Relative Proximity and Orientation of Helices 4 and 8 of the GLUT1 Glucose Transporter*

Running Title: Orientation of Helices 4 and 8 of GLUT1

Arturo Alisio and Mike Mueckler‡

Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri, 63110

‡To whom all correspondence should be addressed:

Mike Mueckler
Department of Cell Biology and Physiology
Washington University School of Medicine
660 South Euclid Ave.
St. Louis, MO 63110
Ph: 314-362-4160
Fax: 314-362-7463
email: mike@cellbio.wustl.edu
*This work was supported in part by a grant from the National Institutes of Health (DK 43695) and by the Diabetes Research and Training Center at Washington University School of Medicine.

The Abbreviations used are: BMH, bismaleimidehexane; o-PDM, 1,4-phenylenedimaleimide; DM, decylmaltoside; C-less GLUT1, GLUT1 molecule in which all 6 native cysteine residues were changed to either glycine or serine; Di-C, C-less GLUT1 in which two native amino acid residues were both replaced with cysteine
SUMMARY

A structure has been proposed for the GLUT1 glucose transporter based on homology modeling that is consistent with the results of numerous mutagenesis studies (Mueckler, M. and Makepeace, C. (2004) J. Biol. Chem., in press). In order to further test and refine this model, the relative orientation and proximity of transmembrane helices 4 and 8 were analyzed by chemical crosslinking of di-cysteine mutants created in a reporter GLUT1 construct. All 6 native cysteine residues of GLUT1 were changed to either glycine or serine residues by site-directed mutagenesis, resulting in a functional C-less Glut1 construct. The Glut1 reporter molecule was engineered from C-less GLUT1 by creating a unique cleavage site for factor Xa protease within the central cytoplasmic loop and by eliminating the site of N-linked glycosylation. Fourteen functional di-cysteine mutants were then created from the C-less reporter construct, each mutant containing a single cysteine residue in helix 4 and one cysteine residue in helix 8. These mutants were expressed in Xenopus oocytes and the sensitivity of each mutant to intramolecular crosslinking by two homobifunctional, thiol-specific, crosslinking reagents, bismaleimidehexane and 1,4-phenylenedimaleimide, was ascertained by protease cleavage followed by immunoblot analysis. Four pairs of cysteine residues, 148/329, 145/328, 148/325, and 145/325, were observed to be in close enough proximity to be susceptible to crosslinking by one or both reagents. All five of the cysteine residues susceptible to
crosslinking are predicted to lie on the same face of helix 4 or 8 and to reside close to the cytoplasmic face of the membrane. These data indicate that the cytoplasmic ends of helices 4 and 8 lie within 6-16 Å of one another, and that the two helices twist or tilt such that they are further than 16 Å apart towards the center and exoplasmic side of the membrane. An updated model for the clustering of the transmembrane helices of GLUT1 is presented based on these data.
INTRODUCTION

The passive exchange of glucose across the membranes of animal cells is mediated by members of the GLUT (SLC2a) protein family [reviewed in (1-3)]. The GLUT family belongs to the Major Facilitator Superfamily (MFS), the largest category of proteins that catalyze the transport of small molecules across membranes (4). GLUT1, the prototype member of the GLUT family and the first eucaryotic member of the MFS Superfamily to be identified and cloned (5), is one of the most extensively studied of all membrane transporters (2,6,7). Kinetic and biophysical studies of glucose transport in the human red blood cell are mostly consistent with a simple alternating conformation mechanism (8-10), a conclusion that appears to be consistent with recent high-resolution structural studies of two bacterial MFS proteins (11,12).

GLUT1 was the first transporter predicted to possess 12 transmembrane helices (5), a characteristic that it appears to share with most if not all MFS proteins (4). This prediction was confirmed by glycosylation-scanning mutagenesis experiments (13) and other biochemical analyses [reviewed in (14)]. The 12 transmembrane helix model for GLUT1 is also consistent with the deduced structures of the lac permease (12) and glycerol-3-P antiporter (11). Several of the twelve proposed transmembrane segments were predicted to form amphipathic α-helices, an observation which
led to the hypothesis that these helices form the walls of a water-filled cavity involved in the binding and transfer of glucose across the membrane (5). It was also suggested that hydroxyl- and amide-containing amino acid side chains within the transmembrane helices form the sugar-binding site of GLUT1 by hydrogen bonding with glucose hydroxyl groups.

