Role of Conserved Arginine and Glutamate Residues on the Cytosolic Surface of Glucose Transporters for Transporter Function†

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Received May 19, 1997; Revised Manuscript Received July 22, 1997⊗

ABSTRACT: The role of conserved arginine and glutamic acid residues at the cytoplasmic surface of the GLUT4 for transporter function was investigated by site-directed mutagenesis and expression of the constructs in COS-7 cells. Reconstituted glucose transport activity, cytochalasin B binding, and photolabeling with the exofacial label 2-*N*4-(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis(D-mannosyloxy)-2-propylamine (ATB-BMPA) was assayed in membranes from transfected cells and corrected for immunoreactivity of expressed transporters. Exchange of Arg 92 (R92L amino acid residues are numbered according to the corresponding residues in the GLUT1) or Arg 333/334 (RR333/4LA) reduced or suppressed transport activity with no or very little effect on photolabeling with ATB-BMPA and cytochalasin B binding. It is suggested that the lack of these residues selectively disturbes the substrate-induced conformational change of the carrier during transport. Exchange of Glu 146 (E146D) or Arg 153 (R153L) markedly reduced transport activity, ATB-BMPA photolabeling, and cytochalasin B binding. Transport activity and ATB-BMPA labeling were abolished in the mutants E329Q, E393D, and R400L, whereas binding of cytochalasin B was normal. Thus, exchange of Glu 329, Glu 393, and Arg 400 appears to arrest the transporter in an inward facing conformation. It is concluded that the conserved arginine and glutamate residues at the cytoplasmic surface of the glucose transporter GLUT4 are essential for its appropriate conformation, and that it is the interaction of charged residues which mediates the oscillation between outward and inward facing states.

Glucose transport into mammalian cells is catalyzed by a family of carrier proteins, GLUT1−4, which differ in expression, K_m, and maximum turnover (*1*−*3*). On the basis of sequence comparisons, the GLUT proteins can be assigned to the large superfamily of transport facilitators (*4, 5*). These transport facilitators are characterized by 12 membrane-spanning helices, a large, highly charged intracellular loop domain, a large glycosylated extracellular loop, and several conserved amino acid motifs. It is assumed that the 12 helices of the transport facilitators form a membrane pore which oscillates between an outward and an inward facing conformation (*6, 7*). This model has been corroborated with mutants of glucose transporters which are arrested in either conformation. Exchange of tyrosine 293 for isoleucine locks the transporter in an outward facing conformation which binds the exofacial photolabel ATB-BMPA,[1] whereas the endofacial site, as assessed by binding of cytochalasin B, is obliterated (*8*). Glu 161, in contrast, appears to be critical for exofacial ligand binding (*9*). Furthermore, results from mutants of Trp 412 (*10*−*12*), in which glucose transport, but not cytochalasin B binding, was affected, suggest that the transporter may also be locked in the inward facing conformation.

We have recently studied the function of a motif (GRR) which is conserved in all members of the transport facilitator family including plant and bacterial carrier proteins. Mutation of the arginine residues in this motif of the GLUT4 abolished glucose transport activity, but failed to affect glucose-inhibitable binding of cytochalasin B or a forskolin-derived photolabel (*13*). Consequently, we suggested that these arginine residues are required for the conformational alteration of the transporter during translocation of the hexose, but not for ligand or glucose binding to the protein. Furthermore, we speculated that the role of the arginines is their interaction with negative charges at the inner surface of the transporter, and that rapid switching of these interactions drives conformational alterations of the protein. In the present study, we tested this hypothesis by investigating the role of conserved charged residues (R92, K93, R153, R333/4, R400, E146, E329, and E393; see Figure 1) on the cytoplasmic surface of the transporters. The function of the mutants was characterized by the parameters glucose transport activity, cytochalasin B binding, and photolabeling with the exofacial label ATB-BMPA. The results indicate that seven of the eight investigated polar residues are essential for the full function of the transporter and that mutation of three of the residues arrests the transporter in an inward facing conformation. Thus, it is suggested that an interaction of Arg and Glu residues on the cytoplasmic surface is

† This study was supported by grants from the Deutsche Forschungsgemeinschaft (Jo 117/8-2 and SFB351) and the German-Israeli Foundation (GIF).

