Effects of Membrane Fatty Acid Composition on Sodium-Independent Phenylalanine Transport in Ehrlich Cells

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ABSTRACT

We have examined Na^+-independent phenylalanine transport in Ehrlich cells having different degrees of membrane fatty acid saturation. These differences were produced by growing the cells in mice fed a fat-free chow supplemented with either sunflower seed oil or coconut oil. Plasma membranes isolated from the cells grown on sunflower oil were enriched with polyenoic fatty acid, especially 18:2, whereas those isolated from the cells grown on coconut oil were enriched in monoenoic fatty acids, primarily 16:1 and 18:1. Arrhenius plots of phenylalanine uptake showed two transitions. The temperatures of these transitions were different in the two cell preparations; 17 C and 24 C for the cells enriched in polyenoic fatty acids, 19 C and 28 C for those enriched in monoenoic fatty acids. Therefore, this transport system is sensitive to changes in the fatty acid composition of the lipid phase in which it operates. The activation energies, however, were the same in both cell preparations; 14, 8 and 4 kcal/mol. There also was no significant effect of the lipid modifications on either the K'_m or V'_{max} of this transport process. The K'_m for phenylalanine uptake from a choline medium remained constant as the temperature was raised from 17 C to 37 C, whereas the V'_{max} showed about a two-fold increase in both cell types. Phenylalanine exodus from the cells into an amino acid-free suspending medium, analyzed using first-order kinetics, also was not influenced by these membrane fatty acid modifications. The changes in the transition temperatures probably reflect differences in the degree of fatty acid unsaturation of lipids that surround and interact with the phenylalanine carrier. Such differences, however, do not appreciably influence the catalytic activity of this transport system.

INTRODUCTION

Fatty acid composition is a major factor that influences the physical state of membrane lipids. We recently have developed a method for producing large modifications in the fatty acid composition of Ehrlich ascites cells (1). Plasma membranes derived from these modified cells showed marked differences in the transition temperatures detected with a spin-labeled stearic acid probe, suggesting that membrane fluidity is altered by these types of fatty acyl modifications (2). Moreover, we observed that the transport activity of the Na^+-independent, short chain neutral amino acid uptake system in Ehrlich cells also is influenced by these changes in the fatty acid composition of the plasma membrane phospholipids (3).

Christensen and his coworkers have shown that there are at least three distinct systems that mediate the transport of neutral amino acids in Ehrlich cells: System A, ASC and L (4,5). The sodium-dependent uptake of α-aminoisobutryic acid by Ehrlich cells, which is influenced by lipid modifications (3), is mediated by System A (4). In order to determine whether other amino acid transport systems are similarly influenced, we have extended our studies on membrane lipid modification to System L, as represented by sodium-independent phenylalanine uptake. The transition temperatures for this transport system showed the expected variations for the changes in fatty acid saturation that were produced. In contrast to System A, however, the kinetic parameters for phenylalanine transport and the activation energies for this process were not appreciably affected by these changes in membrane fatty acid saturation.

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MATERIALS AND METHODS

Cells

A fatty acid-deficient diet was provided by Teklad Test Diets (Madison, WI). Coconut oil was purchased from Ruger Chemical Co. (Irvington, NJ) and sunflower seed oil was obtained from Cargill (Minneapolis, MN). The Ehrlich ascites cells were propagated in CBA mice fed a fatty acid deficient diet supplemented with either 16% coconut oil or 16% sunflower seed oil. The fatty acid composition of these diets has been reported previously (1). Mice were placed on these diets for at least 4 weeks prior to the transplantation of the cells, and the diets were continued during the additional 2 weeks of tumor growth. The cells were transplanted by the intraperitoneal injection of 0.5 ml of a sterile solution containing 1 volume of Ehrlich ascites tumor and 9 volumes of 0.15 M NaCl.