Considerable experimental support has accumulated for this basic structural model. Cysteine-scanning mutagenesis and substituted cysteine accessibility studies implicate transmembrane segments 1 (K. Keller, personal communication), 2 (15), 5 (16), 7 (15,17), 8 (Mueckler and Makepeace, J. Biol. Chem., in press), 10 (18), and 11 (19) of GLUT1 in the formation of a water-accessible cleft within the membrane. Gln$^{161}$ within helix 5 (20) and Gln$^{282}$ within helix 7 (21) appear to participate in forming the exofacial substrate-binding site. Val$^{165}$, which is positioned one helical turn distant from glutamine-161, is accessible to aqueous sulphhydryl reagents and appears to lie near the exofacial substrate binding site based on mutagenesis and inhibitor studies (22). An aromatic side-chain at position 412 within helix 11 appears to be essential for transport activity (23). Finally, hydrogen exchange studies demonstrate that 30% of peptide hydrogen atoms are exposed to water in purified, reconstituted GLUT1, consistent with the formation of an aqueous cleft in the membrane (24).

In this study we utilized chemical crosslinking of di-cysteine (di-C) GLUT1 mutants constructed in a reporter molecule to determine the relative
orientation and proximity of transmembrane helices 4 and 8, both of which are predicted to comprise a part of the inner helical bundle that forms the aqueous translocation pathway. The results are consistent with this prediction and permit modeling of the orientation of both helices in the membrane.

**EXPERIMENTAL PROCEDURES**

*Materials—Xenopus laevis*—Imported African frogs were purchased from Xenopus Express (Homosassa, FL), \(^3\)H-2-deoxyglucose and Diguanosine triphosphate (mRNA cap) were purchased from Amersham Pharmacia Biotech (Arlington Heights, IL), Megascript™ RNA synthesis kit was purchased from Ambion Inc (Austin, TX), Transformer™ Site-Directed mutagenesis kit was obtained from Clontech (Palo Alto, CA). SuperSignal TM West Pico Chemiluminiscent Substrate and Bismaleimidohexane (BMH) were obtained from Pierce (Rockford, IL), 1,4-Phenylenedimaleimide (o-PDM) was purchased from Aldrich Chemical Co. (Milwaukee, WI), and Decylmaltoside (DM) was obtained from Anatrace Inc. (Maumee, OH).

*General Procedures—* Procedures for the site-directed mutagenesis and sequencing of human GLUT1 cDNA and the in vitro transcription and purification of GLUT1 mRNAs (25), isolation, microinjection, and incubation
of *Xenopus* oocytes (26), preparation of oocyte membranes (23), SDS polyacrylamide gel electrophoresis and immunoblotting with GLUT1 C-terminal antibody (20), and 2-deoxyglucose uptake measurements (27), have been described in detail previously.

**Construction of di-Cysteine GLUT1 Mutants-** C-less GLUT1 cDNA subcloned into the oocyte expression vector pSP64T was subjected to site-directed mutagenesis to produce aglyco C-less GLUT1 containing the amino acid motif for Factor Xa protease sensitivity (IEGR). The following changes were introduced into C-less GLUT1 cDNA: Asn\(^{45}\) to Thr, Glu\(^{245}\) to Ile and Ser\(^{248}\) to Gly. This construct (aglyco C-lessXa) was then used as a template to individually change each amino acid residue from 141 to 150 in TMS 4 and 321 to 329 in TMS 8 to a Cys. In order to produce the di-C constructs (one Cys in TMS 4 and the other Cys in TMS 8), restriction fragments produced by Bsp I and Stu I digests of the single-C mutant cDNAs were ligated together.

