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Sodium-Dependent L-Serine Transport in Plasma Membrane Vesicles Isolated from Ehrlich Cells by Two-Phase Compartmentation

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Summary. Plasma membrane vesicles were prepared from Ehrlich cells using two-phase system compartmentation. The highly pure plasma membrane vesicles obtained presented a negligible mitochondrial contamination and were suitable for studies of amino acid transport. L-Serine transport showed a clear ionic specificity, maximum incorporation being observed when an inwardly directed NaSCN gradient was used. Na^+-dependent L-serine transport was dependent on assay temperature and membrane potential, and it seemed to be carried out by two different transport systems. An essential sulfhydryl group seemed to be involved in the transport process.

Key Words serine transport · plasma membrane vesicle · Ehrlich cells · plasma membrane isolation

Introduction

Neoplastic transformation is accompanied by an adaptation of metabolism to an increase in nucleotide and protein synthesis, which requires a continuous supply of substrates. Glucose and glutamine are the main energy and nitrogen sources for these processes (Medina & Núñez de Castro, 1990*a*), although other substrates are also incorporated by tumor cells (Spector & Steinberg, 1965; Baker et al., 1974; Ookhtens & Baker, 1979; Lazo, 1981; Sauer & Dauchy, 1983). The high rates of protein synthesis in rapidly growing tumors require a continuous supply of both essential and nonessential amino acids (Perán, Muñoz & Sainz, 1990*a,b*).

In tumor cells, there is an increase in the activities of the enzymes involved in serine biosynthesis, as well as an increase in the activities of the enzymes that use serine as a precursor for purine biosynthesis (Knox, Herzfeld & Hodson, 1969; Thordinke, Pelliniemi & Beck, 1979; Snell, 1985). L-Serine concentration in plasma of tumor-bearing animals is lower than in plasma of healthy mice (Márquez et al., 1986, 1989). Other authors have also found decreased L-serine plasma concentrations in patients with cancer (Levin & Grevers, 1981*a,b*), as well as in Walker 256 carcinoma-bearing rats (Krause et al., 1979). Furthermore, L-serine concentration in ascitic fluid in Ehrlich ascites tumor-bearing mice is always lower than in plasma (Márquez et al., 1989). These results show that L-serine is consumed by tumor cells (Márquez et al., 1989).

The capacity of tumor cells for using exogenous L-serine must imply the existence of very efficient transport systems. However, the study of transport in whole tumor cells is hampered by the great metabolic capacity of these cells. To avoid the interference of the rapid metabolism of L-serine, the uptake of this amino acid should be studied in isolated plasma membrane vesicles. Currently, there are relatively simple and efficient procedures to obtain plasma membrane vesicles from different sources (Hanning & Heidrich, 1974; Bardin & Johnstone, 1978; Larsson & Andersson, 1979).

The present paper reports the application of a polymeric aqueous two-phase system to obtain highly purified plasma membrane vesicles from Ehrlich ascites tumor cells containing amino acid transport activity. The study of Na^+-dependent L-serine transport in these vesicles is also reported.

Materials and Methods

Ehrlich Ascites Cells

A hyperdiploid Lettré strain was maintained as previously reported (Quesada et al., 1988). Mice were inoculated with 5×10^6 tumor cells from different infested animals; cells were harvested on day 10–11 after tumor transplantation at the beginning of the stationary growth phase (Márquez et al., 1989). Animals were killed by cervical dislocation immediately before the ascitic tumor samples were taken. The cells were washed with 0.9% NaCl.

Isolation of a "Microsomal" Fraction

The isolation of plasma membrane vesicles was carried out in two steps: (i) to obtain an initial "microsomal" fraction, and (ii) to separate plasma membranes by two-phase system compartmentation. Unless otherwise stated, the procedure was carried out at 4°C.

To obtain the initial microsomal fraction, the method described by Colombini and Johnstone (1973) was followed with slight modifications. Briefly, washed cells were resuspended in a volume of 1 mM $ZnCl_2$, 10-fold that of packed cells. Cells were maintained for 10 min at room temperature, and for 10 min in ice. Afterwards they were homogenized with a Polytron PTA 20S at position 3 for 2 min. To separate nonbroken cells, nuclei, and cell debris, the homogenate was centrifuged three times at 1000 × *g* for 1 min, discarding the pellet at each step. The supernatant from the last centrifugation was treated with EDTA at 1 mM final concentration to remove the zinc, and it was centrifuged at 16,300 × *g* for 10 min. The resultant pellet was resuspended in 5 mM potassium phosphate buffer, 0.33 M sucrose, 5 mM KCl, pH 7.4 (S buffer). The suspension was centrifuged once more at 16,300 × *g* for 10 min, and the pellet was resuspended in S buffer. This suspension represented the initial microsomal fraction.

