters. Herein we describe the functional expression of the human HepG2-type and rat adipocyte glucose transporters in oocytes. We demonstrate that these two transporters, when expressed in the oocyte plasma membrane, exhibit distinct half-saturation constants under equilibrium exchange flux conditions, consistent with the kinetic proper-
ties observed for these proteins in their native cellular environments. These results prove the functional identity of the HepG2 and adipocyte cDNA clones and indicate that the distinct kinetic characteristics of glucose flux across the red cell and adipocyte plasma membranes are due to inherent properties of the two glucose transporter species.

**EXPERIMENTAL PROCEDURES**

**Preparation and Injection of Oocytes—**Stage 5 oocytes (1 ± 0.1 mm in diameter) were isolated from excised ovarian tissue using a bent Pasteur pipette and then treated with 2 mg/ml collagenase in modified Barths’ saline (MBS)1 (24) for 30 min at 22 °C. Oocytes were then washed 6 times and maintained in MBS at 22 °C for all subsequent procedures. Healthy oocytes were selected for injection 12 h after collagenase treatment. Oocytes were injected with quantities of either water (sham-injected) or mRNA as described under “Results.” HepG2 glucose transporter mRNA was prepared from Sualinearized pSPGT using a Gilson P-200 Pipetman. Oocyte plasma membrane “ghosts” were isolated from -10 oocytes 3 days after the injection of either water (Sham), HepG2 glucose transporter mRNA (HepG2 mRNA), or adipocyte glucose transporter mRNA (Adip. mRNA). Twenty-five (A) or 15 (B) μg of each sample was subjected to Western blot analysis using affinity-purified anti-erythrocyte glucose transporter antibody (A) or R820 antisera (B) as described under “Experimental Procedures.” Aliquots of purified erythrocyte glucose transporter (A) or rat (R) adipocyte low density microsomal (LDM) protein (B) were run on the same gel as quantitative standards. The numbers above the autoradiograms refer to the quantities of purified transporter (ng, A) or rat adipocyte low density microsomal protein (μg, B) loaded on the gel. The mobilities of soluble protein standards run on the same gel are shown to the left of the autoradiograms.

**Fig. 1. Expression of HepG2 (A) and adipocyte (B) glucose transporter (GT) proteins in the oocyte plasma membrane (PM).** Plasma membrane ghosts were isolated from ~10 oocytes 3 days after the injection of either water (Sham), HepG2 glucose transporter mRNA (HepG2 mRNA), or adipocyte glucose transporter mRNA (Adip. mRNA). Twenty-five (A) or 15 (B) μg of each sample was subjected to Western blot analysis using affinity-purified anti-erythrocyte glucose transporter antibody (A) or R820 antisera (B) as described under “Experimental Procedures.” Aliquots of purified erythrocyte glucose transporter (A) or rat (R) adipocyte low density microsomal (LDM) protein (B) were run on the same gel as quantitative standards. The numbers above the autoradiograms refer to the quantities of purified transporter (ng, A) or rat adipocyte low density microsomal protein (μg, B) loaded on the gel. The mobilities of soluble protein standards run on the same gel are shown to the left of the autoradiograms.

---

1 The abbreviations used are: MBS, modified Barths’ saline; SDS, sodium dodecyl sulfate.
the radioactivity inside the cell at infinite time, and $k$ is the first order rate constant. Assuming that transport under these conditions obeys simple Michaelis-Menten kinetics,

$$k = \frac{V_m}{(K_m + S)}$$

(2)

where $V_m$ equals the maximal velocity of the transport reaction under equilibrium exchange conditions and $K_m$ equals the substrate concentration ($S$) at which the velocity is half-maximal (27).

Transport data were linearized by logarithmic transformation of Equation 1 and the slopes determined by linear regression analysis (27). The negative reciprocals of the slopes were replotted with respect to substrate concentration, and the kinetic parameters were obtained from the slope and intercept of the resulting curve by linear regression analysis. The negative of the $x$ intercept value corresponds to $K_m$ and the inverse of the slope corresponds to $V_m$.

RESULTS

Fig. 1A shows a Western blot of plasma membrane fractions obtained from Xenopus oocytes 3 days after the injection of 25 ng/oocyte of HepG2 glucose transporter mRNA or an equal volume (50 nl) of water. We assume the blot reflects the amount of heterologous glucose transporter expressed in the oocyte plasma membrane, because the oocyte membrane fraction used is free of yolk granules and presumably other intracellular membranes (see "Experimental Procedures"). The blot was probed with affinity-purified antibody against the human erythrocyte glucose transporter. Aliquots of purified erythrocyte glucose transporter were run on the same gel as quantitative standards. Assuming that the antibody reacted with equal affinity to both the erythrocyte transporter and the protein produced in oocytes and that the erythrocyte transporter preparation is 75% pure (28), ~2.0 ng of HepG2 glucose transporter protein were expressed per 25 pg of total plasma membrane protein. This corresponds to roughly 200 pg of transporter/oocyte. Fig. 1B shows a similar Western blot for expression of the adipocyte transporter in the oocyte plasma membrane. The protein expressed in oocytes exhibits a lower mobility by SDS-polyacrylamide gel electrophoresis than does the protein present in rat adipocyte low density microsomes (apparent $M_r$ 48,000 versus 43,000). This discrepancy is most likely due to differential post-translational modification of the protein in certain cell types. For example, the adipocyte transporter expressed in murine 3T3L1 adipocytes comigrates with the protein expressed in oocytes (data not shown). Because the adipocyte transporter has not been purified, it is not possible to directly estimate the actual quantity of this protein expressed in the oocyte plasma membrane.