**Treatment with homobifunctional maleimide crosslinking reagents-** Stage 5 *Xenopus* oocytes were injected with 50 ng of each mutant GLUT1 mRNA. Two days after injection, groups of 15-20 oocytes were incubated for 2 hr with 0.15 mM BMH, 0.15 mM o-PDM or 0.15 mM NEM in 1 mL of Barth’s saline at 22°C. Fifteen millimolar stock solutions of the maleimide reagents were prepared in 100% DMSO. After incubation the reactions were quenched by the
addition of 2 mM cysteine, incubated for 10 min, and then the oocytes were washed twice with 250 mM sucrose, 10 mM HEPES-NaOH pH 7.4 containing a protease inhibitor cocktail. In other experiments, 3.0 µg of freshly isolated total membranes from injected oocytes were incubated with 0.1 mM o-PDM or BMH or 0.2 mM NEM in 50 mM NaCl, 20 mM HEPES-NaOH pH 6.8 for 20 minutes at 22°C. The reaction was quenched by the addition of 2 mM cysteine followed by incubation for 10 min. Total membranes from either treatment protocol were adjusted to 1% Decylmaltoside, 1.5 mM CaCl₂ and then digested overnight at 4°C with 1 µg of Factor Xa protease (New England Biolabs). The digested membranes were then analyzed by SDS PAGE and immunoblot analysis using rabbit polyclonal ab raised against a peptide corresponding to the C-terminal 15 residues of human GLUT1. In some experiments, whole oocytes were treated with crosslinking reagents for 1 hour as described above and then [³H]-2-deoxyglucose uptake (50 µM, 30 min. at 22°C) was measured after quenching the reactions with cysteine.

Statistical Analysis- Uptake data were analyzed for statistical significance using the two-tailed, unpaired Student’s T-test.
RESULTS

A reporter GLUT1 molecule (aglyco-C-lessXa) was engineered in order to facilitate a determination of the relative proximity and orientation of pairs of transmembrane helices using chemical crosslinking of di-cysteine (di-C) mutants, an experimental approach that has been successfully used to analyze the structure of the E. coli lac permease (28). A factor Xa protease recognition site was introduced into the large, central, cytoplasmic loop of C-less GLUT1 (GLUT1 lacking its 6 native cysteine residues), permitting the analysis of pairs of cysteine residues residing in opposite halves of the molecule after chemical crosslinking. The site of N-linked glycosylation was also eliminated in the reporter GLUT1 construct by site-directed mutagenesis in order to simplify the analysis by preventing the appearance of multiple GLUT1 bands on SDS gels.

We have previously demonstrated that C-less GLUT1 exhibits close to wild-type transport activity when expressed in Xenopus oocytes (22). Figure 1a shows that C-less GLUT1 containing the factor Xa site (C-less-Xa) and the aglyco reporter construct (aglyco-C-lessXa) both exhibit robust transport activity in Xenopus oocytes, although aglyco-C-lessXa activity was reduced ~30% relative to that of the parental C-less molecule. Figure 1c shows that this reduced activity was due to a lower level of expression of the reporter construct in oocyte membranes relative to C-less GLUT1. These data indicate that the reporter construct retains functional activity, eliminating
the possibility that the mutations introduced into the molecule may have significantly altered its structure.

According to homology modeling of the GLUT1 structure using the lac permease as a template, helices 4 and 8 of GLUT1 are a part of the inner helix bundle that forms the aqueous substrate-binding cavity and may lie in close proximity to one another. However, homology modeling in this instance might be very imprecise because of the extremely weak sequence similarity between the lac permease and GLUT1 (~10%). Therefore, in order to test the model, we constructed di-C mutants in aglyco-C-lessXa in which a single residue in helix 4 and one residue in helix 8 were both changed to cysteines. Fourteen di-C mutant cDNAs were then constructed from the single-C mutants by restriction enzyme digestion and ligation of the appropriate fragments (see Table 1).