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⊗ Abstract published in *Advance ACS Abstracts,* October 1, 1997.

[1] Abbreviation: ATB-BMPA, 2-*N*4-(1-azi-2,2,2-trifluoroethyl)benzoyl-1,3-bis(D-mannosyloxy)-2-propylamine.

S0006-2960(97)01173-2 CCC: $14.00

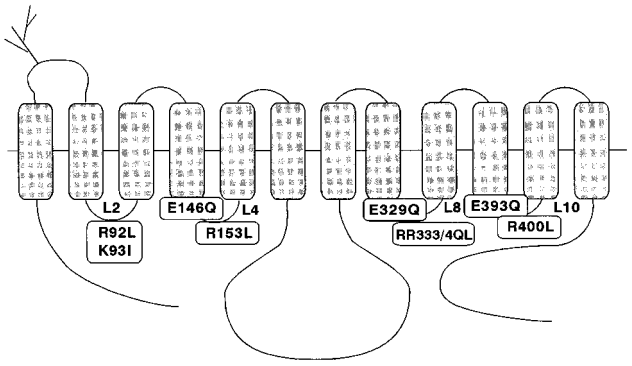

FIGURE 1: Model of the glucose transporter GLUT4 depicting the positions of the mutated residues. The model comprising 12 membrane spanning helices was adopted from Mueckler et al. (*25*). According to a database search and multiple sequence comparisons performed with the CLUSTAL program, these residues are conserved in all known mammalian, yeast, and plant sugar transporters. In order to facilitate comparisons, the numbering of amino acids corresponds with that of the GLUT1.

required for the appropriate conformation of the transporter and its alteration during the transport process.

MATERIALS AND METHODS

Construction of Mutant DNA and Expression Vectors. Mutants were generated by oligonucleotide-directed mutagenesis as described (*14*). Single-stranded DNA of rat GLUT4 (*15*) subcloned into pBluescript was used as template. Mutated constructs were identified by sequencing and subcloned as *Xba*I−*Kpn*I fragments from pBluescript into the mammalian expression vector pCMV, which harbors an SV40 origin, a cytomegalovirus promoter, and a polyadenylation site (*16*).

Transfection of COS-7 Cells. Transfection of COS-7 cells with glucose transporter cDNA was performed with the calcium phosphate precipitation method as described previously (*13*).

Preparation of Membrane Fractions from Transfected Cells. The transfected cells were homogenized and fractionated as previously described (*16*) with a modification of a protocol previously employed in 3T3-L1 cells (*17*). Most of the glucose transporter protein was found in the plasma membrane (13000*g*) and high-density microsomal (45000*g*) fractions which were used for the subsequent assays.

Immunoblotting of the GLUT4. Immunochemical detection and quantitation of the GLUT4 was performed as described previously with antiserum against a dodekapeptide corresponding with the C-terminus of GLUT4 (*17*).

Reconstitution of Glucose Transport Activity from Membrane Fractions. Glucose transport activity reconstituted into lecithin liposomes (L-α-phospatidylcholine type IVS from soybean, catalog no. P3644, Sigma, St. Louis, MO) was assayed as described previously (*18*) with minor modifications (*19*). Glucose transport was assayed at 37 °C with D-[U-^{14}C]glucose (final concentration 1 mM), and initial uptake rates assayed at 10 s were corrected for noncarrier mediated uptake with L-[1-^{3}H]glucose (usually 50% of total uptake in control membranes, 5−10% in membranes from cells transfected with wild-type GLUT4). Uptake rates were linear for at least 20 s. Stereospecific transport is inhibitable by cytochalasin B, phloretin, or $HgCl_2$; the latter was used for termination of uptake. In parallel samples, the abundance of transporters present in the lecithin liposomes was determined by immunoblotting, and the transport rates were corrected for small differences in immunoreactivities. All constructs were reconstituted with identical efficiency.