Transport Measurements

In all solutions used for the cell transport studies, Na^+ was replaced isoosmotically with choline. We have employed the methods developed by Christensen and his coworkers for studying amino acid uptake by and efflux from Ehrlich cells (6,7). Typically, the cells were collected by centrifugation at 200 x g for 2 min and washed once with 0.15 M choline chloride solution. The cells were then suspended in Krebs Ringer-phosphate buffer, pH 7.4, supplemented with 1.5% bovine serum albumin. In this medium, the rate of phenylalanine uptake was not diminished by storage of the cells at 0 C for up to 1 hr. For the uptake measurement, a 0.2 ml aliquot of the cell suspension was added to 1.4 ml of Krebs Ringer-phosphate buffer, pH 7.4, and the reaction was initiated by addition of 0.2 ml uniformly labeled [^{14}C] phenylalanine. At the end of a 45 s incubation, 5 ml of the ice-cold buffer was added, and the cells were pelleted at 10,000 x g for 2 min. The separated cells were extracted with 1 ml of 3% sulfosalicylate for 30 min. After removal of the precipitate, the radioactivity contained in 0.2 ml aliquots of the cell extract and the supernatant fluid from the incubation medium was measured in Packard Tri-Carb 2425 liquid scintillation spectrometer with 15 ml of Tritosol scintillation fluid (8). [^{3}H] Inulin was used to estimate the volume of extracellular water in the cell pellet. Prior to the start of each incubation, both the medium and cells were equilibrated for 5 min at the temperature at which the reaction was to be performed. Without this equilibration, considerable variations in the uptake rate were observed in temperature dependence studies. The weight of intracellular water per 100 mg of the cell pellet was not appreciably affected by changes in incubation temperature.

Krebs Ringer-choline bicarbonate medium supplemented with 5 mM HEPES buffer was used for the efflux studies. The pH of this buffer was 7.6 at 20 C and 7.4 at 37 C with 95% O_2-5% CO_2 as the gas phase. Typically the cells were incubated in the buffer solution containing 20 mM [^{14}C] phenylalanine at 37 C for 10 min. The cells then were washed twice with ice-cold 0.15 M choline chloride solution and suspended in this solution. To measure exodus rates, a 0.5 ml aliquot of the cell suspension was added to 25 ml of the bicarbonate medium with 95% O_2-5% CO_2 as the gas phase. The volume ratio of suspending medium to cellular water was ca. 300. Unless specified otherwise, the decrease in levels of the radioactive amino acid in the cells was measured in the interval from 5 to 15 min after suspending the cells. The rate constant, k, was computed from the first order equation, $A_t = A_o e^{-kt}$.

For Arrhenius plots, rates of uptake were measured from 7° to 42° at intervals of 1.5°. All the data were fitted by the method of least squares using a Hewlett-Packard 98108 programmable calculator.

Lipid Analyses

Washed cells were extracted with a chloroform/methanol solution (2:1, v/v), and the chloroform phase was isolated (9). Polar lipids were isolated from neutral lipids by silicic acid column chromatography (10). Plasma membranes were isolated from Ehrlich cells as described previously (1), and the lipids contained in the membranes were extracted with the chloroform/methanol solution. The phospholipid content of the membranes was measured chemically (11), and the cholesterol content was determined by gas liquid chromatography (12). Other aliquots of the cell and membrane lipid extracts were saponified (13) and methylated (14). The fatty acid composition was determined by gas chromatography using a glass column (1.8 m x 2 mm l.d.) packed with Apolar 10C on 100/200 mesh Gas-Chrom Q (Applied Science Laboratories, Inc., State College, PA). The separations were carried out with a Hewlett-Packard 5710A gas chromatograph equipped with a flame ionization detector, and relative mass amounts were determined with a Hewlett-Packard 3380A automatic integrator (12). Fatty acid methyl ester standards were obtained from Supelco, Inc (Bellefonte, PA).

