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Lipid and temperature dependence of the kinetic and thermodynamic parameters for active amino acid transport in *Escherichia coli* K1060

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The influence of membrane physical state on the kinetic and thermodynamic parameters for the active transport systems for two amino acids has been investigated in *Escherichia coli* K1060, an unsaturated fatty acid auxotrophic mutant. The apparent Michaelis constant (K_m) for the uptake of L-[^{14}C]glutamine (0.05 to 0.08 μM) or L-[^{14}C]proline (1 μM approx.) is invariant with temperature for this mutant grown on elaidate (18:1*t*), palmitelaidate (16:1*t*), oleate (18:1*c*), palmitoleate (16:1*c*) and linoleate (18:2*c*,*c*). Arrhenius plots of the maximum velocities (V_{max}) for L-glutamine transport in cells grown on 16:1*t*, 18:1*c* and 16:1*c* are biphasic within a limited temperature range peculiar to each UFA supplementation. Above an upper temperature limit also displayed by 18:1*t* and 18:2*c*,*c*-cells, V_{max} decreases with temperature. A characteristic temperature (T_b) marks the point of intersection of the biphasic slope of the Arrhenius plots, and activation energy (E_a) is lower above than below T_b. Differential thermal analysis considered with membrane lipid fatty acyl profiles indicates that the upper temperature limit is governed by both membrane lipid acyl chain fluidity and heterogeneity, while T_b is governed by fluidity alone. Data on L-proline transport V_{max} are similar, but the upper temperature limit and T_b are each shifted to lower temperatures relative to L-glutamine. We suggest that membrane defects related to energy-coupling and caused by abnormal fluidity and physical state are responsible for the peculiar temperature dependences of V_{max} for these active transport processes.

Introduction

The state of the membrane polar lipid bilayer affects membrane-associated processes in prokaryotes [1–10] and eukaryotes [11–18]. Most data on the effects of membrane physical state on energy-dependent transport processes in microorganisms have been derived by measuring initial rates of transport at varying temperatures and a fixed, supposedly 'saturating', level of substrate [1–6]. Arrhenius-type treatment of such data has yielded characteristic break temperatures depending on the fatty acid enrichment of the membrane [1–5]. Such data, for *Escherichia coli,* have been used to arrive at different conclusions involving, in the one case, an effect of the membrane physical state on the translocation step of transport [2], and in the other case, an effect on the number of functional carrier proteins [3].

The origin of breaks in Arrhenius plots of membrane-associated reactions has been a matter

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Abbreviations and symbols: E_a, activation energy; M63, medium 63; T_b, Arrhenius plot break temperature; 18:1*t*, elaidate; 16:1*t*, palmitelaidate; 18:1*c*, oleate; 16:1*c*, palmitoleate; 18:2*c*,*c*, linoleate.

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of controversy [19–22]. A change in the Michaelis constant (K_m) with temperature has been reported to be a possible cause of some of these breaks [19,21] in cases where K_m increases as temperature increases, thus underscoring the need to study maximum velocities (V_{max}) rather than initial rates.

The transport system for L-glutamine in *E. coli* is sensitive to mild osmotic shock, while that for L-proline is not [23,24]. The L-proline transport is tightly membrane-bound [23,24] and is energized by the protonmotive force ($\Delta\bar{\mu}_{H^+}$) [23,24] as defined by Mitchell [25–30]. L-Glutamine transport, on the other hand, depends on periplasmic binding protein(s) as well as a membrane-bound component [23,24,31] and is energized by adenosine triphosphate per se or a related phosphate [23,24]. In microorganisms, membrane phase state effects have been reported mainly on the protonmotive force-driven transport of sugars [1,3–5], and amino acids [2,6], as well as on the phospho*enol*-pyruvate-dependent group translocation of sugars [2,4] in *E. coli.* These effects have also been studied for passive permeation and facilitated diffusion of non-electrolytes in *Acholeplasma laidlawii* B [7,8].