Two-Phase System Compartmentation

The polymers used in the two-phase system were polyethylene glycol (PEG) 3350, and dextran (Dx) 500. As the amount of water contained in Dextran-500 widely changes from available commercial batch to batch, for each one the exact concentration should be determined by using a polarimeter, as described by Larsson (1983).

The 24 g two-phase system was prepared by adding the following reagents at the indicated final concentrations: resuspended microsomal membranes in S buffer (6 g), 6.2% (wt/wt) Dx-500, 6.2% (wt/wt) PEG-3350, 0.33 M sucrose, 3 mM KCl, and 5 mM potassium phosphate, pH 7.4. The system was mixed by 30–40 inversions of the tubes by hand. The two phases were separated by centrifugation in a swinging bucket rotor at 1500 × *g* for 2 min; simultaneously, fresh two-phase systems were prepared in which microsomal membranes were omitted. The fresh phases were used to wash the phases obtained in the system containing the microsomal fraction. The upper phase, enriched in plasma membrane, was rewashed twice with fresh lower phase. In the same way, the lower phase was partitioned by adding new fresh upper phase. The final upper phases containing plasma membranes were combined, diluted (1 : 3) with 5 mM HEPES buffer, adjusted with KOH at pH 7.4, and centrifuged at 100,000 × *g* for 20 min. The pellet was suspended in 15% (vol/vol) dimethyl sulfoxide at a final concentration of 12 mg protein/ml and stored at −80°C until use. Stored in such a way, the membranes retained their transport activity for weeks.

Protein Determination

Proteins were measured as described by Bradford (1976).

Enzyme Assays

The following methods were used to determine marker enzyme activities: 5′-nucleotidase (Im & Spector, 1980), K^+-stimulated, ouabain-sensitive paranitrophenyl phosphatase (Kashiwamata et al., 1979), glucose-6-phosphatase (Nordlie & Arion, 1966), succinate dehydrogenase (Im & Spector, 1980), and cytochrome *c* oxidase (Urdiales et al., 1989). The phosphate determination required for the three first enzyme markers was carried out according to the classical Fiske-Subbarow method, as described by Esmann (1988).

Transport Measurements

Membrane preparations stored in 15% DMSO were quickly thawed. To discard the DMSO, the membranes were precipitated by adding 0.1 M NaCl (final concentration), and centrifuging at 16,300 × *g* for 10 min. The pellet was suspended in 5 mM HEPES buffer, 0.1 M KCl, pH 7.4, and centrifuged once more. The final pellet was suspended in the same buffer at a concentration of 4–6 mg protein/ml.

Transport experiments were initiated by mixing, in a 1 : 4 ratio, the membrane suspension with 125 mM NaSCN or KSCN in 5 mM HEPES buffer, pH 7.4, in the presence of 0.2 mM L-(3-^{3}H)-serine (275 dpm/pmol). At each assay time, a 50 μl-aliquot was taken, added to 2 ml of ice-cold stop solution (0.1 M NaCl in 5 mM HEPES buffer, pH 7.4), and rapidly filtered through a glass fiber filter (Whatman GF/C) using a 1225-multiple collector from Millipore connected to a vacuum system. Finally, the filter was immediately washed with 5 ml of ice-cold stop solution. Na^+-dependent L-serine transport is a process that is dependent on assay temperature (Sips, Van Amelsvoort & Van Dam, 1980; Herrero, Giménez & Aragón, 1987). The apparent transport in the presence of KSCN was practically independent of the temperature. Transport experiments were carried out at room temperature.

The filters were dried at 60°C and then placed into scintillation vials containing 5 ml Aquasol-2. Radioactivity was determined using a Rack-Beta liquid scintillation counter from Pharmacia.

All the measurements were carried out at least in triplicate and were corrected for nonspecific binding, calculated from the radioactivity retained in the filters at zero time.

Chemicals

L-(3-^{3}H)-Serine (sp act: 1.85 GBq/mmol) was purchased from Amersham. Aquasol-2 was obtained from NEN-DuPont. All other chemicals were obtained from Merck, or Sigma.

Results

Isolation of Plasma Membrane Vesicles

In the procedures to obtain native plasma membrane vesicles, there are two steps clearly differentiated: The isolation of an initial fraction of membranes (usually called microsomal fraction), and the purification of plasma membrane from this fraction. The first step used in the present work was that reported by Colombini & Johnstone (1973). However, the microsomal fraction was further purified and plasma membrane isolated by using an aqueous two-phase

Table 1. Specific activities of marker enzymes in membrane fractions during the preparation of plasma membranes from Ehrlich cells

Enzymes	Specific activities[a]			RSA[b]	Rec[c]
	F_1	F_2	F_3		
5′-Nase	0.3 ± 0.1	2.8 ± 0.6	11.0 ± 0.8	37	12.8
PNPPase	18.8 ± 6.2	70.3 ± 7.8	299.5 ± 50.8	16	4.4
GPase	3.6 ± 0.1	5.0 ± 0.7	2.1 ± 0.0	0.58	0.2
SDH	51.6 ± 4.1	80.6 ± 10.6	3.3 ± 1.9	0.06	0.018
CCOase	335.7 ± 80.8	1743.0 ± 145.6	41.2 ± 20.5	0.12	0.052