Figure 1b shows that all 14 di-C mutants exhibited significant transport activity above background levels when expressed in Xenopus oocytes, suggesting that the mutations did not produce major structural changes in the transporter. As we have observed for single-C GLUT1 mutants (16-19), most of the variability in transport activity among the different di-C mutants could be attributed to differences in the level of protein expression (data not shown). Total membranes were isolated from oocytes expressing each of the 14 di-C mutants. The membranes were incubated for 20 min in the presence or absence of 0.1 mM of each of two different homobifunctional,
sulfhydryl-specific, crosslinking agents: BMH (bis-maleimidehexane), a
flexible molecule ~16 Å in length, and o-PDM (1,4-Phenylenedimaleimide), a
rigid molecule ~6 Å in length. Control reactions were conducted in the
presence of N-ethylmaleimide (NEM) to replicate the sulfhydryl reaction in
the absence of crosslinking. The reactions were quenched by the addition of
cysteine, the membranes were solubilized in 1% decylmaltoside, digested for
16 h with factor Xa protease, and then subjected to SDS-PAGE and
immunoblot analysis. Analyses of aglyco-C-lessXa, and 7 of the 14 di-C
mutants are shown in Figure 2a. Protease cleavage should result in the
production of a ~20 kD C-terminal fragment of aglyco-C-lessXa that can be
detected with a polyclonal ab raised against the C-terminal 16 residues of
GLUT1. If the two cysteine residues in a di-C mutant are in the proper
orientation and close enough to one another in proximity, the mutant
transporter should show pseudo-protease resistance proportional to the
efficiency of the crosslinking reaction. Figure 2a shows that, as expected,
aglyco-C-lessXa was equally susceptible to protease cleavage in the presence
of NEM, BMH, and o-PDM, indicating that the parental reporter molecule
was not subject to crosslinking. Four di-C mutants, 141/321, 145/325,
146/328, and 148/326, were also not subject to crosslinking by either BMH or
o-PDM. However, mutants 145/328, 148/325, and 148/328 were clearly
susceptible to crosslinking by both BMH and o-PDM. None of the other 7 di-C
mutants listed in Table 1 exhibited detectable crosslinking (data not shown).
Because o-PDM and BMH are permeable to membranes, we next examined whether crosslinking could be achieved in intact oocytes using the protocol described in “Experimental Procedures”. Figure 2b shows that aglyco-C-lessXa and 3 di-C mutants, 141/321, 146/328, and 148/326, were not subject to crosslinking by either o-PDM or BMH, but that 145/325, 145/328, 148/325, and 148/328 were all susceptible to crosslinking in intact oocytes by BMH only. None of the other di-C mutants listed in Table 1 were subject to crosslinking by either reagent in intact oocytes (data not shown).

Given that crosslinking appeared to occur in intact oocytes, we tested the ability of NEM and the two crosslinking reagents to inhibit activity of the four mutants that were subject to crosslinking, along with 4 representative di-C mutants that were not susceptible to crosslinking either in vitro or in vivo. Figure 3 shows that 2-deoxyglucose uptake activity of the parental aglyco C-lessXa transporter was not affected by any of the 3 reagents. However, transport activity of all 8 di-C mutants was affected to some degree by all three sulfhydryl-specific reagents, although the affect of NEM on 145/325 and o-PDM on 145/326 did not reach statistical significance. These data indicate that at least one of the two cysteine residues in all 8 of the di-C mutants was accessible to at least two of the three reagents tested, suggesting that the lack of crosslinking in the majority of the 14 di-C mutants was most likely not due to inaccessibility of the cysteine residues to the reagents, but rather reflects the relative proximity and orientation of the two residues.
**Discussion**

The data presented in this study indicate that 4 of 14 di-C mutant GLUT1 transporters were susceptible to intramolecular chemical crosslinking by homobifunctional, thiol-specific crosslinking reagents (see Fig. 4). The crosslinking data are consistent with our model for the structure of GLUT1 based on studies employing the substituted cysteine accessibility method (SCAM) (15-19) and homology modeling (see Fig. 5). Our data indicate that Gly^{145} and Ser^{148} in helix 4 lie within ~6-16 Å of Leu^{325} and Val^{328} in helix 8. These data are consistent with Leu^{325} and Val^{328} being positioned along the side of helix 8 that faces the aqueous cavity in one or both orientations of the unloaded transporter, in agreement with the results of SCAM analyses (Mueckler and Makepeace (2004), J. Biol. Chem., in press). Helix 4, which has not yet been analyzed by SCAM, can be roughly oriented in the membrane based on the crosslinking data, with Gly^{145} and Ser^{148} facing the aqueous cavity in direct apposition to Leu^{325} and Val^{328} (see Fig. 4 and 5). Consistent with this model, crosslinking was not observed for several pairs of residues (145/326, 145/327, 148/326, 148/327, and 146/328) that are predicted to be positioned on anti-apposing faces of helices 4 and 8 (see Figure 4). Additionally, none of the pairs of residues (141/321, 141/324, and 145/321) predicted to lie near the center or exoplasmic end of the helices, despite apparently lying on directly apposed faces of their respective helices, were
subject to crosslinking, suggesting that helices 4 and 8 are further apart at their exoplasmic ends due to tilting or twisting.