Reported here are the effects of membrane phase state on the K_m and V_{max} as well as on the activation energies (E_a) for [^{14}C]glutamine uptake in *E. coli* K1060, an unsaturated fatty acid auxotroph. This is a more detailed study than that previously reported by Rosen and Hackette [32] on L-glutamine transport in *E. coli.* Results on [^{14}C]proline uptake are also reported in this strain. Our results suggest that interpretation of data derived from studies on the effects of membrane phase state on transport should take cognizance of the complex reactions involved in the energy-coupling to transport, especially in whole cells of *E. coli.*

Materials and Methods

Non-radioactive supplies

Media and non-radioactive chemicals were purchased from sources previously reported [33].

Radioactive supplies

Uniformly labelled L-[^{14}C]proline and L-[^{14}C]glutamine were purchased from New England Nuclear Corporation, Boston, MA, U.S.A. The [^{14}C]proline came in three batches: batch 1, used for preliminary experiments, had a specific activity of 261 mCi/mmol and was 99% pure according to its radiochemical specifications, while batch numbers 2 and 3, used for the main experiments, had a spec. act. of 255.0 mCi/mmol and were each 99.2% pure. The [^{14}C]glutamine was supplied in four batches: batch number 1, used for preliminary experiments had a spec. act. of 213.4 mCi/mmol and was specified as 98.2% pure, while numbers 2 to 4 each had a spec. act. of 251.4 mCi/mmol and a radiochemical purity of 98.8%.

[^{14}C]Glutamine batch numbers 2 and 3 were checked by paper chromatography with authentic L-glutamine and L-glutamic acid standards in two different solvent systems (Ref. 34, and see New England Nuclear quality control brochure). This was followed by paper radiochromatography on a Nuclear Chicago Actigraph III Paper Radiochromatograph equipped with a chart recorder. The purity of these two [^{14}C]glutamine batches was found to conform to the radiochemical specifications by these procedures. These and the other radiochemicals were used without further purification.

Other chemicals

All other standard chemicals were of the highest purity available and were purchased from standards sources. They were used without further purification.

Bacterial strain and growth conditions

E. coli strain K1060, an unsaturated fatty acid auxotrophic mutant, was the generous gift of Dr. David Silbert of University of Washington School of Medicine, St. Louis, U.S.A. Its genotypic and phenotypic characteristics are as previously reported by us [33,35,36]. The cultures were routinely grown with shaking in medium 63 (M63) supplemented with glycerol as carbon and energy source and with the appropriate unsaturated fatty acid as detailed previously [33,35,36].

For the transport experiments an initial 24 h starter culture was diluted 10-fold to form the final starter, which was then grown for another 12 h (approx.). The working culture was a 50-fold dilution of the final starter. This working culture

was typically 300 ml in a 2 litre Erlenmeyer flask. The cells were harvested within the exponential phase ($A_{550} = 0.45–0.6$, as read on a Bausch and Lomb Spectronic 20 spectrometer).

Protein assay

Protein was assayed essentially according to the method of Lowry et al. [37] with bovine serum albumin as standard.

Liquid scintillation cocktail (toluene-based)

This was prepared by stirring overnight a mixture of 15.748 g of 2,5-diphenyloxazol (PPO) and 0.210 g of 1,4-bis[2-(2-methyl-5-phenyloxazolyl)] benzene (POPOP) in 4 litres of toluene [31].

Preparation of cells and [^{14}C]glutamine uptake assays

Cells were harvested by centrifugation at room temperature in a Sorval RC-2B centrifuge at 15 000 × *g* for 5 min. The pellet was washed two times by resuspension in M63 buffer (without any nutrients or other supplementations) and centrifuged at 15 000 × *g* for 5 min each time. The final pellet was then resuspended in M63 buffer to a concentration of 1 g wet cells to 20 ml M63 buffer (1 : 20 cell suspension). Graded dilutions (1 : 40, 1 : 80, 1 : 160, 1 : 320 and 1 : 640 suspensions) of this stock (1 : 20) suspension were made and these were stored at room temperature with occasional stirring to prevent clumping.