Assay of Equilibrium Cytochalasin B Binding. Samples of plasma membranes (19 μg of protein) in 200 μL of tris-buffer (20 mM, pH 7.4) were incubated with 0.05 μCi [^{3}H]-cytochalasin B (NEN, specific activity, 9.8 Ci/mmol), 0.04 μCi [^{14}C]urea (Amersham, 56 mCi/mmol), and the desired concentrations of unlabeled cytochalasin B for 10 min on ice. Membranes were separated by centrifugation in a refrigerated microfuge (13 500 rpm, 30 min). After removal of the supernatants, the tips of the tubes were cut off, and the pellets were solubilized in 0.5 mL tissue solubilizer (BTS-450, Beckman, München, Germany). Bound and free radioactivity were determined in a water-compatible scintillation cocktail (Ready Protein, Beckman). Scatchard plots were evaluated graphically as described previously (*20, 21*). Because of the differences in transfection rates, the number of sites was variable between different series of transfections [range 35−120 pmol/(mg of protein)]. Within a series of transfections, all constructs produced identical numbers of sites [standard deviation (SD) approximately 10%].

Photolabeling of Glucose Transporters with ATB-BMPA. ATB-BMPA was prepared as described (*22*). Photolabeling was performed by a modification of a previously published procedure (*8*). Samples of plasma membranes (100 μL) containing 30 μg of membrane protein in Tris buffer (20 mM, pH 7.4) were incubated with ATB-BMPA (30 μL, 150 μCi, specific activity 10 Ci/mmol) for 2 min on ice. The samples were photolyzed for 1 min in a Rayonet RPR-100 photochemical reactor with 300 nm lamps. Membranes were separated by centrifugation in a refrigerated microfuge (13 500 rpm, 20 min), and the resulting pellets were solubilized in 2% Thesit. GLUT4 was immunoprecipitated (*23*), and the immunoprecipitates were separated by SDS−PAGE (10% gels). The gels were sliced, solubilized, and the incorporated radioactivity was determined by scintillation counting.

RESULTS

Expression of GLUT4 in COS-7 Cells. Transfection of COS-7 cells with cDNA of GLUT1 or GLUT4 in a vector driven by the CMV promoter results in high copy expression of glucose transporters (*16*), which are further enriched by isolation of membrane fractions (plasma membranes and high-density microsomes). The activity of these transporters was assayed after reconstitution of solubilized transporters into lecithin liposomes; this assay yields in a proportional relationship between the abundance of expressed transporters and the activity which is reconstituted from the membrane fractions (data not shown). In intact cells, the transport activity does not increase proportionally with the expression of the transporters, because a substantial portion of the overexpressed foreign transporters does not appear to be inserted into the plasma membranes (*16*). Thus, for the purpose of this study, the reconstitution assay was preferable to the assay of transport activity in cells. The glucose transporter isoform GLUT4 was chosen for mutagenesis and expression, because this isoform can be detected and isolated with specific antiserum in the presence of the constitutive GLUT1, allowing a normalization of transport rates per expressed transporters (*16*).

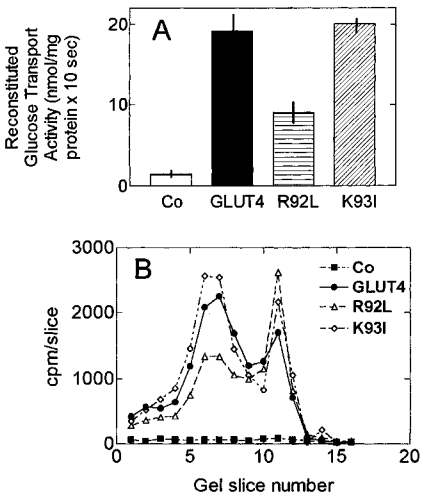

FIGURE 2: Glucose transport activity and ATB-BMPA labeling in mutants of Arg 92 and Lys 93 (R92L, K93I) in the intracellular loop L2 of the GLUT4. (A) Glucose transporters from COS-7 cells transfected with the indicated constructs were solubilized and reconstituted into lecithin liposomes as described. Transport activity was assayed, and data were corrected for differences in the immunoreactivity of GLUT4 as assayed by Western blotting. Co, samples from cells transfected with bland vector. (B) Membranes from cells transfected with the indicated construct were labeled with ATB-BMPA as described in the Materials and Methods. The labeled transporters were immunoprecipitated with specific antiserum and separated by SDS−PAGE. Gels were cut, and slices were solubilized and counted.