RESULTS

Cells and Plasma Membrane Fatty Acid Composition

This dietary approach has been employed previously to modify the Ehrlich ascites cell plasma membrane fatty acid composition, and detailed results from both cell lipids and several subcellular membrane fractions have been reported (1,3,15-17). These analyses were repeated in the present study only to confirm that similar modifications were produced, and they were not carried out in as great detail. As shown in Table I, the fatty acid compositional changes produced in the cell lipid fractions and in the isolated plasma membranes are essentially the same as those reported earlier (1), including those produced in the cells utilized for studies of α-aminoisobutyrate transport (3). Both the polar and neutral lipids of the cells grown in the mice fed the coconut oil-supplemented diet contained more saturated

TABLE I

Fatty Acid Composition of Cell and Plasma Membrane Lipids

	Percentage composition (%)[a]					
	Cells					
	Polar lipids		Neutral lipids		Plasma membranes	
Fatty acid	Sunflower[b]	Coconut	Sunflower	Coconut	Sunflower	Coconut
Classes[c]						
Saturated	44	49	28	38	42	40
Monoenoic	20	31	21	41	19	32
Polyenoic	33	16	49	13	36	23
Major individual acids						
14:0	1.5	3.7	1.7	4.4	1.8	2.9
16:0	19.7	24.7	14.5	20.1	17.7	18.4
16:1	3.9	6.9	3.4	7.8	2.4	4.6
18:0	20.5	18.7	9.6	12.7	22.3	18.6
18:1	16.8	24.8	16.6	30.6	13.7	26.2
18:2	23.5	8.4	24.9	8.2	23.2	9.8
20:4	6.3	5.8	6.2	2.9	6.6	8.4
22:6	3.1	1.7	4.1	1.1	4.3	1.9

[a]Average of two cell and plasma membrane preparations, each analyzed in duplicate. The individual values agreed very closely in each case.

[b]Diet of the mice in which the cells were grown.

[c]These values do not add up to 100% because a small percentage of the fatty acid composition was unidentified.

and monoenoic fatty acids and considerably less polyenoic fatty acids than those from the cells grown in the mice fed the sunflower oil-supplemented diet. In the plasma membranes isolated from cells modified in this way, the differences were confined to the monoenoic and polyenoic fatty acids. Those from the coconut oil-fed animals contained more monoenoic fatty acids, both 16:1 and 18:1, whereas those from the sunflower oil fed animals contained more polyenoic fatty acid, mostly 18:2. As reported previously (1), the plasma membrane fatty acid modifications were not associated with any appreciable change in the phospholipid content of the membrane, 313 (sunflower) vs. 323 μg/mg protein (coconut), and the molar ratio of phospholipid to cholesterol was the same, 1.2, in both cases. Although not tested in the present work, we have shown previously that these fatty acid modifications are not accompanied by any changes in the phospholipid head group composition of the membrane (3).

Phenylalanine Uptake

Figure 1 shows representative time courses of phenylalanine uptake from a choline-containing medium by Ehrlich cells. The phenylalanine concentration was 0.7 mM, and the incubation temperatures were 17 C or 31 C. Values at 0 time represent the uptake that occurred during separation of the cells by

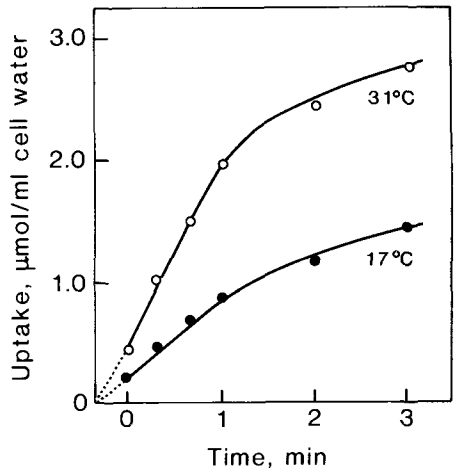

FIG. 1. Time course of phenylalanine uptake from a choline medium. The Ehrlich cells were grown in mice fed a regular diet. The phenylalanine concentration was 0.7 mM, and the incubation temperatures were 17C and 31C.