Immediately before uptake studies, to a 500 μl aliquot of cell suspensions were added 10 μl of 1 M glucose in M63 buffer and 20 μl of 0.2 g% chloramphenicol in M63 buffer. This mixture was then incubated at the desired working temperature for exactly 2 min. A 20 μl aliquot of this treated cell suspension was then quickly mixed with a rapidly stirred 485 μl of [^{14}C]glutamine solution of the appropriate concentration in M63, which contained glucose and chloramphenicol at the same level as in the cell suspension, and which had been pre-incubated at the same working temperature. A sample (200 μl) of this transport assay mixture (total volume 505 μl) was quickly withdrawn at two appropriate time intervals and filtered by suction through a 24 mm nitrocellulose filter (type HA, 0.45 μm pore diameter, Millipore Corporation, Bedford, MA, or Matheson Higgins Incorporated, Woburn, MA) which had been pre-soaked in M63. The trapped cells were immediately washed by suction with 10 ml M63. The filter was then dried over a hot-plate maintained at 40 °C (approx.). The filtration apparatus was a ten-place microfilter assembly equipped with a pressure gauge (Hoffer Scientific Instruments, San Francisco, CA).

During studies on the temperature-dependence of glutamine uptake, an appropriate cell suspension and appropriate sampling times were chosen such that not more than 10% (or about 20% in some cases, see Results) of the total available radioactivity was accumulated in the cells in order to guard against the depletion of substrate. These sampling times ranged from 6.5 s at high temperatures to 3 min at low temperatures.

Liquid scintillation counting

These filters were collectively dried further in scintillation vials in an oven at 50 to 60 °C for 1 h (approx.) before counting in 5 ml of the toluene-based scintillation occktail [31], using any of three Beckman Liquid Scintillation System models (LS-200B, LS-230 and LS-330).

Cells poisoned in energy metabolism (see later) and assayed for transport in the same way as normal cells did not show significantly higher radioactivity (cpm) than control runs. The control runs consisted of filtering and washing 200 μl of the [^{14}C]glutamine or [^{14}C]proline solution in the absence of cells. These controls were run routinely and the experimental cpm at the appropriate [^{14}C]glutamine concentration corrected by subtracting the control value.

The counting efficiency of the liquid scintillation counter was routinely checked by counting in duplicate a known amount of radioactivity (disintegrations per minute, dpm), usually 10 μl of each [^{14}C]glutamine solution, dried on the nitrocellulose filter. This was used to correct the sample cpm during the calculation of the initial rate of transport, i.e., mmol glutamine accumulated per min per mg cell protein.

Preparation of cells and [^{14}C]proline uptake assay

Cells were prepared and transport assays done exactly as detailed for glutamine transport studies above. Scintillation counting was also performed as given for [^{14}C]glutamine uptake.

Energy-poisoning methods employed

Method A. A suspension of K1060 cells grown in the presence of 18 : 1*c* was made 30 mM in NaN_3 and 1 mM in iodoacetate (Na^+ salt) [38]. Chloramphenicol was added to a final concentration of 80 μg/ml and the mixture was incubated at room temperature for 30 min. These cells were used as controls during the glutamine or proline transport assay without any further centrifugation.

Method B. A suspension of K1060 cells grown as for method A above was made 40 mM in NaN_3 and 20 mM in α-methyl glucoside [39]. Chloramphenicol was added as in method A above. The suspension was then incubated at 37°C for 60 min. These energy-poisoned cells were used as controls during the glutamine or proline transport assay without further centrifugation.

Results

[^{14}C]Glutamine and [^{14}C]proline uptake

By adequate manipulation of cell concentration and sampling time as temperature was varied, as much as 10% of the total available radioactivity could be accumulated in the case of [^{14}C]glutamine, without any appreciable change in the initial rate of uptake measured. Subsequently, two sampling times were employed, allowing for less than 10% depletion of radioactivity. However, for mutant K1060 grown in the presence of 18 : 2*c*,*c* or 18 : 1*t*, reproducible results were obtained only when as much as 20% of the total available radioactivity was accumulated. Under these conditions samples removed at the two time intervals gave the same initial rate of uptake.

In the case of [^{14}C]proline uptake, very reproducible initial rates were achieved in all instances at any single temperature at much less than 10% depletion of the total available radioactivity in the reaction mixture. The reason for this may be the fact that the apparent K_m for proline uptake and, therefore, the concentrations employed in the assays, are 10-times higher than in the case of the glutamine transport system.