Table 1: Affinity of Binding of Cytochalasin B in Membranes of Transfected COS-7 Cells[a]

	K_D (nM)
wild-type GLUT4	98 ± 7
R92L	107 ± 19
K93I	79 ± 27
RR333/4LA	107 ± 30
R153L	not detectable
E146D	280 ± 63
E146Q	850 ± 290
wild-type GLUT4	113 ± 3
E329D	144 ± 5
E329Q	99 ± 13
R400L	88 ± 6
wild-type GLUT4	156 ± 40
E393D	126 ± 52
E329Q	152 ± 27

[a] The binding of cytochalasin B was assayed by a centrifugation assay as described in the Materials and Methods. Data were corrected for background binding as determined with cells transfected with bland vector and for small differences in the abundance of the expressed foreign transporter. K_D values were determined as described previously (*21*) and represent means ± SD. Because the K_D of wild-type cytochalasin B was variable (90−150 nM), separate controls were given for each of the three series. The number of binding sites was identical within each of three individual series of transfections (SD approximately 10%).

Mutagenesis of Arg 92 and Lys 93 (GRK Motif) in the Intracellular Loop between Helices 2 and 3 (L2). The intracellular loop L2 harbors a GRR/GRK motif which, like that in L8, is conserved in the family of sugar transport facilitators. A notable exception is the GLUT2 which contains the residues GRI. Mutation of the GRK motif of the GLUT4 to GRI (GLUT4-K93I) failed to affect glucose transport activity (Figure 2, panel A), affinity of cytochalasin B binding (Table 1), or labeling with ATB-BMPA (Figure 2, panel B). Thus, the K93I mutant appeared indistinguishable from the wild-type. In contrast, exchange of the arginine to leucine (GLUT4-R92L; GRK to GLK) reduced transport activity by approximately 60% (Figure 2, panel A) and ATB-BMPA photolabeling by 20% (panel B), whereas the affinity of cytochalasin B binding (Table 1) was unaffected. Thus, the first basic residue in the GRR/K motif appears to be required for the full transport activity, but not for the proper conformation of the endofacial recognition site (cytochalasin B binding).

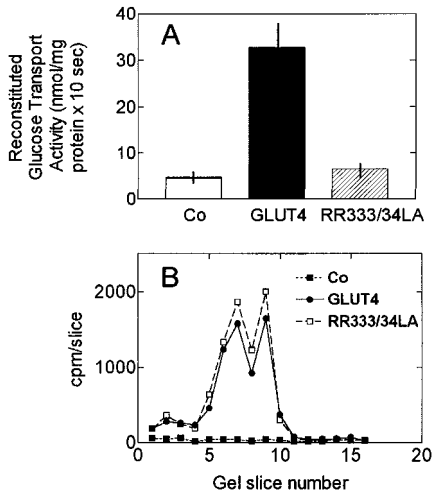

FIGURE 3: Effect of substitution of Arg 333 and 334 (RR333/4LA) in the intracellular loop L8 on glucose transport activity and ATB-BMPA labeling. (A) Glucose transporters from COS-7 cells transfected with wild-type GLUT4 or the RR333/4LA construct were solubilized and reconstituted into lecithin liposomes as described in the Materials and Methods. Transport activity was assayed, and data were corrected for differences in the immunoreactivity of GLUT4 as assayed by Western blotting. Co, samples from cells transfected with bland vector. (B) Membranes from cells transfected with the indicated construct were labeled with ATB-BMPA as described, and incorporation of label was determined by immunoprecipitation and separation of GLUT4 on SDS−PAGE.