TABLE II

Kinetic Parameters for Phenylalanine Uptake at Different Temperatures[a]

Temperature	Coconut[b]		Sunflower	
	K'_m	V'_{max}	K'_m	V'_{max}
°C	mM	μmol/ml cell water•45 s	mM	μmol/ml cell water•45 s
17	0.12	0.84	0.15	0.88
23	0.14	1.14	0.13	1.14
31	0.15	1.51	0.16	1.65
37	0.12 ± 0.03[c]	1.54 ± 0.10	0.14 ± 0.03	1.77 ± 0.14

[a]Lineweaver-Burk plots to derive the constants were based on 8 different substrate concentrations ranging from 0.05 to 0.5 mM.

[b]Diet fed to the mice in which the cells were grown.

[c]Mean ± S.E. of 4 experiments.

centrifugation, and they appear to be equivalent to the uptake that occurs during 20 sec of incubation at the respective temperatures. Phenylalanine uptake deviated from linearity after 1 min of incubation at both temperatures. Because of this, we used a 45 s incubation time for all of the uptake studies.

Table II shows the effect of temperatures on the kinetic constants for phenylalanine uptake from a choline medium by Ehrlich cells grown in mice fed either the sunflower or coconut oil-supplemented diet. The nonsaturable component, estimated by the equation of Inui and Christensen (6), was found to vary from 0.04 to 0.08 min^{-1}, and it did not show any clear dependence on the lipid composition of the cells or the incubation temperatures. We used a value of 0.06 min^{-1} to correct all of the uptake data for the nonsaturable component. We did not observe any significant differences in the K'_m or the V'_{max} values for the two kinds of cells. There was also no appreciable temperature dependence of K'_m values over the range from 17C to 37C.

Temperature Dependence of Phenylalanine Uptake

A fixed substrate concentration, 0.7 mM, was used for the temperature dependence studies since the K'_m for uptake was observed to be temperature-independent over the range of interest. Figure 2 compares Arrhenius plots for phenylalanine uptake (corrected for nonsaturable uptake) in cells grown on either

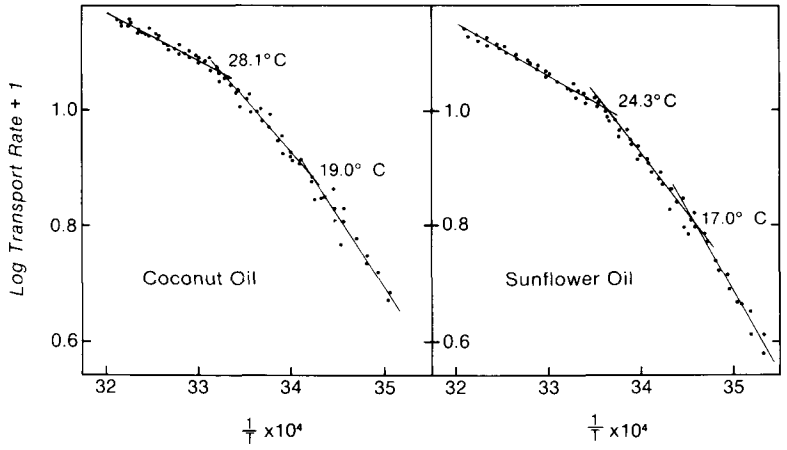

FIG. 2. Arrhenius plots for phenylalanine uptake in the Ehrlich cells grown in mice fed either the sunflower oil or coconut oil diets. Uptake was measured in a medium containing choline, and the phenylalanine concentration was 0.7 mM. Each point represents an average of duplicate values at the temperature. These data were fitted by the method of least squares analysis as described previously (3).

TABLE III

Transition Temperatures and Activation Energies Obtained from Arrhenius Plots of Phenylalanine Uptake

		Phenylalanine uptake				
		T_1	T_2	E_1[a]	E_2	E_3
Diet	Expt. no.	°C		kcal/mol		
Coconut	1	18.6	27.5	12	8	3
	2	19.8	28.5	14	9	5
	3	18.7	28.2	12	9	3
	Mean ± SE	19.0 ± 0.4	28.1 ± 0.3			
Sunflower	1	16.7	25.1	15	8	5
	2	17.5	23.0	18	9	4
	3	16.9	24.9	13	9	4
	Mean ± SE	17.0 ± 0.2[b]	24.3 ± 0.7[c]			

[a] E_1-E_3 are activation energies; E_1 is that below T_1, E_2 is that between T_1 and T_2, and E_3 is that above T_2.