The apparent K_m (henceforth called K_m) and V_{max} values for L-glutamine transport were derived from Hane's plots [31,40] of the variation of the ratio of substrate concentration and initial velocity, [S]/v, with substrate concentration, [S]. The concentration range employed was 0.05–0.5 μM. Fig. 1A embodies some data from K1060 enriched in palmitoleic acid (16 : 1*c*) showing the linearity of these plots at several temperatures. Data not included here indicated that determinations done with L-glutamine concentrations above 1 μM yielded a second (higher) K_m value at the high substrate concentrations. Fig. 1A and Table I also demonstrate that K_m for L-glutamine transport is invariant with temperature and Table I shows that it is reasonably constant with unsaturated fatty acid supplementation, the values being close to 0.08 μM published for *E. coli* strain 7 [31].

For the L-proline transport system, the substrate concentration range employed was 0.1–5.0 μM L-proline. The linearity of the Hane's plots over these concentrations is shown in Fig. 1B for 16 : 1*c*-enriched K1060 cells.

The mean K_m for L-proline uptake at the various unsaturated fatty acid supplements and temperatures (Fig. 1B and Table I) is approx. 1 μM. This K_m closely resembles 0.6 μM for membrane vesicles [41] and 0.44 μM for whole cells [42] of *E. coli* strain W3092. The K_m values reported by Wood and Zadworny [43] for *E. coli* strain CSH4 are much lower, possibly due to strain differences.

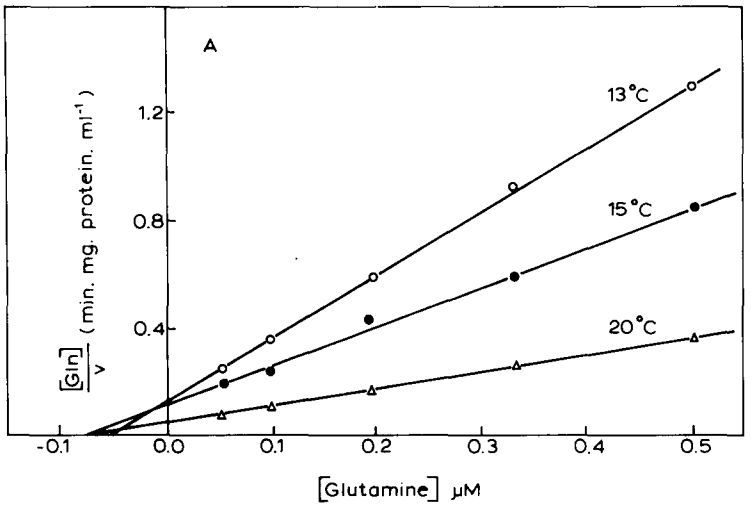
Temperature-dependence of K_m and V_{max} for [^{14}C]glutamine and [^{14}C]proline uptake

The K_m for L-glutamine transport as well as that for L-proline transport in mutant K1060 remained reasonably constant at every unsaturated

TABLE I

MEAN K_m VALUES OVER THE ASSAY TEMPERATURE RANGES FOR [^{14}C]GLUTAMINE AND [^{14}C]PROLINE UPTAKE IN *E. COLI* K1060 GROWN WITH DIFFERENT UNSATURATED FATTY ACID SUPPLEMENTS

Transport substrate	Cells		K_m (μM) (mean ± S.D.)	Temperature range (°C)
L-Glutamine	K1060:	18 : 1*c*	0.050 ± 0.013	9.0–15
		16 : 1*c*	0.061 ± 0.013	11.0–17.5
		18 : 2*c*,*c*	0.076 ± 0.009	17.4–29
L-Proline		16 : 1*t*	1.11 ± 0.33	14.5–38
		16 : 1*c*	1.06 ± 0.22	7.0–41
		18 : 1*c*	0.66 ± 0.19	3.0–38
		18 : 2*c*,*c*	0.66 ± 0.12	5.0–40