Interestingly, the 145/328, 148/325, and 148/328 mutants were differentially susceptible to the two reagents when the reactions were conducted in isolated membranes versus intact oocytes. In isolated membranes, BMH and o-PDM were capable of crosslinking all three pairs of cysteine residues with about equal efficiency, whereas in intact oocytes only the longer molecule, BMH, produced detectable crosslinking of the three pairs of cysteine residues. The former result might reflect differential permeability of the intact oocyte membrane to the two reagents, or it could reflect differential steady-state structures of the mutants in intact oocytes versus isolated membranes. The latter hypothesis is supported by the observation that inhibition of transport activity was observed for 7 of the 8 di-C mutants tested with both BMH and o-PDM (see Figure 3). In the absence of substrate, the transporter will exist primarily in two distinct conformations, its cytoplasmic and exoplasmic substrate-binding forms. One of these forms may be favored in isolated membranes. If so, our data suggest that the interface between helices 4 and 8 near the cytoplasmic face of the membrane is closer together in the favored conformation, because under these conditions the two cysteine residues were subject to crosslinking by the ~6 Å reagent, o-PDM, as well as by BMH. The hypothesis that the transporter exists in different steady-state conformational distributions in isolated membranes versus
intact oocytes is also supported by the observation that 145/325 was only susceptible to crosslinking in intact oocytes (see Figure 2).

Additional SCAM analyses and crosslinking studies should permit further testing and refinement of the structural model shown in Fig. 5. Ultimately, definitive structural information is dependent on the successful crystallization of GLUT1, a feat that has not yet been achieved for any eukaryotic membrane transporter.

Acknowledgment– We thank Paul Hruz for constructing an early version of the GLUT1 reporter cDNA.
FIGURE LEGENDS

FIG. 1. 2-Deoxyglucose uptake activity of GLUT1 mutants. [3H]-2-DOG uptake (50 µM, 30 min. at 22°C) was measured 2 days after injection of oocytes with 50 ng of mRNA. A) The activities of C-less GLUT1 (C-less), C-less GLUT1 possessing the Factor Xa protease site (C-less-Xa) and C-less-Xa lacking the site of N-linked glycosylation (Asp45→Thr) are compared. * p < 0.05 for N45T vs. C-less. B) Immunoblot showing relative levels of expression of C-less, C-less-Xa, and Aglyco-C-lessXa in oocyte membranes. C) Transport activities of di-C mutants. * p < 0.05 for di-C mutant compared to N45T as the control. Results represent the mean ± SE of 3-6 independent experiments with 15-20 oocytes per experimental group. Values observed in sham-injected oocytes were subtracted.

FIG 3. Chemical Crosslinking of di-C Mutants. Stage 5 Xenopus oocytes were injected with 50 ng of mRNA encoding the parental reporter construct (Aglyco-C-lessXa) or the indicated di-C mutant. After incubation of oocytes for 2 days, crosslinking analysis was conducted on either purified oocyte membranes or intact oocytes as described in “Experimental Procedures”. The reactions were quenched by the
addition of 2 mM cysteine and total oocyte membranes were then isolated from the intact oocyte reactions. The total oocyte membranes from both groups were solubilized in 1 % Decylmaltoside (DM), digested with 1 µg Factor Xa protease overnight at 4°C, and then subjected to SDS-PAGE followed by immunoblotting with rabbit polyclonal ab raised against the C-terminal 16 residues of human GLUT1. A) Autoradiogram of immunoblots from crosslinking conducted on purified membranes. B) Autoradiogram of immunoblots from crosslinking reaction conducted on intact oocytes. The position of the ~20 kD C-terminal GLUT1 cleavage product is indicated.