Mutagenesis of the GRR Motif in the Intracellular Loop between Helices 8 and 9 (L8). The GRR motif in the intracellular loop between the membrane spanning helices 8 and 9 is conserved in all known sugar transporters and also in other carrier proteins, e.g., the proton/tetracyclin symporter. We have previously demonstrated that the exchange of this motif (mutant RR333/34LA) markedly reduced glucose transport activity. The data illustrated in Figure 3 extend these results and indicate that photolabeling of the mutant GLUT by ATB-BMPA was unaffected (panel B). As anticipated, transport activity was essentially abolished (panel A), whereas the affinity of binding of cytochalasin B was normal (Table 1). Thus, the exchange of the GRR motif in L8 seems to selectively affect the transport activity, but not glucose binding to the exofacial recognition site, or cytochalasin B binding to the endofacial ligand binding site.

Substitution of Arg 153 and 400 in the Intracellular Loops L4 and L10. Figure 4 illustrates the effects of an exchange of arginine 153 for leucine (GLUT4−R153L). Glucose transport activity (panel A) in this mutant was approximately 50% lower than in the wild-type; ATB-BMPA labeling (panel B) was reduced to a similar extent. Cytochalasin B binding was not detectable in membranes from cells overexpressing the R153L mutant. Thus, the exchange of arginine 153 appears to obliterate the endofacial (cytochalasin B binding) site.

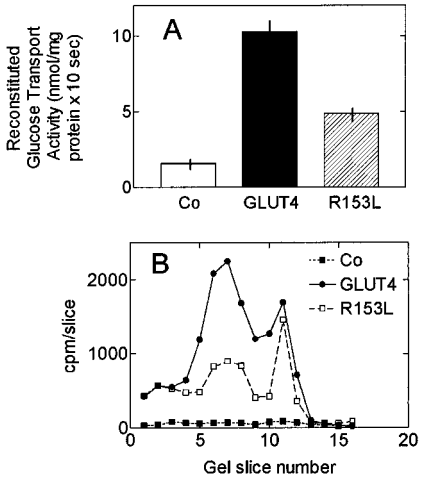

FIGURE 4: Glucose transport activity and ATB-BMPA labeling in a mutant of Arg 153 (R153L) in the intracellular loop L4. (A) Glucose transporters from COS-7 cells transfected with the indicated constructs were solubilized and reconstituted into lecithin liposomes as described. Transport activity was assayed, and data were corrected for differences in the immunoreactivity of GLUT4 as assayed by Western blotting. Co, samples from cells transfected with bland vector. (B) Membranes from cells transfected with the indicated construct were labeled with ATB-BMPA as described, and incorporation of label was determined by immunoprecipitation and separation of GLUT4 on SDS−PAGE.

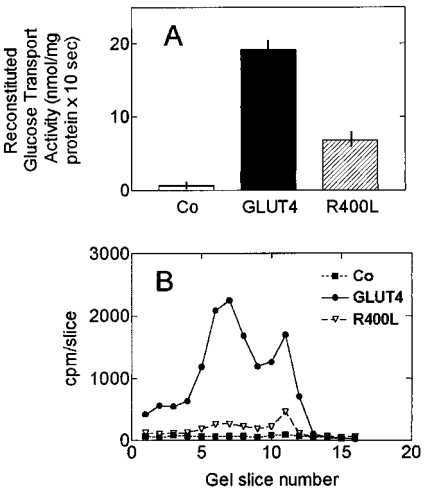

FIGURE 5: Glucose transport activity and ATB-BMPA labeling in a mutant of Arg 400 (R400L) in the intracellular loop L10. (A) Glucose transporters from COS-7 cells transfected with the indicated constructs were solubilized and reconstituted into lecithin liposomes as described. Transport activity was assayed, and data were corrected for differences in the immunoreactivity of GLUT4 as assayed by Western blotting. Co, samples from cells transfected with bland vector. (B) Membranes from cells transfected with the indicated construct were labeled with ATB-BMPA as described, and incorporation of label was determined by immunoprecipitation and separation of GLUT4 on SDS−PAGE.