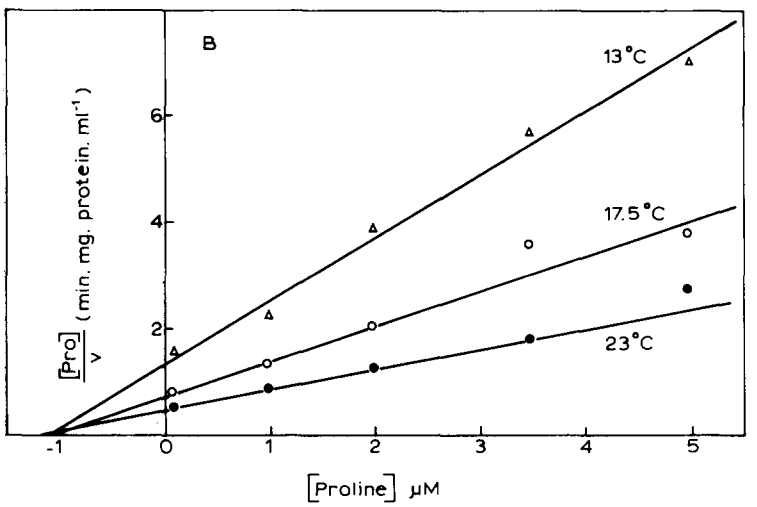


Fig. 1. Hane's treatment of data for the calculation of K_m and V_{max} values for L-[^{14}C]glutamine (A) and L-[^{14}C]proline (B) uptake at various temperatures in palmitoleate (16:1*c*)-enriched *E. coli* K1060 cells. Assays were performed as detailed in Materials and Methods.

fatty acid supplementation within the temperature ranges employed (see Table I).

The V_{max} for either glutamine or proline transport increases with temperature only up to an upper temperature limit. The value of this upper temperature limit for either of the two systems is peculiar to each unsaturated fatty acid supplement and is always below the growth temperature of 37°C (39°C for 18:1*t*-supplemented cells). Above the upper temperature limit, V_{max} starts to decrease with temperature (or otherwise to become aberrant) until at some higher temperature, the transport kinetics no longer follow the Michaelis-Menten equation [40]; in other words, the data cannot be treated in conventional fashion to yield V_{max} and K_m values. The absolute amount of radioactivity accumulated per unit time per unit cell protein within the initial rate period at each glutamine or proline concentration actually decreases with temperature above the upper temperature limit.

Below the upper temperature limit, and down to the minimum temperature at which assays were done, data derived from 16:1*t*-, 18:1*c*- and 16:1*c*-cells yield (biphasic) linear Arrhenius plots (Fig. 2A and B) each of which has a break at a

