Fourier transform infrared spectroscopic study of the structure and conformational changes of the human erythrocyte glucose transporter*

Javier Alvarez‡§, David C. Lee‡†, Stephen A. Baldwin‡∥, and Dennis Chapman‡

From the ‡Department of Biochemistry and Chemistry and the †Department of Chemical Pathology and Human Metabolism, Royal Free Hospital School of Medicine (University of London), Rowland Hill Street, London, United Kingdom NW3 2PF

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Fourier transform infrared spectroscopy has been used to study the secondary structure of the human erythrocyte glucose transporter after purification and reconstitution in erythrocyte lipids. The spectra indicate that the glucose transporter contains, in addition to the predominant α-helical structure, an appreciable amount of β-structure and random coil conformation. A study of the time dependency of H-2H exchange revealed that more than 80% of the polypeptide backbone is readily accessible to the solvent. This result indicates that a portion of the intramembrane-spanning region of the membrane protein is exposed to the solvent, suggesting the existence of an intraprotein aqueous channel. The residual (10–20%) portion of the protein which exchanges slowly includes some α-helical structure, probably situated in a hydrophobic environment inside the membrane. The infrared spectra of transporter preparations were also examined after incubation with substrate and substrate analogues. Compared with the spectra recorded under conditions in which the "inward-facing" form predominates, a small but reproducible shift in the bands assigned to α-helical and β-strand structures is observed after incubation with 4,6-0-ethylidene-D-glucose, which largely fixes the transporter in the "outward-facing" conformation. An increase of temperature, which is known to increase the proportion of transporter in the outward-facing conformation, results in a similar shift in this α-helical absorption band.

The transport of glucose across the human erythrocyte membrane is the best characterized example of a facilitated diffusion process. Recently, the protein responsible for transport has been purified to near homogeneity (1, 2). It is a membrane-spanning glycoprotein of apparent M, 55,000 that bears a large oligosaccharide chain of the poly(N-acetyllactosamine) type on its extracellular domain (3, 4). The isolated protein appears to retain most, if not all, of its functional properties intact. For example, when reconstituted into the membranes of artificial lipid vesicles it catalyzes the uptake of D-glucose with kinetics similar to those seen in the intact erythrocyte (5, 6). It also binds cytochalasin B, a potent reversible inhibitor of glucose transport, with the same high affinity and sensitivity to competition by D-glucose as in the cell (7). The stoichiometry of cytochalasin B binding to preparations of the transporter made using a recently improved procedure (8) is routinely >0.9 sites/polypeptide, indicating that these preparations contain very little denatured protein.

Much evidence from steady state kinetic studies (9–12), from ligand binding studies (13), and from studies of inhibitor specificity (14) supports an alternating conformation model for the mechanism of glucose transport (15, 16). In this model, the membrane-spanning transport protein can exist in two conformations, in which the substrate binding site adopts either an outward-facing or an inward-facing form. Translocation of the sugar after binding is effected by a conformational change between these two forms. A number of glucose derivatives have been described that preferentially bind to one or other forms of the binding site and thus effectively trap the protein largely in a single conformation. For example, 4,6-0-ethylidene-D-glucose, a nontransported glucose derivative, has a 10-fold higher affinity for the outward facing conformation than for the inward-facing conformation of the transporter (17, 18). In contrast, n-propyl-β-D-glucopyranoside binds predominantly to the inward-facing conformation (14, 18). The availability of such derivatives has recently enabled the direct observation of a conformational change in the purified transport protein and determination of its rate by means of fluorescence measurements (19). However, the magnitude of the change in protein conformation remains unknown.

Recently, the amino acid sequence of a glucose transport protein from human HepG2 hepatoma cells was deduced from analysis of a complementary DNA clone (20). Structural analysis of the human erythrocyte glucose transporter by fast atom bombardment mass spectrometry and gas phase sequencing revealed that this protein is very similar if not identical to the hepatoma transporter (20). Examination of the sequence for hydrophobic segments by the procedures of Kyte and Doolittle (21) and of Eisenberg et al. (22), together with the results of vectorial proteolytic digestion experiments, enabled a model to be proposed for the arrangement of the transporter in the membrane (20). In this model, the polypeptide traverses the lipid bilayer 12 times in the form of α-helices made up predominantly of hydrophobic amino acid residues (20). If a membrane-spanning α-helix is taken to contain 21 amino acids, the model would predict that at least 50% of this 492-residue protein is α-helical.

Infrared spectroscopy is now an established technique for the examination of protein secondary structure (23, 24). Many studies, both experimental and theoretical, have shown that

M. T. Cairns and S. A. Baldwin, unpublished observations.
the frequency of the amide I band, and to some extent the amide II band, may be related to the a-helical, $\beta$-strand, and "random" coil conformations in the protein under study (25-28). Recently, the advent of Fourier transform (FT-IR)$^{\text{2}}$ methods of data acquisition has enabled high quality spectra to be obtained for proteins in dilute, aqueous solution using digital subtraction of the background water absorptions (28). The overlapping amide absorptions derived from the different secondary conformations present in large, globular proteins may be observed by deconvolution or derivation of the spectrum (29-31). These techniques have recently been applied to the study of the conformation of membrane proteins such as the Ca$^{2+}$-ATPase and bacteriorhodopsin (32, 33). An additional advantage of the technique is that FT-IR spectroscopy is not subject to the light-scattering artifacts encountered in studies of membranous samples by techniques such as circular dichroism (34). It therefore represents a very powerful tool for the investigation of the secondary structure of membrane proteins. In this paper we examine the secondary structure of the glucose transport protein from human erythrocytes by FT-IR spectroscopy and compare it with the predictions of the model described above. We also investigate the magnitude of the conformational changes associated with glucose transport. A preliminary study of the glucose transporter secondary structure using a dispersive IR spectrometer has been reported (35).

**MATERIALS AND METHODS**

Outdated human blood was provided by the blood bank of the Royal Free Hospital. Endoglycosidase F (endo- $\beta$-N-acetyl glucosaminidase F) was a generous gift of Boehringer Mannheim. Endo- $\beta$-galactosidase from *Bacteroides fragilis* was generously provided by Dr. R. F. Scudier (Clinical Research Centre, Harlow, UK). 4-O-Ethylidene-$\alpha$-D-glucose (ethylidene-$\alpha$-D-glucose) was supplied by Aldrich and n-propyl-$\beta$-D-glucopyranoside (propyl glucoside) was a generous gift from Dr. G. E. Lienhard (Dartmouth Medical School, Hanover, NH). $\alpha$-glucose and $\beta$-glucose were of the highest purity available from Sigma.

The human erythrocyte glucose transporter was purified and reconstituted in erythrocyte membrane lipids by the method of Baldwin et al. (2) with the modifications introduced by Cairns et al. (8). No lipid was added, and the reconstituted material typically contained about 0.3 mg of protein/mg of phospholipid, representing a molar ratio of about 1/250. The purity was routinely tested by SDS-polyacrylamide gel electrophoresis by the procedure of Laemmli (36) using 12% acrylamide gels. The cytochalasin B binding activity was also measured for each preparation by equilibrium dialysis using $4 \times 10^{-6} M$ (4-H)cytochalasin B as previously described (37). Protein concentration was assayed by the method of Lowry et al. (68) including 0.5% SDS in order to solubilize membranous samples.

Deglycosylation of the glucose transporter was carried out by incubating the reconstituted protein first with 0.6 units/ml of endo-$\beta$-galactosidase for 18 h at 57°C, at a protein concentration of 1 mg/ml, in a medium composed of 50 mM sodium phosphate, 1 mM dithiothreitol, 1 mM EDTA, pH 6.2. The transporter was then treated with 2 units/ml of endoglycosidase F for 24 h at room temperature in a medium composed of 100 mM sodium phosphate, 50 mM EDTA, 75 mM 2-mercaptoethanol, pH 6.1. At the end of the digestion period, the deglycosylated transporter was separated from the released oligosaccharides by centrifugation for 1 h at 13,000 $\times g$. The resultant pellet containing the protein was resuspended in 160 mM NaCl, 5 mM HEPES, pH 7.4, and washed twice by centrifugation as above to ensure complete removal of oligosaccharides. A control sample was incubated in parallel without the enzymes. Lipid extraction from the preparation was made by the procedure of Santiago et al. (39).

For deuterium exchange experiments, transporter samples were prepared in $^2$H$_2$O either by dialyzing the glucose transporter against several changes of 5 mM HEPES, 160 mM NaCl, pH 7.0, in $^2$H$_2$O or by dilution of a concentrated sample in this buffer followed by centrifugation (1 h at 13,000 $\times g$) and several washes. The $p$H of the buffer was adjusted to 7.0 with NaOH to yield the deuteron concentration equivalent to the proton concentration in water at pH 7.4 (40). A lyophilized sample of the glucose transporter was also prepared and dissolved either in 10 mM HEPES, pH 7.4, in $^2$H$_2$O, or 10 mM HEPES, pH 7.0, in $^2$H$_2$O, in order to follow the time dependence of the exchange.

The samples for IR spectroscopy were resuspended usually in 5 mM HEPES, 160 mM NaCl, pH 7.4, at a protein concentration of 10-15 mg/ml. Buffer and protein samples were scanned in a Perkin-Elmer FT-IR 1750 spectrometer, using a Perkin-Elmer Model 7300 data station for data acquisition and analysis. Either 200 scans at 2 cm$^{-1}$ resolution or 400 scans at 4 cm$^{-1}$ resolution were averaged. Buffer spectra were recorded under the same temperature and scanning conditions as the corresponding protein spectra. Each sample was equilibrated at the chosen temperature for 10 min before the data acquisition, which itself took about 1 h. Spectra of lyophilized transporter were collected from samples prepared as a KBr disc. 1 mg of lyophilized transporter was mixed with $\sim$100 mg of dried and ground KBr and pressed at 10 tons under vacuum for 20 min. Second-derivative and fourth-derivative spectra were generated from difference spectra by using an OBEY program available from Perkin-Elmer. In order to eliminate noise, the rate of change of slope (second derivative) was calculated over a 13-data point range (13 cm$^{-1}$) and this repeated to yield the fourth derivative. Spectral deconvolution was performed using the Perkin-Elmer ENHANCE function, which is analogous to the method developed by Kauppinen et al. (29).

**RESULTS**

The FT-IR spectrum of the glucose transporter after purification and reconstitution in erythrocyte lipids in H$_2$O is shown in Fig. 1. The difference spectrum reveals a phospholipid carbonyl stretching absorption at 1734 cm$^{-1}$ and amide I and amide II bands at 1656 and 1547 cm$^{-1}$, respectively (Fig. 1a). The second-derivative spectrum obtained from this difference spectrum is shown in Fig. 1b. The carbonyl stretching band now shows two bands at 1744 and 1728 cm$^{-1}$, corresponding to the carbonyl vibrations of the sn-1 and sn-2 chains of the phospholipids (41). Amide I band components appear at 1691, 1681, 1658, 1639, and 1630 cm$^{-1}$. The amide II band presents three components at 1548, 1530, and 1517 cm$^{-1}$. By comparison with previous studies (24, 26, 28, 33) the main amide I band at 1658 cm$^{-1}$ and the amide II band at 1530 cm$^{-1}$ are indicative of a high content of a-helical structure, although part of the absorption at this frequency also corresponds to random coil structure and can only be resolved after deuteration exchange (see below). The presence of amide I components at 1691, 1681, 1658, and 1630 cm$^{-1}$ and the amide II component at 1530 cm$^{-1}$ strongly suggests that some $\beta$-structure is also present and that a portion of it forms antiparallel strands. The weak amide I component at 1681 cm$^{-1}$ cannot be unequivocally assigned, as absorption near this frequency has been reported from $\beta$-structure (30), $\beta$-turns (42, 43) and amino acid side chains (43). Contribution from $\beta$-turns may be present in the amide I components at 1691, 1681, and 1658 cm$^{-1}$ (42, 44). The amide II component at 1517 cm$^{-1}$ may be assigned to random coil peptide group vibrations (23) and/or tyrosine side chain absorption (45). The same features are revealed in the fourth-derivative spectrum that is shown in Fig. 1c. Amide I components appear at 1694, 1684, 1659, 1645, and 1627 cm$^{-1}$ and amide II components at 1544, 1528, and 1516 cm$^{-1}$. These absorption bands can also be demonstrated by deconvolution of the difference spectrum, as shown in Fig. 1d, although the bands are not so well defined as in the second- and fourth-derivative spectra.

*Fig. 2* shows the spectra of the glucose transporter after three washes in $^2$H$_2$O buffer. The difference spectrum (Fig. 2a) shows the carbonyl stretching vibration at 1733 cm$^{-1}$ and

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$^{2}$The abbreviations and trivial names used are: FT-IR, Fourier transform infrared; SDS, sodium dodecyl sulfate; HEPES, N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid; endoglycosidase F, endo- $\beta$-N-acetyl glucosaminidase; ethylidene-$\alpha$-D-glucose, 4,6-O-ethylidene-$\alpha$-D-glucose; propyl glucoside, n-propyl-$\beta$-D-glucopyranoside.
the amide I and amide II bands at 1653 and 1545 cm\(^{-1}\). There is a decrease in the band intensity ratio of amide II/amide I from 0.61 to 0.11 as a result of the isotopic substitution of the peptide bond N-H to N-D, representing an exchange of 82% of these hydrogens. The second-derivative spectrum is shown in Fig. 2b, and the amide I components appear at 1684, 1658, and 1628 cm\(^{-1}\). The high frequency component of the absorption of antiparallel β-sheet in \(^2\)H\(2\)O is known to occur around 1675 cm\(^{-1}\) (23, 30, 43). In the glucose transporter, the two bands present in H\(2\)O at 1691 and 1681 cm\(^{-1}\) are transformed into only one in \(^2\)H\(2\)O at 1684 cm\(^{-1}\), which may represent a combination of absorptions from exchanged and unexchanged portions of the β-structure. The α-helical band does not shift at all after the exchange of 90% of the amide hydrogens, a fact that contrasts with the behavior of soluble α-helical proteins in which a shift of 2–4 cm\(^{-1}\) to lower frequency has been described (23, 43).

A considerable increase can be observed, both in the difference and in the second-derivative spectra, of the absorption intensity in the low frequency portion of the amide I band. The significance of this increase is not known, but it does not reflect a denaturation, as the protein fully retains its cytochalasin B binding activity upon deuterium exchange (data not shown). A shoulder at 1646 cm\(^{-1}\) may be assigned to random coil structure, which absorbs at the same frequency as the α-helices in H\(2\)O and is shifted to this frequency in \(^2\)H\(2\)O medium (26). The fourth-derivative spectrum is shown in Fig. 2c. Amide I components appear at 1684, 1659, 1644, and 1627 cm\(^{-1}\). The absorption peaks which appear between the amide I and amide II band components may be assigned to amino acid side chains (45); bands at 1605, 1584, and 1578 cm\(^{-1}\) may be associated with arginine residues (4.3% of the protein) and bands at 1569 and 1560 cm\(^{-1}\) with glutamic acid residues (4.9% of the protein). Some contribution of aspartic acid residues (1.4% of the protein) may also be present in the band at 1584 cm\(^{-1}\). The increase in intensity of the band at 1529 cm\(^{-1}\) is especially evident in the deconvolved spectrum, shown in Fig. 2d, in which is also apparent the presence of a peak at 1645 cm\(^{-1}\) (due to random conformation, Ref. 26) in between the two main absorptions at 1657 and 1629 cm\(^{-1}\). The second-derivative spectrum of a lyophilized sample is shown in Fig. 3a. The carbonyl stretching band appears as a band at 1743 cm\(^{-1}\) with a weak shoulder at 1730 cm\(^{-1}\). Amide I components can be seen at 1684, 1659, and 1630 cm\(^{-1}\) and amide II components at 1547 and 1517 cm\(^{-1}\). After redissolving in H\(2\)O buffer, the spectrum shown in Fig. 3b was obtained, which is practically identical to the original spectrum (see Fig. 1b), with amide I band components at 1688, 1681, 1657.5, 1639, and 1629 cm\(^{-1}\) and amide II band components at 1548 and 1517 cm\(^{-1}\). A shift in the α-helical band is thus observed upon dissolution with reference to the dry protein, as has been described with other α-helical proteins (28). However, there was almost no shift in the frequency of the main amide II band. Usually the frequency of this band increases between 6 and 13 cm\(^{-1}\) upon dissolution due to greater hydrogen bonding (with water) for the N-H peptide group (28). Our result may be due to the persistence of hydrogen-bonded water inside the protein upon lyophilization.
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The second-derivative spectrum obtained immediately after dissolution of the dry material in $^2$H$_2$O medium is shown in Fig. 3c. The collection of this spectrum took 1 h, and it may be considered as an average of the spectra of the glucose transporter at different stages of the exchange during this time. The rate of exchange is extremely fast, 81% of the hydrogens being already exchanged as calculated from the amide II/amide I band intensity ratio. Similar results were obtained with other H-2H exchange procedures, such as dilution followed by washing in 2H$_2$O buffer or dialysis against 2H$_2$O buffer. The percentage of exchange in all the cases was about 75% from the first hour and increased very slowly with time at room temperature (82% after 3 days; see Fig. 2). In order to obtain a greater exchange, the glucose transporter was incubated for 90 min at 50 °C. The exchange increased to 90%, but no shift was detected in the position of the peaks in the second-derivative spectrum. The last 10% of the amide hydrogens have a very slow rate of exchange, and another spectrum taken after a further 16 h incubation at 37 °C showed a slightly greater exchange of 93%, although in this case a small shift of the α-helical band from 1558 to 1657 cm$^{-1}$ was observed (not shown). The cytochalasin B binding activity of the transporter was assessed after every treatment, and no loss of activity was observed.

In order to confirm the assignment of these bands to protein absorptions and not to absorptions of the lipid or carbohydrate present, both the spectra of the extracted lipid and of the deglycosylated protein were recorded. No significant absorption was detected in the spectral region of the amide I and II bands in the spectrum of the extracted lipid. Deglycosylation of the transporter was performed by treatment with endoglycosidases. It has previously been reported that complete removal of the asparagine-linked oligosaccharide chain from the transporter by cleavage with endoglycosidase F can be achieved only if the protein is first solubilized in detergent (46). The harsh conditions required lead to denaturation of the transporter. In contrast, endo-β-galactosidase acts on the membrane-embedded transporter, preserving its activity, but does not remove all the carbohydrate (4). We have now found that prior treatment of the transporter with endo-β-galactosidase renders the residual carbohydrate susceptible to endoglycosidase F digestion in the absence of detergent. Treatment of the transporter in this fashion yielded a sharpening of the protein band on SDS-polyacrylamide gels similar to that reported for endoglycosidase F treatment of detergent-solubilized transporter (46). The cytochalasin B binding activity demonstrated that 70% of the protein was still active after glycosidase treatment, the same as a control treated in parallel without the glycosidases. This indicates that the deglycosylation does not produce by itself any loss in activity. The second-derivative spectrum of the deglycosylated glucose transporter was identical to the spectrum of the original glucose transporter within our resolution.

Spectra showing the effect of temperature on the structure of the glucose transporter are shown in Fig. 4. Second-derivative spectra recorded at 8, 20, 26, and 50 °C are presented, and the peak frequencies in the fourth derivatives are given in Table I. There are no major changes in the spectra in the range studied, but some small shifts can be detected. There is only one peak at 1684 cm$^{-1}$ below 20 °C which splits above
this temperature into two peaks at 1680 and 1690 cm\(^{-1}\). The temperature of the transition seems to be variable in different preparations, since in the majority of the recorded spectra at 20 °C in this work two peaks are seen (see Fig. 1). A less apparent gradual shift in the α-helical band can also be appreciated in the fourth-derivative spectra (see Table I).

The effect of the binding of glucose and glucose analogues on the structure of the glucose transporter is presented in Fig. 5. In Fig. 5, a and b the second-derivative spectra of the glucose transporter in the presence of 200 mM D-glucose and L-glucose, respectively, are shown. Because L-glucose is not able to bind to the transporter, it represents a useful control for nonspecific sugar effects at this high concentration. Both spectra can be considered identical within our resolution, and they are also identical to the untreated glucose transporter (the difference obtained was \(-0.4 \pm 0.4 \text{ cm}^{-1}\) for L-glucose, two experiments, and \(-0.3 \pm 0.5 \text{ cm}^{-1}\) for D-glucose, three experiments, with respect to the untreated transporter). On the other hand, the differential effect of 200 mM ethylidene-D-glucose (outward-facing conformation ligand) as compared with 200 mM propyl glucoside (inward-facing conformation ligand) is shown in Figs. 5c and 5d, respectively. The spectrum in the presence of propyl glucoside is identical to the untreated transporter (0.0 ± 0.6 cm\(^{-1}\), four experiments), but the addition of ethylidene-D-glucose produces a shift of the α-helical band of 1.1 ± 0.4 cm\(^{-1}\) in four experiments done with different preparations at 2 cm\(^{-1}\) resolution. There is also a shift in the band at 1629 cm\(^{-1}\) in the same direction, whose magnitude varied between 1 and 3 cm\(^{-1}\).

**DISCUSSION**

This is the first FT-IR spectroscopic study of the structure of the human erythrocyte glucose transporter to employ deconvolution calculations to assign overlapping absorption bands. Our results show that this membrane protein, when reconstituted in erythrocyte lipids, adopts a mainly α-helical structure, although some β-strand and random coil conformation is also present. An earlier short report using a dispersive IR spectrometer gave similar results (35). Whether β-turns are present is not certain, because the assignment of the amide I component in H\(_2\)O at 1681 cm\(^{-1}\) is uncertain. On H-2H exchange a shift in the β-turn absorption to the 1660 cm\(^{-1}\) region has been described (43), but in our case the presence of the strong α-helical band at 1658 cm\(^{-1}\) makes it impossible to resolve this band.

A recent model deduced from the amino acid sequence (20) did not predict the presence of β-structure. The model proposed that the polypeptide chain crosses the membrane 12 times in the form of α-helices of 21 amino acid residues each, accounting for about 50% of the 492 amino acid residues of the protein. The presence of β-turns in the hydrophilic loops linking the membrane-spanning segments was also predicted.

A recent FT-IR spectroscopic study failed to detect β-structure in this protein (47). This study, however, used a Lorentz band fitting procedure to separate the overlapping amide band components. The usefulness of this method is limited by the requirement for extensive input information. A combination of Lorentzian bands is fitted to the experimental spectra with no independent evidence for the presence of each component. We have used derivative spectroscopy and Fourier self-deconvolution to identify the band components in the experimental spectra, obtaining by both methods evidence for an absorption around 1630 cm\(^{-1}\), corresponding to β-structure. The presence of this band both in the native and the deglycosylated protein and the absence of absorption in this region by the erythrocyte lipids confirm that this band is due to the protein and not to the carbohydrates nor to the lipids and constitutes strong evidence for the presence of a proportion of β-structure in this protein.

When we compare IR spectroscopic data with predictive methods of secondary structure, it is important to note that far less is known about the structure of membrane proteins than of soluble proteins. Prediction methods have been extensively used to obtain the secondary structure from the amino acid sequence (48). However, computer algorithms like the ones that were used to predict the structural model of the glucose transporter (21, 22) are designed to seek membrane-spanning α-helices, and other types of membrane-bound protein secondary structure, such as β-strands, may elude detection (48). Bacteriorhodopsin has long been the only membrane protein about which detailed knowledge of structure was available. However, the presence of β-structure even in this membrane protein is still unclear. Electron diffraction studies did not detect any β-structure in this protein (49), but infrared spectroscopy has suggested the presence of a significant proportion of antiparallel β-strand (32). Recently, the molecular structure of the protein subunits in the photosynthetic reaction center of *Rhodopseudomonas viridis* has been elucidated at high resolution, showing a predominant α-helical structure along with an average 10% of β-structure in the three subunits (50). Sarcoplasmic reticulum Ca\(^{2+}\)-ATPase has been studied by infrared spectroscopy, its secondary structure being a mixture of α-helical and random conformations, with some β-strand also present (32). The structure of the lactose permease of *Escherichia coli*, a protein of similar molecular weight and related function to the glucose transporter, has been determined from Raman spectroscopy (51). The α-helix content is around 70%, the β-strand content below 10%, and β-turns contribute 15%. A model with 10–14 membrane-span-
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Fig. 4. Temperature dependence of the FT-IR spectrum of the glucose transporter protein in H₂O buffer. Second derivative spectra at 8 °C (a), 20 °C (b), 26 °C (c), and 50 °C (d). 400 scans were averaged at 4 cm⁻¹ resolution. Scale divisions are 0.0008 absorbance units/cm⁻².

TABLE I

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<th>Temperature (°C)</th>
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The study of the time-dependent H²H exchange has shown that 75-80% of the protein exchanges within the 1st h as calculated from the ratio of amide II/amide I band intensity. This fact contrasts with the behavior of other membrane proteins like rhodopsin, bacteriorhodopsin, or sarcoplasmic reticulum Ca²⁺-ATPase which exchange much more slowly (53, 54). Our result means, according to the structural model (20), that more than half of the intramembrane residues are directly exposed to the solvent, as opposed to only the five amphipathic α-helices which were predicted. The absence of shift in the amide II band upon lyophilization is also consistent with the presence of a strong intraprotein network of hydrogen-bonded water. The presence of an aqueous pore in the human red cell membrane has long been postulated (55), but its exact location is unclear. Although it has been associated with band 3 protein or anion transporter (56), the possible implication of the band 4.5 (glucose transporter) was not excluded, as the evidence was mainly based on the effects on the water and anion permeability of some sulfhydryl reagents that are known also to act on the glucose transporter (57).

The exchange experiments also provide information about the localization of the different structures. An increase in the amount of exchange above 90% produced a shift to lower frequency in the α-helical band. This observation suggests that the 10-20% of slowly exchanging residues include a proportion of the α-helical structure, which is probably situated in a hydrophobic environment inside the membrane.

The effect of the binding of glucose and glucose derivatives was studied in order to obtain direct information about the conformational change which occurs during transport. At the concentrations used (200 mM), ethylidene-D-glucose and propyl glucoside should trap approximately 80% of the transporter in the outward-facing or inward-facing conformations, respectively (14, 17–19). The effect of temperature was also studied. Recent kinetic studies of transport in the intact erythrocyte have shown that the proportion of outward-facing transporters increases as the temperature is raised (12). At 20 °C, the temperature used for most of our study, about 80% of the transporter in the erythrocyte is in the inward-facing conformation in the absence of glucose, and about 70% of the transporter is in this conformation when complexed with glucose (12).

The spectra of both the transporter and the transporter in the presence of L-glucose, D-glucose, or propyl glucoside are

2 D. C. Lee and D. Chapman, unpublished observations.
the same. This is consistent with the fact that in all these circumstances the transporter is mainly in the inward-facing conformation. However, in the presence of ethylidene-D-glucose there is a shift to lower frequency in both the α-helical and β-structure bands, corresponding with a transporter in the outward-facing conformation. As may be expected, a large change in the protein secondary structure between the inward- and the outward-facing conformations does not occur. Nevertheless, changes in tertiary structure such as changes in the relative orientation of some α-helical segments cannot be excluded. These changes could also result in a small distortion in the α-helical or the β-structure, enough to produce the observed shifts.

There are no major changes in the spectrum when varying the temperature from 8 to 50 °C. However, a small shift to lower frequency in the main α-helical band can be detected when increasing the temperature. The shift is better appreciated in the fourth-derivative spectra. This shift, in the same direction but less intense than the one produced by ethylidene-D-glucose, may be related to the fact that the proportion of outward-facing transporters varies from about 10% at 8 °C to about 60% at 50 °C in the intact erythrocyte (12).

CONCLUSIONS

Second- and fourth-derivative FT-IR provide evidence that the human erythrocyte glucose transporter contains β-structure and random coil conformation in addition to the predominant α-helical structure. A portion of the α-helical structure is buried in a hydrophobic portion of the membrane, but most of the protein is freely accessible to the solvent. This accessibility contrasts with the behavior of other membrane proteins and is consistent with the presence of an aqueous channel in the protein, which could contribute to the water permeability of the human erythrocyte. The effects of sugar and sugar analogues as well as the temperature studies support the alternating conformation model for transport. However, the molecular basis of the conformational change that occurs during the process of transport between the outward- and the inward-facing conformations is still unclear.

REFERENCES