Exchange of the arginine 400 in L10 (R400L) reduced glucose transport activity by approximately 60% and nearly suppressed photolabeling with ATB-BMPA (Figure 5, panels A and B). The affinity of cytochalasin B binding was unaffected in this mutant. Thus, exchange of arginine 400 appears to selectively affect the exofacial recognition site of glucose.

Mutagenesis of Glu 146 (GLUT4−E146D and E146Q) in the Intracellular Loops between Helices 4 and 5 (L4). Exchange of glutamate 146 for glutamine fully abolished

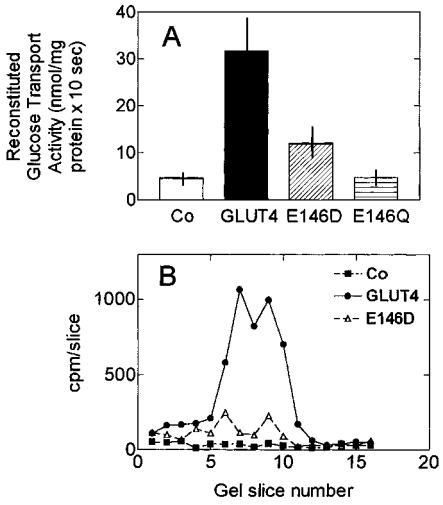

FIGURE 6: Glu 146 is essential for both glucose transport activity and ATB-BMPA photolabeling. (A) Glucose transporters from COS-7 cells transfected with the indicated constructs were solubilized and reconstituted into lecithin liposomes as described. Transport activity was assayed, and data were corrected for differences in the immunoreactivity of GLUT4 as assayed by Western blotting. Co, samples from cells transfected with bland vector. (B) Membranes from cells transfected with the indicated construct were labeled with ATB-BMPA as described, and incorporation of label was determined by immunoprecipitation and separation of GLUT4 on SDS−PAGE.

the glucose transport activity of the GLUT4, whereas a marked reduction was seen in the E146D mutant (Figure 6, panel A). Binding of cytochalasin B was markedly reduced in both mutants (Table 1). Photolabeling with ATB-BMPA was abolished in E146D, and markedly reduced in E146Q. Thus, the negative charge of glutamate 146 as well as its proper distance to the peptide chain appear essential for the formation of both exo- and endofacial recognition site of glucose.

Mutagenesis of Glutamate 329 (GLUT4-E329D and E329Q) in the Intracellular Loop L8. Figure 7 illustrates the effects of a substitution of the conserved glutamate residue in the intracellular loop L8. It should be noted that this glutamate is present in all so far identified mammalian hexose transporters, and that it is substituted for aspartate in most yeast and plant transporters/symporters. Exchange of this residue to aspartate failed to reduce the glucose transport activity or ATB-BMPA labeling. In contrast, elimination of the negative charge (in E329Q) fully abolished the glucose transport activity and ATB-BMPA labeling, whereas cytochalasin B binding was not affected by the exchange of the carboxyl group. Thus, the negative charge of glutamate 329 appears to be required for the formation of the exofacial, but not of the endofacial recognition site of glucose.

Substitution of Glutamate 393 (GLUT4-E393Q and E393D) in the Intracellular Loops between Helices 10 and 11 (L10). The exchange of glutamate 393 for glutamine or aspartate markedly reduced glucose transport activity and, in approximate proportion, also the binding of ATB-BMPA (Figure 8). In contrast, there was no apparent difference in the affinities (K_D) of cytochalasin B binding.