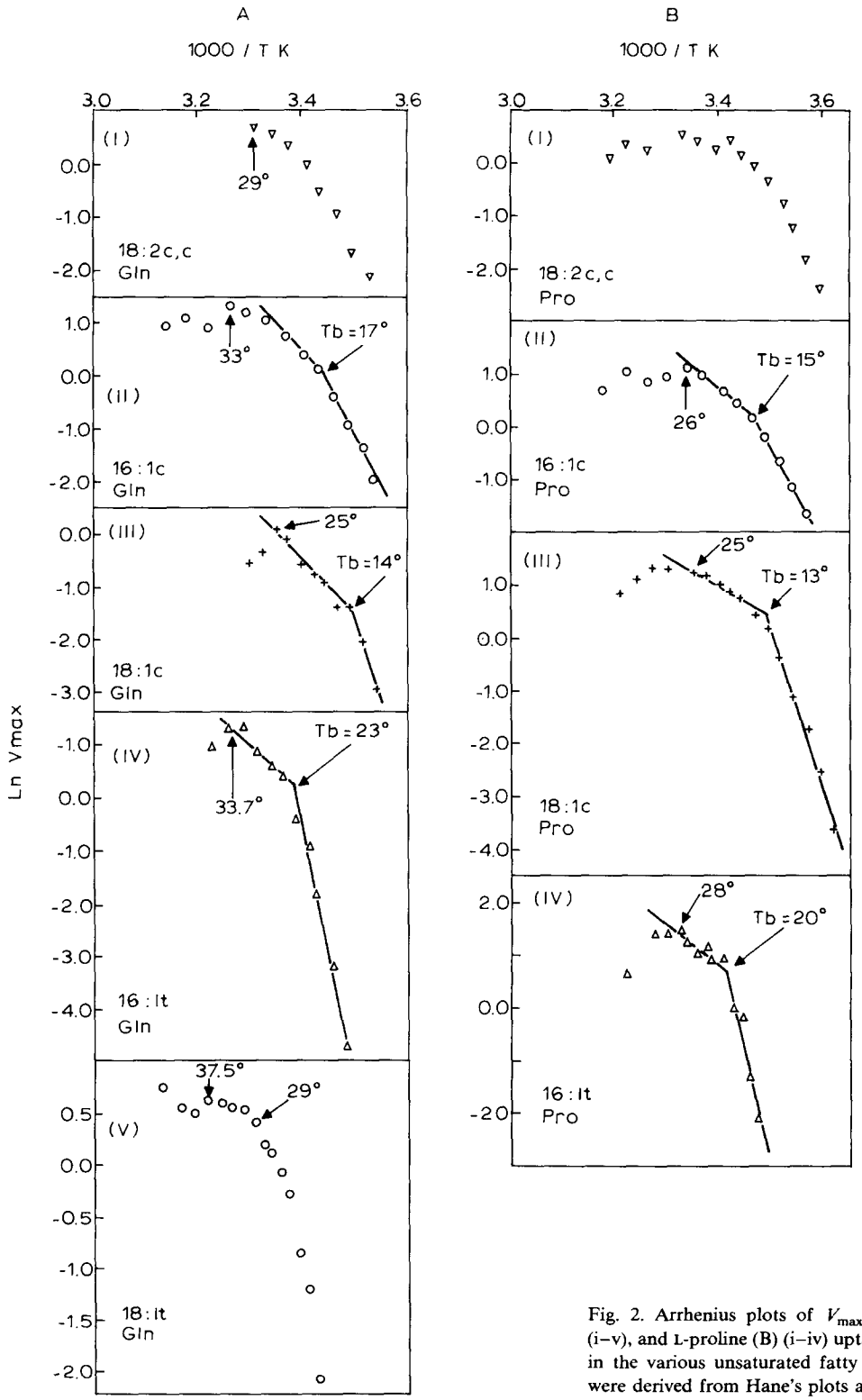


Fig. 2. Arrhenius plots of V_{max} values for L-glutamine (A) (i–v), and L-proline (B) (i–iv) uptake in *E. coli* K1060 enriched in the various unsaturated fatty acids indicated. V_{max} values were derived from Hane's plots as given in Fig. 1.

TABLE II

ARRHENIUS PLOT TEMPERATURE BREAKS (T_b), ACTIVATION ENERGIES (E_a) AND TEMPERATURES AT WHICH V_{max} STARTS TO DECREASE WITH TEMPERATURE (UTL) FOR L-GLUTAMINE AND L-PROLINE TRANSPORT IN RELATION TO MEMBRANE UFA (UNSATURATED FATTY ACID) ENRICHMENT (mol%) AND PHASE TRANSITION RANGES (T_s TO T_l) AND MIDPOINTS (T_m) IN *E. COLI* K1060

Derived from Fig. 2A and B except as otherwise indicated.

Cells	UFA supplement	UFA supplement incorporated into membrane (mol%) [a]	Transition range T_s to T_l (°C)	Temp. at which 50% of membrane lipids are liquid crystalline T_m (°C) [a]	T_b (°C)		UTL (°C)		Activation energy, [a] E_a (kcal/mol)			
									Above T_b		Below T_b	
					glutamine	proline	glutamine	proline	glutamine	proline	glutamine	proline
E. coli	18:1*t*	66–71	+29 to +40	37	29	n.d. [b]	37.5	n.d. [b]				
K1060	16:1*t*	80–85	+10 to +34	27	23	20	33.7	28	17	14	85	80
	18:1*c*	52–58	+2 to +21	12.5	14	13	25	25	20	10	60	66
	16:1*c*	39–44	−10 to +20	7	17	15	33	26	18	14	40	40
	18:2*c*,*c*	48–50	−16 to +22	−2			29	27				

[a] Derived from Refs. 35 and 36.

[b] n.d., not determined.

characteristic temperature (T_b). For L-glutamine transport, T_b values are respectively 29°C, 23°C, 14°C and 17°C for 18 : 1*t*-, 16 : 1*t*-, 18 : 1*c*- and 16 : 1*c*-enriched cells, while for L-proline transport they are 20°C, 13°C and 15°C for 16 : 1*t*-, 18 : 1*c* and 16 : 1*c*-enriched cells, respectively (Table II). The Arrhenius plot (Fig. 2A(i) and 2B(i) for either glutamine or proline transport in 18 : 2*c*,*c*-enriched K1060 cells, however, shows a rather gentle curve below the upper temperature limit.