[b] $0.01<P<0.02$.

[c] $P<0.01$.

coconut or sunflower oil. Table III lists the transition temperatures for the transport process and the activation energies obtained from three separate experiments of this type. In all cases, the transition temperatures observed with the cells grown on sunflower oil were from 2° to 4° lower than those for the cells grown on coconut oil. The activation energies, however, were not significantly different for the two cell preparations, being ca. 14, 8 and 4 kcal/mol in both cases.

Phenylalanine Efflux

Figure 3 shows the time course of [^{14}C]-phenylalanine exodus from the two cell preparations into an amino acid-free suspending medium. Two internal phenylalanine concentrations were tested, 4 and 17 mM. A first-order rate constant, 0.04 min^{-1}, appears to fit all of these situations. These data indicate that phenylalanine exodus at 37 C was not influenced by the types of membrane fatty acid modifications that we have produced.

DISCUSSION

Two temperature transitions for Na^+-independent phenylalanine uptake, which is mediated by System L in Ehrlich cells, were observed in the Arrhenius plots. These temperatures were influenced by the modifications in membrane fatty acid composition that were produced, and enrichments with polyenoic fatty acids was associated with a 2-3° lowering of both transition temperatures. A similar type of change was noted for Na^+-dependent, short chain neutral amino acid transport which is mediated by System A in Ehrlich cells (3). Therefore, both of these neutral amino acid transport systems are sensitive to changes in the fatty acid composition of the membrane lipids in which they are embedded. Studies with *Escherichia coli* indicate that the transition temperatures for transmembrane transport

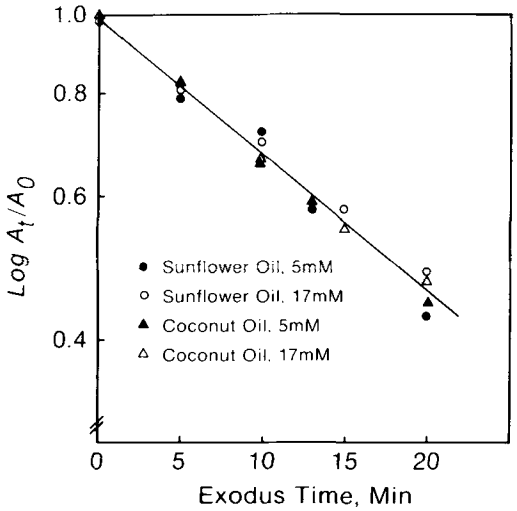

FIG. 3. Efflux of phenylalanine from the Ehrlich cells grown on either coconut oil or sunflower oil. The radioactive phenylalanine concentration inside of the cells was either 5 mM or 17 mM at the beginning of the incubation. These studies were done at 37 C, and each value is the average of two determinations.

systems are caused by phase transitions of the membrane lipids (18,19). In isolated Ehrlich cell plasma membranes, spin-labeled stearic acid probes have demonstrated the presence of two major lipid transitions (2,20). The temperatures of these transitions, however, differ by several degrees from those noted for the amino acid transport systems. Likewise, the temperatures of the activity transitions for each of the amino acid transport systems differ by several degrees (3). Therefore, unlike the *E. coli* transport systems (18,19), the neutral amino acid transport system transitions in the Ehrlich cell are not a direct reflection of the membrane bulk lipid phase transitions. A likely possibility is that the lipid microenvironments of the two transport systems differ and that the activity transitions of each transport system reflects the composition of its microenvironment. It should be noted that the activity transition of the Ehrlich cell plasma membrane (Na^++K^+)-ATPase also is not consistent with the bulk lipid transitions when the fatty acid composition of the membrane was modified by these dietary procedures (21).