[a] Units of specific activity: nmol/mg protein-min. Values are mean ± SEM for three different determinations.
[b] RSA or enrichment is obtained as specific activity in F_3 divided by specific activity in F_1.
[c] Recoveries in F_3 fraction are expressed as percentages of the activities found in the first homogenate.
F_1 is the homogenate, F_2 is the microsomal fraction, and F_3 is the purified plasma membrane fraction.

system containing 6.2% (wt/wt) PEG-3350, and 6.2% (wt/wt) Dx-500.

Table 1 shows the specific activities of the marker enzymes used to characterize the obtained plasma membrane vesicles: 5′-nucleotidase, and K^+-stimulated, ouabain-sensitive paranitrophenyl phosphatase, as plasma membrane markers; glucose-6-phosphatase, as endoplasmic reticulum marker; and succinate dehydrogenase, and cytochrome *c* oxidase, as inner mitochondrial membrane markers. Specific activities were determined in the homogenate (F_1 fraction), in the microsomal fraction (F_2 fraction), and in the purified plasma membrane vesicles (F_3 fraction). The enrichment obtained for 5′-nucleotidase (37-fold higher in F_3 than in F_1), and paranitrophenyl phosphatase (16-fold enrichment) reflects the purity of the plasma membranes obtained. The enrichment of plasma membranes obtained with gradient centrifugation methods is usually less (Van Amelsvoort, Sips & Van Dam, 1978; Zafra & Giménez, 1986). Previously reported procedures to isolate plasma membrane vesicles from Ehrlich ascites tumor cells yield approximately a 20-fold enrichment in the plasma membrane fraction (Colombini & Johnstone, 1973; Kilberg & Christensen, 1979; Im & Spector, 1980). However, the degree of contamination by internal membranes (especially, mitochondrial membranes) in these preparations is greater than that obtained by the two-phase system compartmentation used in the present work. In fact, our plasma membrane preparations contained an endoplasmic reticulum contamination of less than 2%, and an inner mitochondrial membrane contamination of less than 0.4%. The purity of the plasma membrane obtained was greater than 95%.

Table 2 shows the recoveries for the different marker enzymes in F_2 and F_3 fractions. These data reveal valid information on the specificity of each one of the two steps in the procedure followed to isolate plasma membrane vesicles. The data clearly show that the isolation of a microsomal fraction is the less efficient step. However, 2-phase system compartmentation yielded such a good result that it compensated for the inefficient first step. Nonetheless, the global procedure might be easily optimized by changing the homogenization and centrifugation conditions used in the first step.

Table 2. Recovery of enzyme activities

Enzymes	F_2/F_1(%)	F_3/F_2(%)
5′-Nase	24	50
GPase	3.8	5
CCOase	24	0.25

There is something that is worthwhile to stress. As Table 2 shows, in the first step there was no selective elimination of the mitochondrial fraction which was completely eliminated after two-phase compartmentation. This fact reveals that 2-phase compartmentation is such a selective method that it is not necessary to obtain an initial membrane fraction essentially free of mitochondria, a condition that is almost indispensable when density gradient centrifugation methods are used.

Ionic Specificity of The Na^+-Dependent L-Serine Transport

Table 3 shows that L-serine accumulation in native vesicles depends on ionic gradients: maximum uptake was obtained with a Na^+ gradient. A K^+ or choline-ion gradient did not stimulate L-serine transport. However, L-serine transport in the presence of an NH_4^+-ion gradient was 41% of that obtained in the presence of Na^+. This surprising effect of NH_4^+ was previously found in the transport of L-

Table 3. Ionic specificity of Na^+-dependent L-serine transport

Added compound	Transported L-serine (%)
NaCl	100
NH_4Cl	41
Choline chloride	25
KCl	6
Mannitol	10
NaSCN	137
NaH_2PO_4	52
Sodium citrate	78

Na^+-dependent L-serine transport was determined with 160 μM L-(3-^{3}H)-serine in the presence of different salts at 100 mM concentration (except sodium citrate, at 33 mM), or mannitol at 200 mM. Transport was stopped after 15 sec. Results are expressed as percentages of transport taking the transport in 100 mM NaCl as 100%. Standard deviations for the mean values reported were within the 20% range.