For both L-glutamine and L-proline transport, the activation energy (E_a) below T_b, as shown in Table II, is higher for K1060 enriched in the *trans*-unsaturated fatty acid, 16 : 1$_t$, which is higher-melting, than for this auxotroph enriched in the lower-melting *cis*-unsaturated fatty acid species. A similar behaviour was reported for the sodium-independent component of α-aminoisobutyric acid uptake in Ehrlich ascites cells [17], in which the E_a below T_b was higher the more the saturated fatty acid content of the membranes. Generally also (see Fig. 2A and B; Table II), E_a below T_b is higher than above it, but no definite discernible trend exists in these E_a values above T_b with respect to fatty acid supplementation. A striking similarity exists between E_a below T_b for L-glutamine transport and that for L-proline transport in the same cell type (Table II). For instance, for K1060 enriched in 16 : 1*t*, the values are 85 and 80 kcal/mol, respectively, for glutamine and proline uptake. Also, the E_a above T_b is higher for L-glutamine than for L-proline transport at all unsaturated fatty acid supplements (Table II).

Fig. 2A(v) is the rather peculiar Arrhenius plot of V_{max} for glutamine transport in 18 : 1*t*-enriched K1060 cells. The plot shows the general phenomena seen in the other K1060 cases. It also resembles that published for β-glucoside transport by Linden et al. [1] for *E. coli* 30Eβox$^-$, another unsaturated fatty acid auxotroph.

The most conspicuous difference between the data for glutamine transport and those for proline transport is that in each case, for K1060 grown with various unsaturated fatty acid supplements (except for the upper temperature limit for 18 : 1*c*-enriched cells), both the upper temperature limit and the T_b values observed for proline are shifted to lower temperatures with respect to these same parameters for glutamine transport (Table II).

Discussion

The data presented herein indicate a number of similarities while highlighting, at the same time, some striking differences between the binding protein-dependent L-glutamine transport and the energized membrane- (i.e., protonmotive force-) dependent L-proline transport in *E. coli* K1060. The results clearly demonstrate a profound dependence of the maximum velocity (V_{max}) of each of L-glutamine and L-proline transport on the physical state of the membrane lipids. The apparent Michaelis constant (K_m) is invariant with temperature and is reasonably unchanged with unsaturated fatty acid supplementation. These same observations on the V_{max} and K_m are also true for L-proline transport in K1060.

The transport activation energy (E_a) values in Table II demonstrate that, for both the L-glutamine and the L-proline transport, E_a below T_b (the break temperature of the biphasic Arrhenius plot) is higher for mutant K1060 enriched in the *trans*-unsaturated fatty acid, 16 : 1*t* (palmitelaidate), which is higher melting, than for this auxotroph enriched in the lower-melting *cis*-unsaturated fatty acid species. Similar behaviour was reported for the sodium-independent component of α-aminoisobutyric acid uptake in Ehrlich ascites cells [17] in which E_a below T_b was higher the more the saturated fatty acid content of the membranes. Generally also (see Fig. 2A and B; Table II), E_a below T_b is higher than above it, but no definite discernible trend exists in these E_a values above T_b with respect to fatty acid supplementation. A striking similarity exists between E_a below T_b for L-glutamine transport and that for L-proline transport in the same cell type (Table II). For instance, for K1060 enriched in 16 : 1*t*, the values are 85 and 80 kcal/mol, respectively, for glutamine and proline uptake. Also, the E_a above T_b is higher for L-glutamine than for L-proline transport at all unsaturated fatty acid supplementations (Table II). No reasonable rationalizations have so far been found for these trends in E_a values.