**FIG 3.** *Effect of maleimide reagents on transport activity of di-C mutants.* Two days after injection of 50 ng of the indicated mRNA, oocytes were incubated for 1 hour at 22°C with DMSO, 0.15 mM N-ethylmaleimide (NEM), 0.15 mM o-phenylenedimaleimide (o-PDM), or 0.15 mM bismaleimidhexane (BMH). [³H]-2-DOG uptake (50 µM, 30 min. at 22°C) was then measured. * p < 0.05 for mutants treated with maleimide compared to DMSO as a control. Results represent the mean ± SE of 3-4 independent experiments with 15-20 oocytes per experimental group.
FIG 4. Proposed orientation of transmembrane helices 1, 4, 5 and 8. Helices 1, 4, 5 and 8 are represented as cylinders. Atoms comprising amino acid side chains in helices 4 and 8 are shown as colored spheres. Lines connecting residues G145-L325, G145-V328, S148-L325, and S148-V328 represent observed crosslinking with a homobifunctional maleimide in the corresponding di-C mutants. The orientation of the helices is based on homology modeling using the lac permease as the template.

FIG 5. Cross-sectional model of GLUT1 in the membrane as viewed from the cytoplasmic face. Amino acids are represented by the single letter code. Lines between residues G145-L325, G145-V328, S148-L325, and S148-V328 represent observed crosslinking with a homobifunctional maleimide reagent in the corresponding di-C mutants. * indicates residues that are accessible to p-chloromercuribenzenesulfonate (pCMBS) in SCAM studies.
TABLE 1

Construction of Di-Cysteine Mutants in a C-less GLUT1 Reporter Molecule

cDNA encoding C-less human GLUT1 was subjected to oligonucleotide-mediated, site-directed mutagenesis, changing Asn \(^{45}\) to Thr, Glu \(^{246}\) to Ile and Ser \(^{248}\) to Gly to produce aglyco C-less containing the Factor Xa protease recognition motif (aglyco-C-lessXa). This construct was then used as the template to individually change amino acid residues in TMS 4 or in TMS 8 to a cysteine. Di-C mutants were then constructed from the single-C mutants by ligation of restriction fragments.

| Residue # | Amino Acid Change | Codon Change |
|-----------|-------------------|--------------|
| **Aglyco C-less GLUT1 Xa** | | |
| 45        | Asn/Thr           | AAC/ACA      |
| 246       | Glu/Ile           | GAA/ATA      |
| 248       | Ser/Gly           | AGT/GGT      |

| Double Cys Mutants | |
|--------------------|-----------------|-----------------|-----------------|-----------------|
| (TMS4/TMS8)        | TMS4 | TMS8 | TMS4 | TMS8 |
| 141/321            | Pro/Cys | Thr/Cys | CCC/TGC | ACT/TGT |
| 141/324            | Pro/Cys | Ser/Cys | CCC/TGC | TCG/TGT |
| 145/321            | Gly/Cys | Thr/Cys | GGT/TGT | ACT/TGT |
| 145/325            | Gly/Cys | Leu/Cys | GGT/TGT | CTG/TGT |
| 145/326            | Gly/Cys | Phe/Cys | GGT/TGT | TTT/TGT |
| 145/327            | Gly/Cys | Val/Cys | GGT/TGT | GTG/TGT |
|    | Amino Acid 1 | Amino Acid 2 | Nucleotide 1 | Nucleotide 2 |
|----|-------------|-------------|-------------|-------------|
| 145/328 | Gly/Cys | Val/Cys | GGT/TGT | GTG/TGT |
| 146/328 | Glu/Cys | Val/Cys | GAA/TGT | GTG/TGT |
| 148/325 | Ser/Cys | Leu/Cys | TCA/TGT | CTG/TGT |
| 148/326 | Ser/Cys | Phe/Cys | TCA/TGT | TTT/TGT |
| 148/327 | Ser/Cys | Val/Cys | TCA/TGT | GTG/TGT |
| 148/328 | Ser/Cys | Val/Cys | TCA/TGT | GTG/TGT |
| 149/328 | Pro/Cys | Glu/Cys | CCC/TGC | GAG/TGT |
| 150/328 | Thr/Cys | Val/Cys | ACA/TGT | GTG/TGT |
REFERENCES