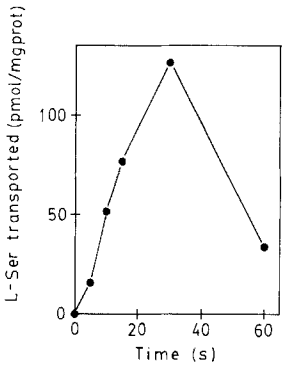

Fig. 1. Time course of Na^+-dependent L-serine transport in plasma membrane vesicles isolated from Ehrlich cells. Values of transport in 100 mM NaSCN were corrected with those in 100 mM KSCN. A typical result is represented

glutamine (*unpublished results*), and its possible causes deserve further study. In the absence of ionic gradient, L-serine uptake was minimal. It could be concluded from these data that L-serine is transported into plasma membrane vesicles from Ehrlich cells mainly via Na^+-dependent systems.

The counter-ion accompanying Na^+ influences L-serine accumulation. Total uptake was correlated with the penetrability of the anion (Table 3). Thus maximal uptake was obtained with a NaSCN gradient. The anion thiocyanate easily diffuses across the phospholipidic bilayer and gives rise to a membrane potential change (negative inside), which constitutes an additional driving force for Na^+-dependent L-serine transport. This effect of thiocyanate has been previously described (Lücke, Haase & Murer, 1977). The effect of the anions on L-serine transport could only be observed in the presence of a Na^+ gradient; this fact rules out the possibility that the observed L-serine incorporation arose from osmotic changes in the medium. Similar results have been obtained in plasma membrane vesicles from other sources (Sips et al., 1980; Sips, DeGraaf & Van Dam, 1982; Lynch & McGivan, 1987).

Valinomycin is a selective ionophore for K^+. When valinomycin is added to a membrane vesicle suspension, a dissipation of the transmembrane potential takes place (Medina & Núñez de Castro, 1990*b*). Na^+-dependent L-serine transport in the presence of 10 μM valinomycin was twice that obtained in control vesicles with no valinomycin added (results not shown). This finding shows that this transport activity responds to changes in transmembrane potentials. The increase was similar to that observed by Colombini and Johnstone (1974) in native vesicles and greater than that obtained in reconstituted vesicles from Ehrlich cells (McCormick, Tsang & Johnstone, 1984).

Time Course of Na^+-Dependent L-Serine Transport

Na^+-dependent L-serine transport reached a maximum at 30 sec, and it was almost linear for this period of time (Fig. 1). The decrease observed from the 30th sec on reflects the dissipation of the Na^+ gradient. Im and Spector (1980) also observed a decrease in Na^+-dependent AIB transport after the first minute, reaching equilibrium values at 10 min.

Kinetic Characteristics of Na^+-Dependent L-Serine Transport

Na^+-dependent L-serine transport showed an apparently hyperbolic kinetics (Fig. 2). To determine the possible existence of a positive cooperativity, the Hill plot was represented (Fig. 2, insert), yielding an apparent $S_{0.5}$ = 0.45 mM, and a Hill coefficient very close to unity (1.1). Na^+-dependent L-serine transport might be mediated by, at least, two different carriers, most probably A, and ASC systems (Christensen et al., 1965; Christensen, Liang & Acherd, 1967; Christensen, 1990). To elucidate the kinetic features of these two carriers, methyl-AIB is usually employed as a selective inhibitor for A system (Christensen, 1989). However, we could not obtain congruent results upon the utilization of methyl-AIB (results not shown). For this reason, only "total" kinetics could be evaluated.

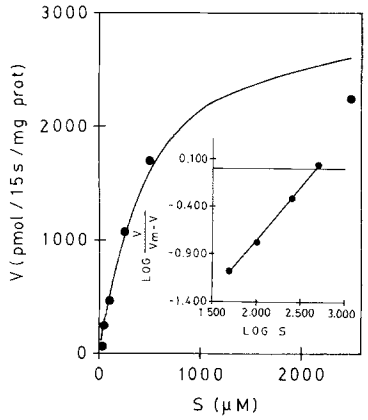

Fig. 2. Kinetics for total Na^+-dependent L-serine transport. Transport was determined at different L-serine concentrations in 100 mM NaSCN and 100 mM KSCN. Transport was stopped after 15 sec. Corrected values are represented. *Insert:* Hill plot for the kinetics

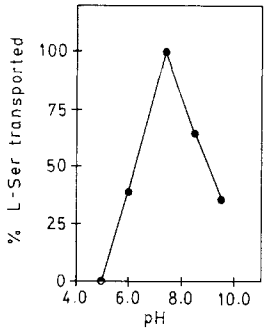

Fig. 3. Effect of pH on Na^+-dependent L-serine transport. L-Serine transport was determined at different pH values. Buffers used (all of them at 5 mM concentration) were: carbonate at pH 9.5; Tris-HCl at pH 8.5; HEPES or phosphate at pH 7.4; phosphate at pH 6; and acetate at pH 5. Transport was stopped after 15 sec. Values obtained in the presence of 100 mM NaSCN were corrected by subtracting the correspondent values in the presence of 100 mM KSCN. Data are presented as percentage of L-serine incorporated, taking the amount of L-serine incorporated at pH = 7.4 as 100%

Table 4. Effect of some amino acids on the Na^+-dependent L-serine transport

Added amino acids	Na^+-dependent L-serine transported (%)
None	100 ± 3
L-Glutamate	69 ± 9
L-Leucine	94 ± 25
L-Lysine	99 ± 2
L-Serine	21 ± 19
L-Threonine	39 ± 13

Membrane suspensions were incubated in the presence of 160 μM L-(3-^{3}H)-serine and nonlabeled amino acids at 5 mM concentration. Transport was stopped after 15 sec. Values of transport in NaSCN were corrected with those in KSCN. Results are expressed as percentages and are means ± SD for three different determinations.