Differential thermal analysis thermograms earlier published [35,36] revealed that relative fluidities decrease in the order 18 : 2*c*,*c*- > 16 : 1*c*- > 18 : 1*c*- > 16 : 1*t*- > 18 : 1*t*-enriched K1060 cells. For L-glutamine transport, as shown in Table II

and the differential thermal analysis thermograms [35,36], T_b occurs at T_s, the onset temperature for the membrane transition (i.e., at 100% gel state lipid) for K1060 grown on elaidate (18 : 1*t*), but occurs at a point when 21%, 55% and 97% of the membrane lipids are in the liquid-crystalline state for palmitelaidate (16 : 1*t*)-, oleate (18 : 1*c*)- and palmitoleate (16 : 1*c*)-grown K1060 cells, respectively. Thus T_b is governed by fluidity. This correlation also holds true for L-proline transport. For L-glutamine transport, T_b is usually higher by 1–3 centigrade degrees than for L-proline transport (with the possible exception of 18 : 1*c*-grown K1060 cells).

Homeoviscous regulatory mechanisms in microorganisms lead to an increase in the proportion of higher-melting fatty acids in the membrane lipids at high growth temperatures [44]. This would confer stability to the membrane and the organism. There is thus an upper as well as a lower limit to the degree of fluidity compatible with normal functioning of cellular processes, e.g., growth of microorganisms [9,10,44–46]. Organisms unable to regulate the fatty acid composition of their membrane lipids as temperature increases exhibit lower optimum and maximum growth temperatures.

The upper temperature limit seems to be a peculiarity of this *E. coli* strain employed in these studies. It might have arisen, firstly due to a decrease in the heterogeneity of the membrane lipid fatty acyl chains, and secondly, due to the abnormal increase in fluidity with temperature in those circumstances. This inference derives from the fact that even though 18 : 1*t*-cells exhibit the highest upper temperature limit and that the upper temperature limit decreases as follows, 18 : 1*t*- > 16 : 1*t*- > 18 : 1*c*- > 16 : 1*c*- > 18 : 2*c*,*c*-enriched cells (i.e., as fluidity increases), the upper temperature limit values for the more homogeneous (18 : 1*t* and 16 : 1*t*) membranes [25,36] are lower than would be predicted from the phase transition midpoints (T_m), while UTL for the more heterogeneous (18 : 1*c*, 16 : 1*c* and 18 : 2*c*,*c*) membranes exhibit higher values. These upper temperature limit values correlate directly with heterogeneity in the membrane lipid acyl chains [35,36]. Thus, for L-glutamine transport, the upper temperature limit is higher than T_m by 0.5, 6.7, 12.5, 26 and 31 Cdeg for 18 : 1*t*-, 16 : 1*t*-, 18 : 1*c*-, 16 : 1*c*- and 18 : 2*c*,*c*-grown cells, respectively. This indicates that membrane stability increases with the supplementations in the order, 18 : 1*t* < 16 : 1*t* < 18 : 1*c* < 16 : 1*c* < 18 : 2*c*,*c*. These arguments also apply well to proline transport. Other factors such as a possible differential loss of viability at high temperatures could also be responsible for the upper temperature limit.

In these same *E. coli* K1060 cells, glycerol facilitated diffusion, which is protein mediated but not energy coupled, does not display the upper temperature limit [35]. Moreover, *E. coli* strain 7, another derivative of K12 [31,36] which may not be isogenic with K1060 but is nonetheless prototrophic in fatty acid metabolism, does not display an upper temperature limit for glutamine or proline uptake (data not included here). In addition, the main difference which is experimentally detectable between L-glutamine transport and L-proline transport in *E. coli* is the mode of energy-coupling. Even though some studies of inhibition of L-proline and L-glutamine transport in *E. coli* by colicin K [47] tend to show that the two transport systems have some features in common, these transport systems do differ sufficiently in energy coupling. Thus the upper temperature limit may be explained from the point of view of defect in energy-coupling. An increase in membrane lipid acyl homogeneity coupled with temperature effects would lead to leakage of ions [48,49]. The magnitude of protonmotive force across the K1060 membrane would decrease if protons leak. Thus, proline transport, which depends directly on protonmotive force, would be affected as leakage starts. The effect on glutamine transport would be at a higher temperature at which the higher decrease in $\Delta\bar{\mu}_{H^+}$ would lead to depletion of even glycolytically derived ATP. Since glutamine transport depends on phosphate bond energy per se, this would explain why the upper temperature limit is higher for glutamine than for proline transport in most instances.

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