Although the transport transition temperatures were altered, the fatty acid modifications that were produced had no appreciable effect on the activation energies or the K'_m or V'_{max} of phenylalanine transport. By contrast, the same dietary modifications altered the activation energy and K'_m of α-aminoisobutyrate transport in the Ehrlich cell (3). Therefore, in contrast to our previous findings with System A, the present results indicate that membrane lipid composition does not exert a modifying effect on the function of System L in the Ehrlich cell. In this regard, the K'_m for α-aminoisobutyric acid uptake in the GF-14 lymphocyte line shows a sharp transition at around 29° (22). As the temperature was raised from 20.5C to 28.9C, the K'_m decreased from 1.78 to 0.68 mM, and it then remained constant between 29C and 42C. On the other hand, the K'_m for phenylalanine uptake in Ehrlich cells showed no transition over the same temperature range. One possible explanation is that the binding site of the α-aminoisobutyric acid carrier, System A, is influenced by changes in the physical state of the surrounding lipid, whereas the binding site of the phenylalanine carrier, System L, is not affected by these kinds of membrane lipid changes. Alternatively, it is possible that the lipid dependence of the transport parameters in System A is exerted on the Na^+-dependent component of this system, perhaps on the (Na^++K^+)-ATPase (21), and therefore not directly on the amino acid carrier component. Independently of mechanism, these results demonstrate that membrane lipid modifications do not have a uniform effect on carrier mediated transport systems in a eucaryotic cell, even on those systems which transport closely related metabolites.

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REFERENCES

1. Awad, A.B., and A.A. Spector, Biochim. Biophys. Acta 426:723 (1974).
2. King, M.E., B.W. Stavens, and A.A. Spector, Biochemistry 16:5290 (1977).
3. Kaduce, T.L., A.B. Awad, L.J. Fontenelle, and A.A. Spector, J. Biol. Chem. 252:6624 (1977).
4. Oxender, D.L., and H.N. Christensen, J. Biol. Chem. 23:3686 (1963).
5. Christensen, H.N., M. Liang, and E.G. Archer, J. Biol. Chem. 242:5237 (1967).
6. Inui, Y., and H.N. Christensen, J. Gen. Physiol. 50:203 (1966).
7. Christensen, H.N., and M.E. Handlogten, J. Biol. Chem. 243:5428 (1968).
8. Pande, S.V., Anal. Biochem. 74:25 (1976).
9. Folch, J., M. Lees, and G.H. Sloane-Stanely, J. Biol. Chem. 226:497 (1957).
10. Burns, C.P., D.G. Luttenegger, S.-P.L. Wei, and A.A. Spector, Lipids 12:747 (1977).
11. Raheja, R.K., L. Kaur, A. Singh, and S. Bhatia, J. Lipid Res. 14:695 (1973).
12. Spector, A.A., R.E. Kiser, G.M. Denning, S.-W.M. Koh, and L.E. DeBault, J. Lipid Res. 20:536 (1979).
13. McGee, R., and A.A. Spector, Cancer Res. 34:3355 (1974).
14. Morrison, W.R., and L.N. Smith, J. Lipid Res. 5:600 (1964).
15. Liepkalns, V.A., and A.A. Spector, Biochem. Biophys. Res. Commun. 63:1043 (1975).
16. Awad, A.B., and A.A. Spector, Biochim. Biophys Acta 450:239 (1976).
17. Brenneman, D.E., T. Kaduce, and A.A. Spector, J. Lipid Res. 18:582 (1977).
18. Overath, P., H.U. Schairer, and W. Stoffel, Proc. Natl. Acad. Sci. USA 67:606 (1970).
19. Wilson, G., and C.F. Fox, J. Mol. Biol. 55:49 (1971).
20. King, M.E., and A.A. Spector, J. Biol. Chem. 253:6493 (1978).
21. Solomonson, L.P., V.A. Liepkalns, and A.A. Spector, Biochemistry 15:892 (1976).
22. Finkelstein, M.C., and E.A. Adelberg, J. Biol. Chem. 252:7101 (1977).

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