2-Deoxyglucose uptake into water-injected oocytes was very low (<3 pmol/oocyte/30 min, Fig. 2A). Uptake into oocytes injected with glucose transporter mRNAs was much higher and varied in proportion to the amount of RNA injected. That the increased 2-deoxyglucose uptake was due to translation of the injected mRNA is indicated by the correlation between the amount of 32P-gGppG added to the in vitro transcription reaction and the extent of the increased sugar uptake (Fig. 2B). The increased 2-deoxyglucose uptake observed in mRNA-injected oocytes was blocked by cytochalasin B, a well characterized inhibitor of the facilitated diffusion glucose transporters (29-31) (Fig. 3A). Increased 3-O-methylglucose transport was inhibited by D-glucose but not by L-glucose, thus demonstrating stereospecificity of the carrier systems (Fig. 3, B and C). We concluded from these data that functional HepG2 and adipocyte-type glucose transporter proteins were produced from synthetic mRNA transcripts injected into oocytes.

In order to be useful for the comparative characterization of different members of the facilitated diffusion glucose transporter gene family and for structure-function studies, it is necessary for the expression system to be sufficiently sensitive to allow determination of kinetic parameters. We analyzed transport progress curves using an integrated rate equation in order to avoid potential problems with initial rate data. Equilibrium exchange conditions were used to avoid problems due to anomeric specificity and cellular volume changes (22). Determination of equilibrium exchange $K_v$ values should also allow us to determine whether the two transport systems behave kinetically in the oocyte membrane as they do in their native membrane environments, because these two transporters appear to exhibit different equilibrium exchange $K_v$ values in their native cell types (see "Discussion").

Transport progress curves were followed between 0.25 and 30 min, the latter time point generally corresponding to less than 2 half-lives for an equilibrium exchange concentration of 4 mM. Fig. 4, A and B, shows the logarithmic transformation of transport progress curves for the HepG2 and adipocyte transporters, respectively. These data indicate that radioactive sugar uptake followed first order kinetics for at least 30 min. The slopes of the regression lines represent first order rate constants, which correspond to half-times of 16, 23, 30,
Expression of Mammalian Glucose Transporters

FIG. 3. Glucose uptake into mRNA-injected oocytes is inhibited by cytochalasin B and is stereospecific. Oocytes were injected with 25 ng of HepG2 or 50 ng of adipocyte glucose transporter mRNA or water (Sham). A, three days after injection 3H-labeled 2-deoxyglucose (25 μM) uptake was measured either in the absence or presence of a saturating amount (50 μM) of cytochalasin B (CB). Values represent the mean ± S.D. of data from 5-6 oocytes. B and C, 3H-labeled 3-O-methylglucose (25 μM) transport in the presence of D- or L-glucose (Gluc, 50 mM) was followed for up to 2 h. Values represent the mean of data from 4-8 oocytes. Error bars have been omitted for clarity. ZRGT, adipocyte glucose transporter.

and 49 min (HepG2 transporter) and 28, 58, 106, and 208 min (adipocyte transporter) for equilibration of isotope in the presence of 4, 10, 30, and 50 mM 3-O-methylglucose, respect-

FIG. 4. Estimation of the kinetic parameters for 3-O-methylglucose transport in mRNA-injected oocytes under equilibrium exchange conditions. Oocytes were injected with 25 ng of HepG2 (A) or 50 ng of adipocyte (B) glucose transporter mRNA or an equal volume of water (Sham). Two days after injection, the oocytes were transferred into MBS containing the indicated concentrations of 3-O-methylglucose. Twenty-four hours thereafter oocytes were transferred into 3H-labeled 3-O-methylglucose of the same concentration, and influx was measured as described under "Experimental Procedures." Data from transport progress curves were linearized by logarithmic transformation. Time points were between 0.25 and 30 min. The equilibrium values were determined from long time points (≥24 h, see "Experimental Procedures"). Each value is derived from the mean of data for 6-11 oocytes. C, Hanes plot of the negative reciprocals of the slopes of the lines in A and B, corresponding to transport rate constants plotted against substrate concentration.
Expression of Mammalian Glucose Transporters

Assuming that transport under these conditions follows simple Michaelis-Menten kinetics (e.g., if the glucose transporters behave like simple carriers), a plot of the inverse of the rate constants versus substrate concentrations should allow determination of the equilibrium exchange maximal velocity \(v_{max}\) and half-saturation constant \(K_m\) \(\left(23\right)\). These values were derived from the plot shown in Fig. 4C, yielding values for \(K_m\) and \(v_{max}\) of 21.3 mM, 16 \(\mu\)mol/liter/s (HepG2 transporter) and 1.8 mM, 2.7 \(\mu\)mol/liter/s (adipocyte transporter).