DISCUSSION

We have initiated the present study on the basis of the commonly accepted model which pictures the glucose

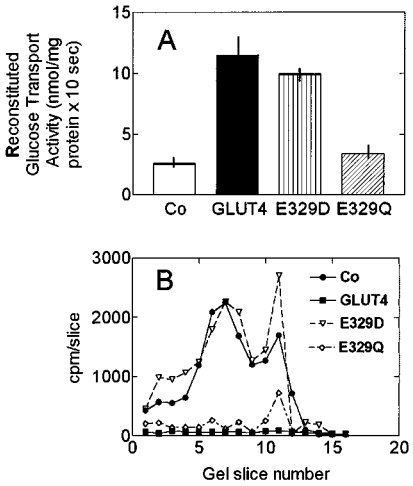

FIGURE 7: Glucose transport activity and ATB-BMPA photolabeling in mutants of Glu 329 (E329D and E329Q) in the intracellular loop L8. (A) Glucose transporters from COS-7 cells transfected with the indicated constructs were solubilized and reconstituted into lecithin liposomes as described. Transport activity was assayed, and data were corrected for differences in the immunoreactivity of GLUT4 as assayed by Western blotting. Co, samples from cells transfected with bland vector. (B) Membranes from cells transfected with the indicated construct were labeled with ATB-BMPA as described, and incorporation of label was determined by immunoprecipitation and separation of GLUT4 on SDS−PAGE.

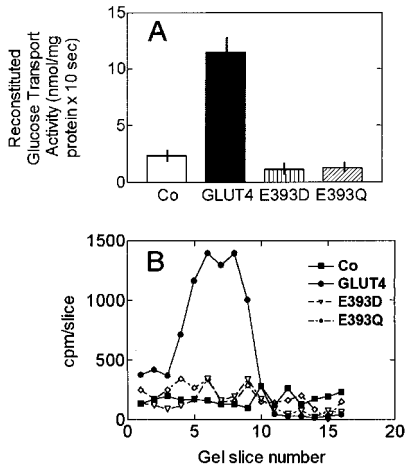

FIGURE 8: Glucose transport activity and ATB-BMPA photolabeling in mutants of Glu 393 (E393D and E393Q) in the intracellular loop L10. (A) Glucose transporters from COS-7 cells transfected with the indicated constructs were solubilized and reconstituted into lecithin liposomes as described. Transport activity was assayed, and data were corrected for differences in the immunoreactivity of GLUT4 as assayed by Western blotting. Co, samples from cells transfected with bland vector. (B) Membranes from cells transfected with the indicated construct were labeled with ATB-BMPA as described, and incorporation of label was determined by immunoprecipitation and separation of GLUT4 on SDS−PAGE.

transporter as an oscillating pore formed by its membrane spanning helices (*6*, *7*). This model is supported by the present data which indicate that the proper function of the protein is dependent on residues adjacent to eight of the 12 helices and that mutation of some of these residues may arrest the transporter in an inward facing conformation. Furthermore, we assumed that the proper arrangement of the helices requires the interaction of residues by hydrophobic, semipolar, or ionic interactions and that the transport process is

Table 2: Summary of the Alterations Observed in Mutants of Arginine and Glutamate Residues at the Cytosolic Surface of the GLUT4[a]

		recognition site	
	glucose transport	exofacial	endofacial
Arg 92	↓	(↓)	normal
Arg 333/4	↓↓	normal	normal
Glu 146	↓↓	↓↓	↓↓
Arg 153	↓	↓	↓↓
Glu 329	↓↓	↓↓	normal
Glu 393	↓↓	↓↓	normal
Arg 400	↓↓	↓↓	normal

[a] The symbols summarize the effects shown in Figures 2−7 [(↓), 20% reduction; ↓, 50% reduction; ↓↓, 90−100% reduction of glucose transport activity, ATB-BMPA labeling or cytochalasin B binding].

mediated by an alteration of these interactions. The present data show that the function of the glucose transporter GLUT4 is indeed dependent on several charged residues at the cytoplasmic surface of the transporter. These glutamate and arginine residues are conserved throughout the family of sugar transport facilitators and, therefore, do not determine the substrate specificity of the transporters. We conclude from the data that it is the interaction of acidic and basic residues on the cytoplasmic surface of the transporters which provides the structural basis for the correct arrangement of the helices.