Another useful criterion could be the inhibition by L-threonine, a specific inhibitor for system ASC (Handlogten et al., 1981). Table 4 shows that Na^+-dependent L-serine transport was not totally inhibited in the presence of an excess of L-threonine. This result suggests that both Na^+-dependent systems A and ASC transport L-serine are present in Ehrlich cells. Na^+-dependent L-serine transport was not inhibited by basic (lysine), or branched-chain (leucine) amino acids. On the other hand, there was a partial inhibition by the acidic amino acid glutamate. As expected, the greatest inhibition was obtained using nonlabeled L-serine as a competitive inhibitor. In the presence of KSCN, a significant increase in the values of radioactivity recovered in filters was observed when L-serine transport was studied in the presence of the charged amino acids glutamate and lysine, but not in the presence of neutral amino acids.

Effect of pH

Figure 3 shows the effect of pH on Na^+-dependent L-serine transport. Uptake was maximal at pH = 7.4. There was a stepwise decrease of transport activity with increasing pH. On the other hand, the decline in the transport was more pronounced at acidic pH, in such a way that at pH 5 Na^+-dependent L-serine transport was not observed. First, Handlogten et al. (1981) reported that both transport systems A and ASC behave dissimilarly against the acidification of the medium. System ASC is described to be less sensitive to decreases in pH than system A; in fact, system ASC slightly decreases at pH 6, whereas system A is almost totally ineffective at pH 6. Since the observed activity decrease at pH 6 was 60%, it is possible to conclude that both systems A and ASC are functional in the transport of L-serine in Ehrlich cells. These data are in good agreement with the partial inhibition observed in the presence of L-threonine (Table 4). However, the Na^+-dependent L-serine transport activity completely ceased at pH 5 (Fig. 3); this result agrees with Makowske and Christensen (1982), who showed that the transport activity of system ASC sharply drops at pH below 6.

Table 5. Inhibitory effect of sulfhydryl reagents on Na^+-dependent L-serine transport

Added inhibitor	Na^+-dependent L-serine transported (%)
None	100 ± 12
DTNB	37 ± 6
PCMBS	28 ± 21
Mersalyl	7 ± 12

Plasma membrane suspensions were preincubated for 5 min at room temperature in the presence of different inhibitors at 0.5 mM. Transport was started by adding L-(3-^{3}H)-serine in NaSCN, or KSCN, at final amino acid concentration of 160 μM, and inhibitor concentration of 0.1 mM. Transport was stopped after 15 sec. Results are percentages and are means ± SD for three different determinations.

Effect of Sulfhydryl Group Reagents

Table 5 shows the effect of mersalyl, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), and *para*-chloromercurobenzenesulfonate (PCMBS) on Na^+-dependent L-serine transport in native plasma membrane vesicles. All of them produced an inhibition greater than 50%. These compounds are inhibitors that act on sulfhydryl groups. The inhibition observed could be explained as an evidence for the existence of essential sulfhydryl groups for the activity of the transporter molecules. In the absence of an electrochemical gradient of Na^+, the amount of L-serine accumulated into the vesicles was not altered by the presence of organomercurials (PCMBS and mersalyl). This fact might suggests that the inhibition produced by these compounds is a specific effect, and not a consequence of nonspecific damage or alterations in membrane permeability (Quesada & McGivan, 1988). On the contrary, DTNB produced an increase in the apparent L-serine incorporation in the presence of KSCN. It could be concluded that the inhibition produced by DTNB is less specific than that produced by organomercurials and, at least in part, it could be the result of an increase in the membrane permeability (Biber & Haüser, 1979).

As PCMBS is weakly penetrating (Driessen & Konings, 1990), and yet it produces an inhibition greater than 50%, it could be suggested that the sulfhydryl groups involved in the transport activity are exposed at the external surface of the plasma membrane. Very recently Pola et al. (1990) confirm the different characteristics of system A and ASC transport activities to thiol-group-modifying reagents in vesicles isolated from hepatocyte plasma membranes. Nonetheless, to know the actual role played by the sulfhydryl groups in the carriers, as well as their spatial disposition, it would be necessary to carry out additional experiments of inhibition on purified transporters and protection experiments with compounds like DTT (Driessen & Konings, 1990; McCormick & Johnstone, 1990).

Discussion

The importance of transport processes in connection with tumor growth and proliferation was stressed by Holley (1972), who suggested that the crucial change in the malignant transformation would be due to alterations in plasma membrane. Owing to the elevated nourishing needs and the limited blood supply, as a consequence of the scarce vascularization of tumors, these cells must have very efficient transport systems to compete with the normal tissues of the host.

The study of amino acid transport in whole cells is difficult to carry out owing to the great metabolic capacity of neoplastic cells; thus isolated plasma membrane vesicles must be used (Sips et al., 1980; Bardin & Johnstone, 1978; Im & Spector, 1980; Zafra & Giménez, 1986). Nonetheless, in the last years some authors describe an alternative method using whole cells adhered to an inert bed, perfused in a column, and applying a highly resolutive technique of double isotopic labeling (Perán et al., 1990*a*).

The first aim in the present work was to develop a new method of isolation and purification of plasma membrane vesicles from Ehrlich cells. Several different methods are described (Colombini & Johnstone, 1973; Kilberg & Christensen, 1979; Im & Spector, 1980; Medina, 1989) which suffer from slowness, poor recovery, and/or contamination by other subcellular fractions. The good results obtained with the method used in the present work reveal the great capabilities of 2-phase compartmentation to clearly and efficiently separate different subcellular fractions (Flanagan, 1985). The effects of including $ZnCl_2$ in the homogenization buffer or in the 2-phase system have been systematically investigated (Gruber et al., 1984). It seems that plasma membrane recovery increased significantly when $ZnCl_2$ was omitted in the homogenization medium and included in the 2-phase system (Gruber et al., 1984).

The main problem in transport studies, particularly when the accumulation ratio is low, is the discrimination between unspecific binding of the substrate to the membranes and their transport. It can be assumed that the "incorporation" measured in the present work is really the consequence of a transport into the intravesicular compartment due to the following observations: (i) L-serine uptake is ion-dependent (Table 3); (ii) L-serine uptake depends on

its extravesicular concentration, and it is saturable (Fig. 2); (iii) the process depends on assay temperature; and (iv) the inhibitory effect of organomercurials is dependent on the cation present in the external medium.

L-Serine transport in native plasma membrane vesicles from Ehrlich cells can be separated into two components: one Na^+ independent and the other Na^+ dependent. The first one represents a small percentage of total L-serine transport (Table 3). Na^+-dependent L-serine transport showed an apparently Michaelian kinetics. However, the double reciprocal plot did not completely fit a straight line, which suggests that Na^+-dependent L-serine transport could be carried out by more than one transport system. Moreover, three converging evidences for the functionality of both systems in the transport of L-serine are presented: (i) L-threonine, a specific inhibitor for system ASC, inhibited, but not totally, Na^+-dependent L-serine transport; (ii) at pH 6, the transport activity decreased by 60%, but not totally, as it should be expected if only A system is functional; on the other hand, this decrease was greater than that expected if only ASC system is functional; in fact, at pH 6 it is reported that the activity of system ASC decreases less than 20% (Makowske & Christensen, 1982); and (iii) there is a decrease in the incorporation of L-serine at pH higher than 8, behavior that is characteristic of system ASC (Handlogten et al., 1981).

The next step for a further characterization of L-serine transport in Ehrlich cells should be the study in functional reconstituted vesicles.

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References

Baker, N., Sandborg, C., Morris, D., Ookhtens, M. 1974. Competition for host essential and nonessential fatty acid in Ehrlich ascites carcinoma in mice. *Cancer Res.* **37:**2218–2225

Bardin, C., Johnstone, R.M. 1978. Sodium-dependent amino acid transport in reconstituted membrane vesicles from Ehrlich ascites cell plasma membranes. *J. Biol. Chem.* **253:**1725–1732

Biber, J., Haüser, H. 1979. The role of SH-group in the concentrative transport of D-glucose into brush border membrane vesicles. *FEBS Lett.* **108:**451–456

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:**248–254

Christensen, H.N. 1989. Distinguishing amino acid transport system of a given cell or tissue. *Methods Enzymol.* **173:**576–616

Christensen, H.N. 1990. Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol. Rev.* **70:**43–77

Christensen, H.N., Liang, M., Archerd, E.G. 1967. A distinct Na^+-requiring transport system for alanine, serine, cysteine, and similar amino acids. *J. Biol. Chem.* **242:**5237–5246

Christensen, H.N., Oxender, D.L., Liang, M., Vatz, K.A. 1965. The use of N-methylation to direct the route of mediated transport of amino acid. *J. Biol. Chem.* **240:**3609–3616

Colombini, M., Johnstone, R.M. 1973. Preparation and properties of the (Na^+ + K^+)-ATPase of plasma membranes from Ehrlich ascites cells. *Biochim. Biophys. Acta* **323:**69–86

Colombini, M., Johnstone, R.M. 1974. Na^+-gradient-stimulated AIB transport in membrane vesicles from Ehrlich ascites cells. *J. Membrane Biol.* **18:**315–334

Driessen, A.J.M., Konings, W.N. 1990. Reactive exofacial sulfhydryl-groups on the arginine-ornithine antiporter of *Lactococcus lactis*. *Biochim. Biophys. Acta* **1015:**87–95

Esmann, M. 1988. ATPase and phosphatase activity of Na^+, K^+-ATPase: Molar and specific activity, protein determination. *Methods Enzymol.* **156:**105–115

Flanagan, S.D. 1985. Partitioning of animal membranes and organelles. *In:* Partitioning in Aqueous Two-Phase Systems. H. Walter, D.E. Brooks, and D. Fisher, editors. pp. 453–495. Academic, London

Gruber, M., Cheng, K., Lepock, J.R., Thompson, J.E. 1984. Improved yield of plasma membrane from mammalian cells through modifications of two-phase polymer isolation procedure. *Anal. Biochem.* **138:**102–118

Handlogten, M.E., García-Cañero, R., Lancaster, K.T., Christensen, H.N. 1981. Surprising differences in substrate selectivity and other properties of system A and ASC between rat hepatocytes and the hepatoma cell line HTC. *J. Biol. Chem.* **256:**7905–7909

Hanning, K., Heidrich, H.G. 1974. The use of continuous preparative free-flow electrophoresis for dissociating cell fractions and isolation of membranous components. *Methods Enzymol.* **31:**746–761

Herrero, E., Giménez, C., Aragón, M.C. 1987. Chronic administration of lithium modulates tryptophan transport by changing the properties of the synaptosomal plasma membrane. *Life Sci.* **41:**643–650

Holley, R.W. 1972. Unifying hypothesis concerning the nature of malignant growth. *Proc. Natl. Acad. Sci. USA* **69:**2840–2841

Im, W.B., Spector, A.A. 1980. Sodium-dependent neutral amino acid transport in native and reconstituted membrane vesicles from Ehrlich cells. *J. Biol. Chem.* **255:**764–770

Kashiwamata, S., Goto, S., Semba, R., Suzuki, F. 1979. Inhibition by bilirubin of (sodium-potassium ion)-activated adenosine triphosphatase and potassium ion-activated *p*-nitrophenylphosphatase activities of sodium iodide-treated microsomes from young rat cerebrum. *J. Biol. Chem.* **254:**4577–4584

Kilberg, M.S., Christensen, H.N. 1979. Electron-transferring enzymes in the plasma membrane of the Ehrlich ascites tumor cell. *Biochemistry* **18:**1525–1530

Knox, W.E., Herzfeld, A., Hodson, J. 1969. Phosphoserine phosphatase distribution in normal and neoplastic rat tissue. *Arch. Biochem. Biophys.* **132:**397–405

Krause, R., James, J.H., Humphrey, C., Fisher, J.E. 1979. Plasma and brain amino acids in Walker 256 carcinosarcoma-bearing rats. *Cancer Res.* **39:**3065–3069

Larsson, C. 1983. Partition in aqueous two-phase systems. *In:* Isolation of Membranes and Organelles from Plant Cells. J.L. Hall and A.L. Moore, editors. pp. 277–309. Academic, London

Larsson, C., Andersson, B. 1979. Two-phase methods for chloroplasts, chloroplast elements and mitochondria. *In:* Plant Or-

ganelles: Methodological Surveys. B. Biochemistry. E. Reid, editor. Vol. 9, pp. 498–527. Academic, London

Lazo, P. 1981. Amino acid and glucose utilization by different metabolic pathways in ascites tumour cells. *Eur. J. Biochem.* **117:**19–25

Levin, L., Gevers, W. 1981*a*. Metabolic alterations in cancer: I. *S.A. Med. J.* **59:**518–521

Levin, L., Gevers, W. 1981*b*. Metabolic alterations in cancer: II. *S.A. Med. J.* **59:**553–556

Lücke, H., Haase, W., Murer, H. 1977. Amino acid transport in brush-border-membrane vesicles isolated from human small intestine. *Biochem. J.* **168:**529–532

Lynch, A.M., McGivan, J.D. 1987. Evidence for a single common sodium-dependent transport system for alanine, glutamine, leucine, and phenylalanine in brush-border membrane vesicles from bovine kidney. *Biochim. Biophys. Acta* **899:**176–184

Makowske, M., Christensen, H.N. 1982. Hepatic transport system interconverted by protonation from service for neutral to service for anionic amino acids. *J. Biol. Chem.* **257:**14635–14638

Márquez, J., Quesada, A.R., Sánchez-Jiménez, F., Núñez de Castro, I. 1986. Determination of 27 dansyl-amino acid derivatives in biological fluids by reversed-phase high-performance liquid chromatography. *J. Chromatogr. Biomed. Appl.* **380:**275–283

Márquez, J., Sánchez-Jiménez, F., Medina, M.A., Quesada, A.R., Núñez de Castro, I. 1989. Nitrogen metabolism in tumor bearing mice. *Arch. Biochem. Biophys.* **268:**667–675

McCormick, J., Johnstone, R.M. 1990. Evidence for an essential sulfhydryl group at the substrate binding site of the A system transport of Ehrlich cell plasma membranes. *Biochem. Cell Biol.* **68:**512–519

McCormick, J., Tsang, D., Johnstone, R.M. 1984. A simple and efficient method for reconstitution of amino acid and glucose transport systems from Ehrlich ascites cells. *Arch. Biochem. Biophys.* **231:**355–365

Medina, M.A. 1989. Glutamina y Tumor: Transporte y Metabolismo. Ph.D. Thesis, Málaga University (*in Spanish*)

Medina, M.A., Núñez de Castro, I. 1990*a*. Glutaminolysis and glycolysis interactions in proliferant cells. *Int. J. Biochem.* **22:**681–683

Medina, M.A., Núñez de Castro, I. 1990*b*. Plasma membrane oxidoreduction and amino acid transport. *In:* Oxidoreduction in the Plasma Membrane: Relation to Growth and Transport. Vol. 1, pp. 247–255. F.L. Crane, D.J. Morré, and H. Löw, editors. CRC, Boca Ratón

Nordlie, R.C., Arion, W.J. 1966. Glucose-6-phosphatase. *Methods Enzymol.* **9:**619–625

Ookhtens, M., Baker, N. 1979. Fatty acid oxidation to H_2O by Ehrlich ascites carcinoma in mice. *Cancer Res.* **39:**973–980

Perán, S., Muñoz, M., Sainz, M.T. 1990*a*. Rapid and steady-state amino acid transport in perfused human fibroblast and colon adenocarcinoma cells: Effects of methotrexate. *Biochim. Biophys. Acta* **1024:**233–240

Perán, S., Muñoz, M., Sainz, M.T. 1990*b*. New approach to the study of transport of biomolecules by microcarrier cell cultures perfused in column applying a high resolution paired tracer technique. *In:* Cell Membrane Transport: Experimental Approaches and Methodologies. D.L. Yudilevich, I. Cabanchik, R. Devés, and S. Perán, editors. Plenum, London (*in press*)

Pola, E., Beltrán, J., Roca, A., Palacín, M., Zorzano, A., Testar, X. 1990. Sensitivity of system A and ASC transport activities to thiol-group-modifying reagents in rat liver plasma-membrane vesicles. *Biochem. J.* **271:**297–303

Quesada, A.R., McGivan, J.D. 1988. A rapid method for the functional reconstitution of amino acid transport system from rat liver plasma membranes. *Biochem. J.* **255:**963–969

Quesada, A.R., Medina, M.A., Márquez, J., Sánchez-Jiménez, F., Núñez de Castro, I. 1988. Contribution by host tissues to circulating glutamine in mice inoculated with Ehrlich ascites tumor cells. *Cancer Res.* **48:**1551–1553

Sauer, L.A., Dauchy, R.T. 1983. Ketone body, glucose, lactic acid and amino acid utilization by tumors *in vivo* in fasted rats. *Cancer Res.* **43:**3497–3503

Sips, H.J., De Graaf, P.A., Van Dam, K. 1982. Transport of L-aspartate and L-glutamate in plasma-membrane vesicles from rat liver. *Eur. J. Biochem.* **122:**259–264

Sips, H.J., Van Amelsvoort, J.M.M., Van Dam, K. 1980. Amino acid transport in plasma membrane vesicles from rat liver. *Eur. J. Biochem.* **105:**217–224

Snell, K. 1985. Enzymes of serine metabolism in normal and neoplastic tissues. *Biochem. Biophys. Acta* **843:**276–281

Spector, A.A., Steinberg, D. 1965. The utilization of unsterified palmitate by Ehrlich ascites tumor cells. *J. Biol. Chem.* **240:**3747–3753

Thordinke, J., Pelliniemi, T.T., Beck, W.S. 1979. Serine hydroxymethyltransferase activity and serine incorporation in leukocytes. *Cancer Res.* **39:**3435–3440

Urdiales, J.L., Medina, M.A., Núñez de Castro, I., Sánchez-Jiménez, F. 1989. Early systemic effect on the hepatic mitochondria of tumour bearing mice. *Cancer Lett.* **44:**179–183

Van Amelsvoort, J.M.M., Sips, H.J., Van Dam, K. 1978. Sodium-dependent alanine transport in plasma membrane vesicles from rat liver. *Biochem. J.* **174:**1083–1086

Zafra, F., Giménez, C. 1986. Characterization of glycine uptake in plasma membrane vesicles isolated from cultured glioblastoma cells. *Brain Res.* **397:**108–116

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