**Discussion**

Previous studies have demonstrated increased uptake of the phosphorylated substrate, 2-deoxyglucose, into cells expressing heterologous facilitated diffusion glucose transporters, without directly demonstrating increased transport rates \(\left(9, 11, 32\right)\). The data presented in this paper unequivocally demonstrate functional expression of the human HepG2-type and rat adipocyte glucose transporters in Xenopus oocytes. 2-Deoxyglucose uptake was blocked by cytochalasin B, and inhibition of the transport of the nonmetabolized analogue, 3-O-methylglucose, was shown to be stereospecific for D-glucose.

The expression system was sufficiently sensitive to allow an estimation of kinetic parameters for the expressed heterologous transporters under equilibrium exchange conditions. The \(K_m\) value for the exchange of 3-O-methylglucose via the HepG2 transporter determined in the present study (21 mM) is in the middle of the range of values reported for glucose exchange in human erythrocytes at room temperature (8.1–38 mM) \(\left(33-36\right)\) and is remarkably similar to the value of 22 mM reported for 3-O-methylglucose exchange at 16°C \(\left(37\right)\). The reason for the wide range of \(K_m\) values obtained for glucose exchange in different laboratories is unclear. It has been suggested that the higher \(K_m\) values were due to insufficient time resolution of the methods used \(\left(36\right)\). The value of \(K_m\) for 3-O-methylglucose exchange via the adipocyte transporter (1.8 mM) was strikingly different than the HepG2 transporter value and was in reasonable agreement with the corresponding \(K_m\) observed for this transporter in the rat adipocyte \(\left(2.5–5\right)\) \(\left(19, 20\right)\). Both transporters when expressed in the oocyte plasma membrane exhibited half-saturation constants in agreement with the transport kinetics observed in their native cell types. The most likely explanation for the supraphysiological exchange \(K_m\) value for the HepG2 transporter observed in the present study is the phenomenon of accelerated exchange, which is reflected in an equivalent increase in both \(v_{max}\) and \(K_m\) over those values observed in net flux experiments \(\left(2, 22\right)\). The adipocyte transporter does not exhibit accelerated exchange in its native cell type \(\left(20\right)\), and the experiments presented here would suggest that this is true when the protein is expressed in the oocyte plasma membrane as well. If so, this would indicate that accelerated exchange is an inherent property of a specific glucose transporter protein and is independent of the cellular environment.

We consistently observed higher \(v_{max}\) values for transport via the HepG2 protein as compared with the adipocyte transporter. However, a direct comparison of the \(v_{max}\) values observed in these experiments for exchange via the HepG2 and adipocyte transporters is not meaningful, because they depend on the level of expression of the two proteins as well as their intrinsic activities, and it is not possible to directly determine the quantity of adipocyte transporter protein expressed in oocytes. The \(v_{max}\) value obtained in the present study for transport via the HepG2 transporter (0.016 mmol/liter/s) can be used to obtain an estimate of the turnover number under equilibrium exchange conditions for the HepG2-type glucose transporter in the oocyte plasma membrane. From the data in Fig. 1, ~0.2 ng of glucose transporter protein were expressed per oocyte. Thus,

\[
0.2 \times 10^{-3} \text{ g} \times 2 \times 10^{10} \text{oocytes} \times 1 \text{ mol} \times \frac{1 \text{ mole}}{55,000 \text{ g}} \times 6 \times 10^{22} \text{ molecules} \approx 4.4 \times 10^{15} \text{ molecules liter water}^{-1}
\]

There are 9.6 × 10^{15} molecules of glucose in 0.016 mmol, which gives a turnover of ~2.2 × 10^{15}/s. This value is ~3-fold higher than the turnover for glucose exchange estimated for the erythrocyte transporter at 20°C \(\left(23\right)\) and ~6-fold higher than the value that can be calculated from the \(v_{max}\) for 3-O-methylglucose exchange in erythrocytes at 16°C \(\left(37\right)\), assuming 7.9 \(\mu\)mol of transport/liter of cell water \(\left(23\right)\). Whether the discrepancy is due to a genuine difference in the kinetic properties of the polyol expressed in two different environments, the assumptions inherent in the calculations, or simply the accuracy of the kinetic data cannot be determined at present.

**Acknowledgments**—We are deeply indebted to Dr. Robert Marcer, Gwyn Gould, and Gustav Lienhard for their assistance and advice during the early stages of this work.

**References**

Expression of Mammalian Glucose Transporters