1. Baldwin, S. A. (1993) *Biochim. Biophys. Acta* **1154**, 17-49

2. Mueckler, M. (1994) *Eur. J. Biochem.* **219**, 713-725

3. Pessin, J. E., and Bell, G. I. (1992) *Annu. Rev. Physiol.* **54**, 911-930

4. Pao, S. S., Paulsen, I. T., and Saier, M. H., Jr. (1998) *Microbiology & Molecular Biology Review (Washington, DC)* **62**, 1-34

5. Mueckler, M., Caruso, C., Baldwin, S. A., Panico, M., Blench, I., Morris, H. R., Allard, W. J., Lienhard, G. E., and Lodish, H. F. (1985) *Science* **229**, 941-945

6. Mueckler, M. (1989) in *Red Blood Cell Membranes* (Agre, P., and Parker, J. C., eds), pp. 31-45, Marcel Dekker, Inc., New York

7. Mueckler, M., Hresko, R. C., and Sato, M. (1997) *Biochem. Soc. Trans.* **25**, 951-954

8. Lowe, A. G., and Walmsley, A. R. (1989) in *Red Blood Cell Membranes* (Agre, P., and Parker, J. C., eds) Vol. 11, pp. 597-634, Marcel Dekker, Inc., New York

9. Appleman, J. R., and Lienhard, G. E. (1985) *J. Biol. Chem.* **260**, 4575-4578

10. Appleman, J. R., and Lienhard, G. E. (1989) *Biochemistry* **28**, 8221-8227

11. Huang, Y., Lemieux, M. J., Song, J., Auer, M., and Wang, D. N. (2003) *Science.* **301**, 616-620
12. Abramson, J., Smirnova, I., Kasho, V., Verner, G., Kaback, H. R., and Iwata, S. (2003) *Science*. **301**, 610-615

13. Hresko, R. C., Kruse, M., Strube, M., and Mueckler, M. (1994) *J. Biol. Chem.* **269**, 20482-20488

14. Hruz, P. W., and Mueckler, M. M. (2001) *Molecular Membrane Biology* **18**, 183-193

15. Olsowski, A., Monden, I., Krause, G., and Keller, K. (2000) *Biochemistry* **39**, 2469-2474

16. Mueckler, M., and Makepeace, C. (1999) *J. Biol. Chem.* **274**, 10923-10926

17. Hruz, P. W., and Mueckler, M. M. (1999) *J. Biol. Chem.* **274**, 36176-36180

18. Mueckler, M., and Makepeace, C. (2002) *J. Biol. Chem.* **277**, 3498-3503

19. Hruz, P. W., and Mueckler, M. M. (2000) *Biochemistry* **39**, 9367-9372

20. Mueckler, M., Weng, W., and Kruse, M. (1994) *J. Biol. Chem.* **269**, 20533-20538

21. Hashiramoto, M., Kadowaki, T., Clark, A. E., Muraoka, A., Momomura, K., Sakura, H., Tobe, K., Akanuma, Y., Yazaki, Y., Holman, G. D., and et, a. l. (1992) *J. Biol. Chem.* **267**, 17502-17507

22. Mueckler, M., and Makepeace, C. (1997) *J. Biol. Chem.* **272**, 30141-30146
23. Garcia, J. C., Strube, M., Leingang, K., Keller, K., and Mueckler, M. M. (1992) *J. Biol. Chem.* **267**, 7770-7776

24. Jung, E. K., Chin, J. J., and Jung, C. Y. (1986) *J. Biol. Chem.* **261**, 9155-9160

25. Hresko, R. C., Murata, H., Marshall, B. A., and Mueckler, M. (1994) *J. Biol. Chem.* **269**, 32110-32119

26. Marshall, B. A., Murata, H., Hresko, R. C., and Mueckler, M. (1993) *J. Biol. Chem.* **268**, 26193-26199

27. Keller, K., Strube, M., and Mueckler, M. (1989) *J. Biol. Chem.* **264**, 18884-18889

28. Kaback, H. R., Voss, J., and Wu, J. (1997) *Current Opinion in Structural Biology* **7**, 537-542
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Relative proximity and orientation of helices 4 and 8 of the GLUT1 glucose transporter
Arturo Alisio and Mike Mueckler

J. Biol. Chem. published online April 7, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M402303200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts