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Comparison of the kinetics and thermodynamics of the carrier systems for glucose and leucine in human red blood cells

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Kinetic data for the transport of glucose and leucine in human red blood cells are fitted to the conventional carrier model and the thermodynamics of the two carrier mechanisms are compared. In the absence of the carried molecule both carriers exist mainly in the inward-facing conformation at low temperatures and the outward-facing conformation at physiological or supra-physiological temperatures, this finding reflecting the strongly endothermic process involved in changing from the inward- to outward-facing forms. Reorientations from inward- to outward-conformations also involve substantial increases in entropy for both carriers. In contrast, substrate binding to the glucose carrier involves little change in enthalpy and an increase in entropy, while leucine binding is strongly exothermic and associated with a decrease in entropy. Application of transition state theory to glucose carrier kinetics reveals that the entropy of formation of the transition state of the carrier is much greater than that for the transition state of the carrier-glucose complex.

Introduction

Knowledge of the molecular structures and chemico-kinetic properties of membrane-bound proteins such as the $(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase in higher animals, the lactose and phosphotransferase transport systems of *Escherichia coli*, and bacteriorhodopsin has advanced rapidly in recent years leading to models by which these relatively complex energy-transducing systems appear to operate. Facilitated diffusion systems like the glucose carrier of the human red blood cell catalyse simpler, non-energy transducing transport processes but their detailed mechanisms tend to be more difficult to elucidate because they lack intermediates such as phosphorylated enzyme derivatives, which provide a useful entry point for mechanistic studies of systems like the $(Na^+ + K^+)$-ATPase. In consequence, most research into simple facilitated diffusion systems like the glucose carrier has been directed primarily at documenting and characterising the kinetics of transport and the affects of inhibitors, and at demonstrating which of the proteins in, for instance, the red cell membrane, is responsible for

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Abbreviations: maximum rates of transport under conditions of equilibrium exchange, zero trans influx and zero trans efflux are, respectively, V^{ee}, V_{oi}^{zt}, and V_{io}^{zt}. Michaelis constants (K_m) under conditions of equilibrium exchange, zero trans influx and zero trans efflux are, respectively, K^{ee}, K_{oi}^{zt}, and K_{io}^{zt}. V^{ee} and K^{ee} refer to measurements of transport of radiolabelled glucose when unlabelled glucose is at equilibrium across the cell membrane. Dissociation constants for inward- and outward-facing carrier conformations are, respectively, K_{si} and K_{so}.

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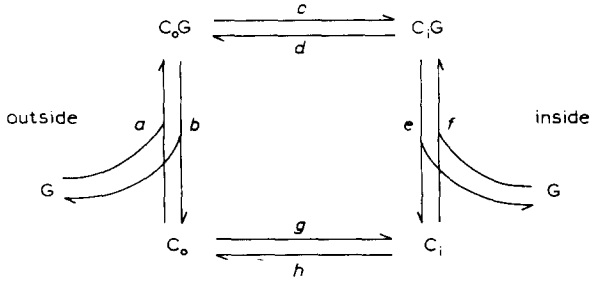
transport [1,2]. Detailed kinetic schemes have also been worked out for the four-state carrier model [3,4] but analyses of kinetic data in accordance with these has, until recently, made it possible only to put plausible ranges on the values of the rate constants governing the operation of, e.g., the nucleoside carrier [5], and to give estimates of the extent of the asymmetry of distribution of, e.g., the glucose carrier across the membrane [6].

More recently a better understanding of the mode of operation of the glucose carrier of the human red cell has been made possible by the determination of the amino acid sequence of the carrier protein [7] and the derivation of the individual rate constants which control carrier reorientation [8]. In this paper we show that our previous kinetic analysis of the glucose carrier [8] can be applied with similar success to the leucine carrier of the human red cell. In addition we show that the kinetic data can be used to derive detailed thermodynamic descriptions of both the glucose and leucine carrier systems, and discuss these in relation to the mechanisms of transport.

Model and Results

Derivation of the rate constants governing transport

The 4-state carrier model (Scheme I), where C_i and C_o represent the inward- and outward-facing forms of the carrier, G is glucose and a–h are rate constants, has been widely used to describe the operation of facilitated diffusion systems such as the carriers for glucose and nucleosides in human red blood cells. Provided that the rates of substrate association with and dissociation from the carrier are not rate limiting the kinetic equations derived from this model [3,4] simplify to the forms



Scheme I.

$$V_{oi}^{zt} = \frac{[C]}{(1/c + 1/h)}, \quad V_{io}^{zt} = \frac{[C]}{(1/d + 1/g)},$$

and

$$V^{ee} = \frac{[C]}{(1/c + 1/d)}$$

where [C] is the carrier concentration and each of the rate constants (c, d, g and h) has a temperature-dependence governed by the Arrhenius equation [9,10],

$$k = A \cdot e^{-E_A/RT}$$

here k is one of the rate constants (c, d, g, h), E_A is the Arrhenius activation energy and the pre-exponential term (A) can be considered constant within the temperature range studied.

The one assumption necessary to reach these simplified equations from those of, e.g., Lieb and Stein [4] is that the rates of glucose association with and dissociation from the carrier are substantially faster than the rates of carrier reorientation across the membrane. This can be expected if glucose binding to the carrier is an essentially diffusion-controlled process for which rates would be expected to be several orders of magnitude faster than the observed rates of carrier turnover. Furthermore the existence of a large (approx. 100-fold) trans-effect at 0 °C proves that, at least at this temperature, the rates of glucose binding to and dissociation from both the outward and inward-facing carrier conformations must be at least 100-times faster than the rate of reorientation of the unloaded carrier from inward to outward-facing conformations. Recent NMR studies [11] have in fact confirmed that binding and dissociation of glucose from the carrier are much faster than the overall maximum rates of transport.

In a previous paper [8] we showed that it was possible to obtain best fit values for the individual rate constants, c, d, g and h (and the corresponding transport rates) together with the dissociation constants, $K_{so}(=b/a)$ and $K_{si}=(e/f)$ from measurements of the rates of glucose transport into and out of human red blood cells under conditions of 'zero trans' influx and efflux and equilibrium exchange over a wide range of tempera-

TABLE I

THE RATES OF REORIENTATION OF THE GLUCOSE AND LEUCINE CARRIERS AND THEIR SUBSTRATE COMPLEXES AT 0 °C

Reorientation rates are the products of rate constants c, d, g and h ($= k$, s^{-1}) and the concentrations of the respective carriers [C] (mmol·(l cell water)$^{-1}$). Parameters were obtained by fitting transport data [8,12] to the conventional 4-state carrier model by the method of Lowe and Walmsley [8].

Rate constant	Glucose carrier		Leucine carrier	
	k[C](mmol·l^{-1}·s^{-1})	E_A (kJ·mol^{-1})	k[C](μmol·l^{-1}·s^{-1})	E_A (kJ·mol^{-1})
c	7.42 ±3.29	31.7±5.11	2.41 ±1.64	64.0±13.0
d	0.602 ±0.0231	88.0±6.17	0.236 ±0.0409	185 ±28.9
g	0.0809 ±0.00654	127 ±4.78	0.249 ±0.0588	93.8± 5.6
h	0.00484±0.000332	173 ±3.10	0.0185±0.00203	183 ± 7.65

tures. Few other membrane transport systems have been examined in the same detail as the glucose carrier, but Hoare [12] did make a comprehensive study of the temperature dependence of leucine transport in the human red cell, and we have also been able to fit Hoare's data [12] to the 4-state carrier model using the same non-linear regression techniques used for glucose transport [8]. The results of this analysis of leucine transport are given in Table I, which also gives rates of reorientation of the glucose carrier for comparison.

The exact procedures followed in order to obtain definite values for the rates of carrier reorientation have been described previously [8] but the key to finding the solutions is as follows. Near 0 °C the rates of equilibrium exchange transport of both glucose and leucine are very much more rapid than the 'zero trans' transport rates both into and out of the cell. Consequently measurements of 'zero trans' fluxes at low temperatures give very good approximations to rate constants g and h (the corresponding rates of carrier reorientation) and their associated activation energies, while the rates of 'equilibrium exchange' transport can be used to set lower limits to rate constants c and d at these low temperatures. The range of possibilities for the values of c and d (and their activation energies) is further restricted by the need to account for the curved Arrhenius plots for V^{ee} (determined by c and d), V_{oi}^{zt} (determined by c and h) and V_{io}^{zt} (determined by d and g). When these are taken into account non-linear least-squares fitting procedures converge to the solutions shown in Table I. The only other possible means of fitting the data to the 4-state carrier model is to invert the values of rate constants c and d at 0 °C. This leads to a negative activation energy for rate constant d, and the fitted parameters indicated in Table I are therefore unique in the sense that the alternative solution is physically unrealistic. It should be noted, however, that the fitted parameters in Table I and the following thermodynamic analysis do depend on the rates of substrate association with and dissociation from the carriers being much faster than the rates of carrier reorientation.

Carrier reorientation

Table II gives the enthalpy and entropy changes accompanying reorientations of the glucose and leucine carriers and their substrate complexes. The enthalpy changes were obtained as the differences in activation energies for the rate constant pairs, c/d and g/h, while the entropy changes were calculated from the relationship

$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$$

where the standard Gibbs free energies of carrier reorientation were obtained from the corresponding equilibrium constants ($\Delta G^\circ = -RT \ln K$, where $K = d/c$, g/h, $1/K_{so}$ or $1/K_{si}$. The corresponding basic Gibbs free energy changes are also given in Table II and were calculated from these data by the method of Hill [13]. These represent the differences in Gibbs free energy for the various carrier processes at near physiological concentrations of the carried molecules (5 mM glucose or 0.2 mM leucine at both surfaces of the transporting membrane). The basic Gibbs free

TABLE II

THERMODYNAMIC PARAMETERS FOR THE TRANSPORT SYSTEMS FOR GLUCOSE AND LEUCINE IN HUMAN RED BLOOD CELLS

Standard enthalpies and entropies were calculated as described in the text. Basic Gibbs free energies and the proportions of carrier states present were calculated for a glucose concentration of 5 mM or a leucine concentration of 0.2 mM. C_i and C_o are the inward- and outward-facing carrier states, while G and L represent glucose and leucine.

Glucose carrier	$\Delta H°$ ($kJ \cdot mol^{-1}$)	$\Delta S°$ ($J \cdot K^{-1} \cdot mol^{-1}$)	Basic Gibbs free energy change ($kJ \cdot mol^{-1}$)		Carrier state	Proportion present (%) at equilibrium	
			0 °C	37 °C		0 °C	37 °C
Substrate binding to the							
outward-facing carrier	5.51 ± 3.39	58.1 ± 24.0	1.7	1.2	C_o	4.1	28.1
inward-facing carrier	−4.95 ± 3.73	17.3 ± 21.9	2.3	3.3	C_i	68.9	42.3
Carrier reorientation from inside to outside							
unloaded carrier	46.0 ± 5.69	145 ± 21.0	6.4	1.05	C_oG	1.9	17.7
carrier-glucose complex	56.3 ± 8.01	185 ± 30.7	5.8	−1.05	C_iG	25.0	11.8
Leucine carrier	$\Delta H°$ ($kJ \cdot mol^{-1}$)	$\Delta S°$ ($J \cdot K^{-1} \cdot mol^{-1}$)	Basic Gibbs free energy change ($kJ \cdot mol^{-1}$)		Carrier state	Proportion present (%) at equilibrium	
			0 °	37 °C		0 °C	37 °C
Substrate binding to the							
outward-facing carrier	−31.7 ± 2.99	−64.7 ± 19.7	5.4	10.2	C_o	6.5	87.5
inward-facing carrier	−61.8 ± 3.32	−178 ± 12.8	6.1	15.2	C_i	87.0	10.7
Carrier reorientation from inside to outside							
unloaded carrier	89.2 ± 9.48	305 ± 35.1	5.9	−5.4	C_oL	0.60	1.7
carrier-leucine complex	120.9 ± 31.7	423 ± 117	5.2	−10.4	C_iL	5.9	0.033

energies were used to calculate the distributions of the carriers between the four possible states (free carrier and carrier-substrate complexes in outward- and inward-facing conformations) indicated in Table II.

Substrate binding affinities

Given that the rates of carrier-substrate complex formation and dissociation are not rate-limiting the Michaelis constants for transport via the 4-state carrier model are given by

$$K_{oi} = \frac{b}{a} \cdot \frac{(1+(g/h))}{(1+(c/h))}, \quad K_{io} = \frac{e}{f} \cdot \frac{(1+(h/g))}{(1+(d/g))},$$

$$\text{and } K^{ee} = \frac{b}{a} \cdot \frac{(1+(g/h))}{(1+(c/d))}$$

so that the values of the dissociation constants, $K_{so} = (b/a)$ and $K_{si} (= e/f = (b/a) \times [dg/ch])$ can be calculated from measured values of the Michaelis constants and the rates of carrier reorientation corresponding to rate constants c, d, g and h indicated in Table I. When this is done for a range of temperatures the enthalpies and entropies associated with substrate binding can be obtained from the van't Hoff equation

$$R \cdot \frac{d(\ln K)}{d(1/T)} = -\Delta H°$$

and using the relationship

$$-RT \ln K = \Delta G° = \Delta H° - T\Delta S°$$

Fig. 1 shows van't Hoff plots of the data for both carriers and Table II gives the derived values for the entropies and enthalpies of binding of glucose and leucine to the inward- and outward-facing conformations of their respective carriers.

Glucose transport and the transition state

Knowledge of the rate constants and activation energies associated with carrier reorientations

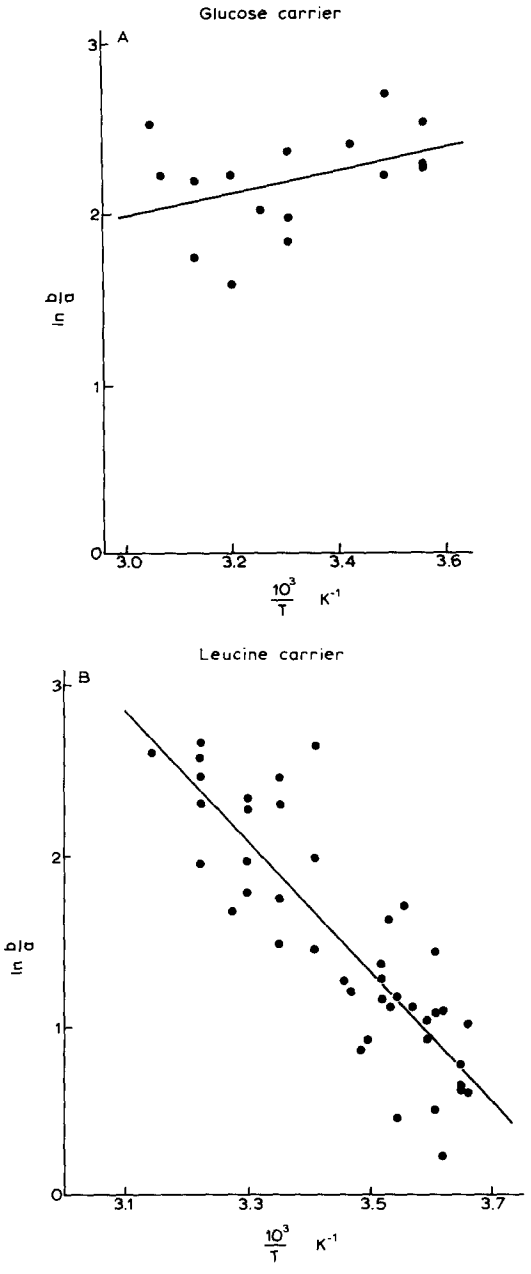


Fig. 1. Van't Hoff plots for the dissociation constants ($K_{so} = b/a$) for glucose and leucine from the outward-facing conformations of their respective carrier complexes.

makes it possible to calculate the Gibbs energy, entropy and enthalpy changes associated with transition states of the glucose carrier and its glucose complexes from the equations:

TABLE III

ENTHALPIES AND ENTROPIES OF ACTIVATION FOR CONFORMATIONAL TRANSITIONS OF THE GLUCOSE CARRIER AT 0 °C

	Rate constant	$\Delta H^{\ddagger}$ (kJ·mol^{-1})	$\Delta S^{\ddagger}$ (J·K^{-1}·mol^{-1})
Glucose-carrier complex	c	29.4±5.11	−78.2±23.3
	d	85.7±6.17	107 ±22.8
Free carrier	g	125 ±4.78	233 ±17.5
	h	170 ±3.10	379 ±11.4

$$E_A = \Delta H^{\ddagger} + RT$$

$$k = \frac{k_B T}{\hbar} e^{\Delta S^{\ddagger}/R} \cdot e^{-\Delta H^{\ddagger}/RT}$$

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$$

where k can be rate constant c, d, g or h, E_A is the corresponding activation energy, k_B is the Boltzman constant, T is the absolute temperature, $\hbar$ is Planck's constant, R is the gas constant and $\Delta G^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ are the standard Gibbs free energy, entropy and enthalpy changes associated with formation of the transition state [9,10]. The calculated thermodynamic parameters for the transition states of the loaded and unloaded glucose carrier at 0 °C and 37 °C are given in Table III, and Fig. 2 illustrates the changes in basic Gibbs free energy, enthalpy and entropy(×temperature) occurring during carrier turnover.

Discussion

Thermodynamics of substrate binding

Comparison of the thermodynamics of binding of glucose and leucine to their respective carriers reveals marked differences between the processes involved in binding the two substrates. In the case of glucose binding, the enthalpy change is very small (endothermic outside and exothermic inside) and there is a fairly large increase in entropy on binding. These findings are qualitatively at least in partial agreement with those of Zala et al. [14] who reported an endothermic apparent enthalpy of binding (which would include a contribution from the enthalpy of carrier reorientation because of the design of the direct calorimetric measure-

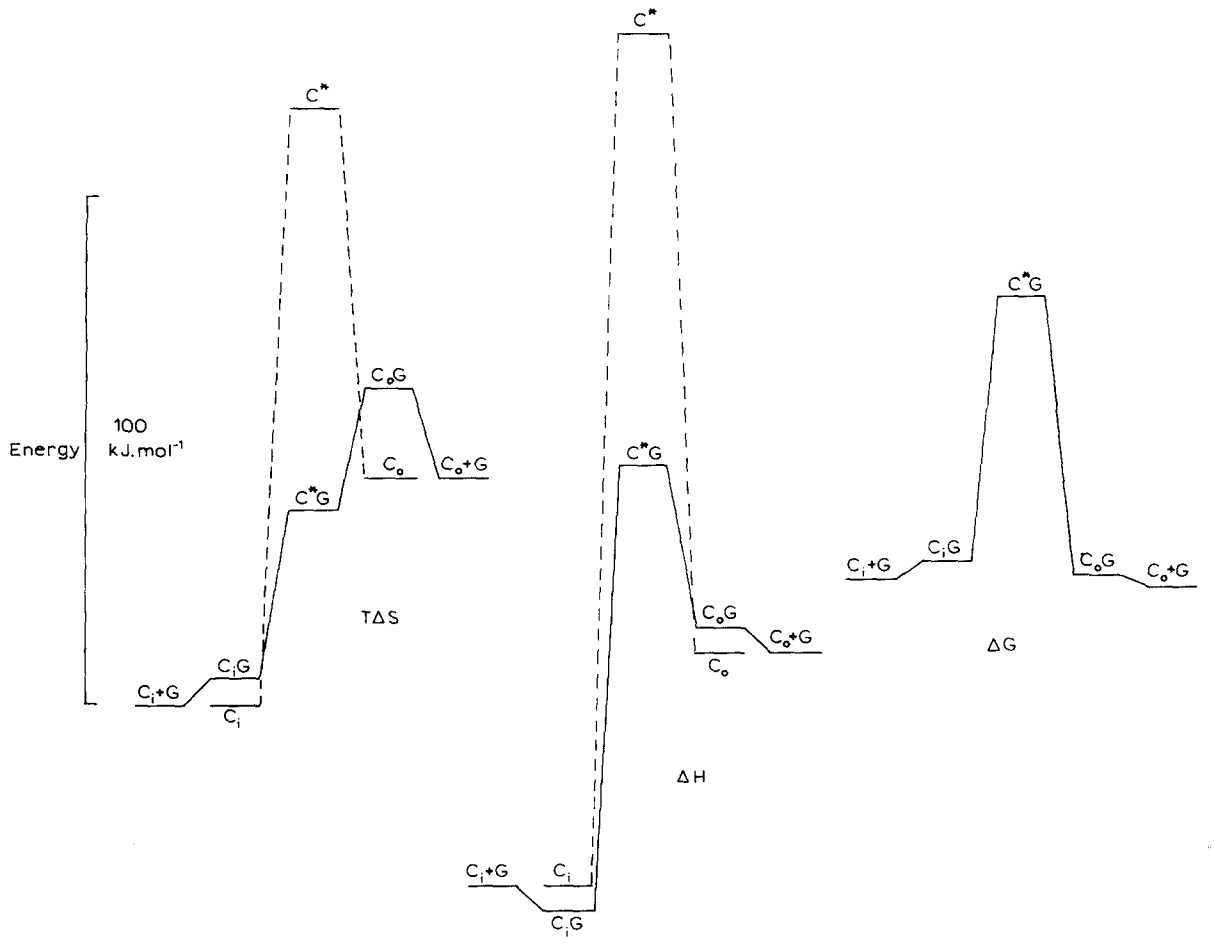


Fig. 2. Gibbs free energy, enthalpy and entropy diagrams associated with transport of glucose via the glucose carrier at 37 °C. Standard Gibbs free energies are shown except for glucose binding, for which basic Gibbs free energies (with glucose at 5 mM on both sides of the membrane) are given. Data were calculated from the dissociation constants of the glucose complexes of the inward- and outward-facing carriers, and the rate constants and activation energies for carrier reorientation using the transition state theory.

ments). Also Levine et al. [15], who measured the temperature dependence of the K_i for inhibition of sorbose transport in human red cells by glucose, found a small enthalpy of binding which was endothermic at low temperatures and exothermic at high temperatures.

At first glance the small enthalpy change for glucose binding to the carrier appears unexpected in view of the work of Barnett et al. [16] who showed that several hydrogen bonds (each presumably associated with an enthalpy change of up to about 20 $kJ \cdot mol^{-1}$) are involved in binding glucose to the carrier. However, the amino acid sequence of the glucose carrier [7] reveals that the largely hydrophobic membrane-spanning α-helices of the carrier contain groups of amino acid residues capable of hydrogen bonding (serine, threonine and asparagine residues) and these could well form part of the binding site for glucose and exist in a hydrated state in the absence of bound glucose. Since glucose is also hydrated in aqueous solution it seems at least possible that, during the binding process, water dissociates from both carrier and glucose. Thus glucose may effectively exchange hydrogen bonds with water for a similar number of hydrogen bonds with the carrier, in a process that would be consistent with the observed small net change in the enthalpy of binding. This

would also be consistent with the observed positive entropy of binding since the loss of translational freedom associated with glucose binding would be outweighed by the increased entropies arising from dissociation of water from both glucose and the carrier. A similar process appears to occur on binding of glucose to hexokinase [17].

The above interpretation constitutes a simple way of describing the chemical interactions that underlie the thermodynamic parameters governing glucose binding, but it should be born in mind that this analysis is not definitive and other explanations are possible. For instance glucose binding could lead to secondary changes in other parts of the carrier protein so that the observed enthalpy and entropy changes could represent the sums of the energies involved in two or more separate sets of bonding changes. However, we prefer the explanation presented because the reported rapid rates of carrier-glucose association and dissociation [11] seem more consistent with a simple binding process than a more complex event involving major changes in protein structure.

Leucine binding to both the inward- and outward-facing conformations of its carrier is strongly exothermic and associated with a large decrease in entropy (Table II), so processes very different from those described for glucose binding appear to be involved. It seems probable that leucine binding involves occupation of a hydrophobic pocket in the carrier, with displacement of water from the binding site balanced by loss of 'ordered' water when leucine leaves the aqueous phase. The way in which the carboxyl and amino groups of leucine are accommodated at the binding site is uncertain, but formation of two salt links between the zwitterionic form of the molecule and amino acid side chains in a hydrophobic region of the carrier could be expected to give a negative enthalpy change of about 24–36 $kJ \cdot mol^{-1}$ [18], which is similar to the calculated enthalpy of leucine binding to the outward-facing carrier (Table II).

Carrier reorientation

As shown in Fig. 3 the rates of carrier reorientation are such that both the glucose and leucine carriers (and their substrate-bound complexes) exist mainly in the inward-facing conformations at

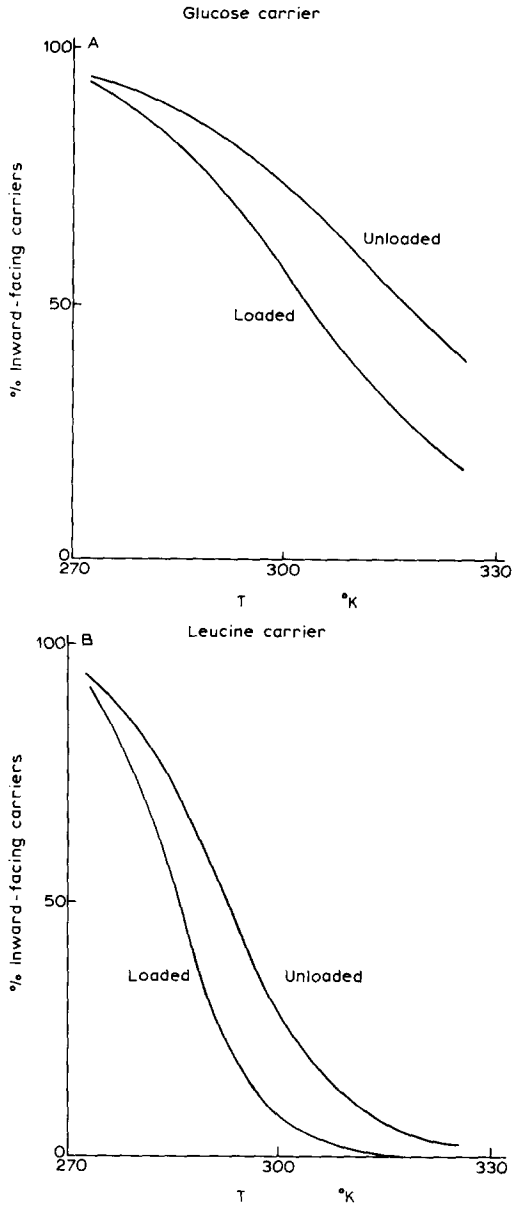


Fig. 3. The effect of temperature on the transmembrane orientations of the glucose and leucine carriers and the carrier-substrate complexes. The curves represent the states of the carriers at equilibrium in the absence of substrate, and with substrates present at saturating concentrations. Curves were calculated from the rate constants (*c, d, g, h*) and activation energies given in Table I.

0 °C, whereas the outward-facing conformations are favoured by increasing temperature and, to some extent, by binding of the carrier substrate. Temperatures at which inward- and outward-fac-

ing conformations are equally populated are 44.5 °C for the unloaded glucose carrier, 30.8 °C for the glucose-carrier complex, 19.4 °C for the unloaded leucine carrier and 12.5 °C for the leucine-carrier complex. These temperature-dependent distributions of carrier states arise from the large endothermic enthalpy and positive entropy changes associated with reorientations from inward- to outward-facing conformations, with outward-facing conformations predominating only when the temperature is sufficiently high for the Gibbs free energy to be dominated by the entropy term.

Notably, under near physiological conditions (37 °C with 5 mM glucose on both sides of the membrane) the basic Gibbs free energies are such that all four states of the glucose carrier are present as substantial proportions of the total carrier population (Table II). This may be significant in that the carrier system is poised to respond to any change in the (external) glucose concentration by re-equilibrating glucose across the membrane by net transport in either direction. Although the red cell itself metabolises glucose very slowly, rapid outward transport may be of physiological importance in supplying glucose to the brain from the red cells (via the plasma), while rapid inward transport may be significant at the placenta where transport into fetal red cells provides an additional sink for glucose. In contrast the basic free energy calculations for the leucine carrier indicate that, at equilibrium, the outward-facing free carrier predominates under the (near physiological) conditions chosen. This may reflect the fact that the leucine carrier normally functions as a system for carrying leucine into cells especially in the liver where the portal blood may contain much higher leucine concentrations after a meal. Naturally, under conditions when net transport is occurring the equilibrium distributions of both carriers will differ from those indicated in Table II.

It is not possible to establish the types of chemical interaction underlying carrier reorientation with any certainty, but the large positive entropies accompanying changes from inward- to outward-facing conformations indicate that the glucose and leucine carriers exist in more ordered (perhaps more highly hydrated) states when facing inwards, and intramolecular interactions of some kind could make a contribution to this. In the case of the glucose carrier the bonds stabilising the inward-facing carrier conformations are associated with enthalpy changes equivalent in energy to a few hydrogen bonds or salt bridges. One interesting possibility is that salt bridges between ionised amino acid residues in the globular (intracellular) part of the carrier molecule could be responsible for determining the orientation of the intra-membrane parts of the carrier (and hence presumably the glucose binding site). In addition interactions between charged amino acid residues and lipid head groups would provide a molecular basis for the observation that the turnover rate of the glucose carrier in lipid vesicles is dependent on the type of phospholipid present [19].

In the case of the leucine carrier the enthalpy and entropy changes associated with carrier reorientation are similar in sign, but much larger than, those for the glucose carrier. The chemical changes underlying carrier reorientation may therefore be qualitatively similar for the two carriers. It is also possible that the large enthalpies involved in conformational changes of the leucine carrier may be overestimates since the measured rates of leucine transport were low (in relation to rates of passive diffusion of leucine) and transport data for leucine are therefore probably less reliable than those for the much more rapidly transported glucose.

Glucose transport and the transition state

An important outcome of the application of transition state theory to the kinetics of glucose transport is that formation of the transition state for the unloaded carrier is strongly endothermic and associated with a large increase in entropy, whereas these enthalpy and entropy changes are much smaller for the carrier-glucose complex (Table III, Fig. 2). The substantial endothermic enthalpy changes indicate that attainment of the transition state of the free carrier (with the carrier poised 'half way' between its inward- and outward-facing conformations) involves breakage of bonds, while the large increases in entropy on formation of the transition state would be consistent with a major contribution from breakage of hydrogen bonds between water and the carrier, and consequent dissociation of water. Some of this

water could well be bound to the carrier at the glucose binding site since it would be displaced by glucose and hence could at least partially account for the lower entropies and enthalpies associated with the transition state of the carrier-glucose complex. In addition their relative entropies suggest that the inward-facing carrier and carrier-glucose complex may be more highly hydrated than the outward-facing forms. Some evidence for the importance of water in the binding of glucose to the carrier is provided by the finding that high concentrations of ammonium sulphate substantially increase the affinity of the carrier for glucose [20]. Withdrawal of water by this salt would be expected to facilitate formation of the carrier-glucose Michaelis complex by removing water from the glucose binding site, and might also stabilise the transition state thereby giving rise to an 'occluded' glucose complex comparable to the 'occluded' rubidium complex described for the $(Na^+ + K^+)$-ATPase [21]. A change in the carrier conformation allowing strengthening of hydrogen bonds with bound glucose may also contribute to the fact that the enthalpies of formation of the transition state of the carrier-glucose complex are much smaller than those for the transition state of the free carrier (Table III).

The thermodynamics of conformational transitions of the leucine carrier cannot be analysed in so much detail because the number of carriers per red cell, and hence the rate constants for the conformational changes associated with transport transitions are not known. However, the pattern of activation energies for the carrier transitions is similar to that for the glucose carrier and it seems reasonable to suggest that the transition state of the leucine carrier may be stabilised in an analogous way.

Conclusion

The overall picture emerging from this kinetic and thermodynamic analysis of the glucose carrier is as follows. Transport appears to involve (i) binding of glucose to and dissociation of water from hydrogen-bonding residues at sites (either at the inside or outside, but not simultaneously at both sides of the membrane) formed by the conjunction of some of the membrane-bound α-helices of the carrier molecule, (ii) reorientation of these α-helices, leading to a transition state in which hydrogen bonds between the carrier α-helices and the glucose are strengthened at the expense of bonds (such as salt bridges) elsewhere in the carrier, and (iii) a further reorientation of the α-helices such that the glucose molecule reaches the binding site at the opposite side of the membrane. Perhaps the most important feature of this scheme is that, in reaching the transition state, the carrier α-helices undergo a rearrangement which both strengthens the hydrogen bonds with the bound glucose and occludes the glucose within the membrane. Many features of this scheme may well be generally applicable to other carrier systems since the galactose carrier of *E. coli* may have substrate binding sites involving transmembrane α-helices [22] and the $(Na^+ + K^+)$-ATPase involves occluded complexes of both sodium [23] and potassium [21].

In addition to the transport-related events discussed above the conformation of the glucose carrier (with or without bound substrate) appears to be influenced by other factors (intra-carrier bonds and/or hydration state) which favour an inward-facing conformation at low temperature and roughly equal proportions of inward- and outward-facing carriers at physiological temperatures. One possibility is that the bonds stabilising the inward-facing conformation are salt bridges in the globular (cytoplasmic) part of the carrier molecule (or perhaps between the carrier and the headgroups of membrane lipids), which could have the function of holding the intra-membrane parts of the carrier in one of two distinct conformations. These inward- and outward-facing carrier conformations can be regarded as analogous to the tight and relaxed (T- and R-) forms of haemoglobin and other proteins involved in metabolic regulation (such as phosphofructokinase) or energy transduction (such as myosin). This possibility is interesting in relation to active transport systems such as the $(Na^+ + K^+)$-ATPase since it provides a mechanism by which phosphorylation or binding of ATP to the globular part of the carrier could influence the properties of the intra-membrane domain and hence drive the active transport process.

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