In order to provide a detailed analysis of the mutants, three different functional parameters were assessed: glucose transport activity, binding of glucose as determined with the photoreactive mannose derivative ATB-BMPA, and binding of cytochalasin B. The results indicate that these parameters may be selectively affected in the mutants and that different patterns of alterations were produced (see Table 2). Firstly, a selective reduction of glucose transport activity with no or very little alteration of the cytochalasin B and ATB-BMPA binding was observed with the mutants R92L and RR333/4LA. In these mutants, the function of the carrier, i.e., the capability to undergo the conformational rearrangements required for the movement of the hexose through the pore, appears to be obliterated, whereas the glucose and cytochalasin B binding site appear only marginally affected. This result seems to indicate that the exchange of Arg 92 and 333/334 selectively disturbes the substrate-induced conformational change of the carrier during transport, but not the exofacial or endofacial glucose recognition site.

A second pattern of alterations was seen with the mutants E146D and R153L in which both binding of cytochalasin B and labeling with ATB-BMP were markedly reduced. It should be noted that both proteins retained a residual transport activity and binding activity for the mannose photolabel R153L somewhat more than E146D. These residues appear crucial for all conformations of the transporter; their lack seems to produce a profound disruption of the tertiary structure. We speculate, therefore, that the interaction of E146 and R153 is required for all conformational states of the transporter and that the mutation of either one of these residues releases the helices into a conformation that is unable to bind ligands or to transport glucose normally. Thirdly, a reduction in transport activity and ATB-BMPA binding with no apparent alteration of the cytochalasin B binding was observed in the mutants R400L, E329Q, and E393D. These residues are obviously required to maintain

the helical arrangement which forms the exofacial glucose recognition site, and their mutation locks the transporter in an inward facing conformation which exposes the cytochalasin B binding site but not the exofacial glucose (ATB-BMPA) binding site.

In this study, the activity of the mutant transporters was assessed after reconstitution into artificial membranes. This in vitro system provides information as to the molecular function of the carriers without their proper insertion into plasma membranes. Assessment of the activity of mutants with intact cells was not feasible, because overexpression of the GLUT4 (and also of large amounts of GLUT1) in COS-7 cells does not target the transporter into the plasma membrane but into a vesicle population which is isolated with the PM and HDM fractions (*16*). It has to be considered, therefore, whether proper insertion of the mutant transporters in plasma membranes might have altered the results. It seems unlikely that mutants which exhibit reduced activity in the reconstitution assay can be normal when properly inserted into plasma membranes of cells. However, the possibility cannot fully be excluded that a mutant which is normal in the reconstitution assay exhibits reduced activity in intact cells. This caveat would pertain to the mutants K92I and E329D, which exhibited normal transport activity and ligand binding.

An inherent problem of the mutagenesis approach is the possibility that amino acid exchanges may disrupt the proper folding of a peptide chain, leading to a nonspecific disruption of the secondary structure. The following arguments support our view that the alterations observed here reflect specific effects, i.e., that the exchanged amino acids are involved in the arrangement of the helices. Firstly, very small alterations like the exchange of Glu for Asp in E146D or E393D produced marked effects on the function of the protein; it appears unlikely that these exchanges fully disrupted the protein folding. Secondly, all mutants retain residual or even full activity in at least one of the investigated parameters. Thirdly, the position of the mutated residues is strictly conserved within the family of hexose transporters; substitutions which are found in homologues of other species could be made without effect (K93I and E329D). Thus, it is concluded here that the conserved arginine and glutamate residues at the cytoplasmic surface of the glucose transporter GLUT4 provide the structural basis for the formation the glucose-transporting pore and its conformational alteration during transport.

ACKNOWLEDGMENT

A.S. and H.D. contributed equally to this paper. The authors are indebted to Geoff Holman for the exofacial photolabel ATB-BMPA, to Morris Birnbaum for the cDNA of GLUT4, and to Petra Mühl-Zürbes and Simone Prinz for skillful technical assistance.

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BI971173C