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HEXOSE AND AMINO ACID TRANSPORT BY CHICKEN EMBRYO FIBROBLASTS INFECTED WITH TEMPERATURE-SENSITIVE MUTANT OF ROUS SARCOMA VIRUS

COMPARISON OF TRANSPORT PROPERTIES OF WHOLE CELLS AND MEMBRANE VESICLES

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Summary

The effect of transformation on hexose and amino acid transport has been studied using whole cells and membrane vesicles of chicken embryo fibroblasts infected with the temperature-sensitive mutant of the Rous sarcoma virus, TS-68. In whole cells, TS-68-infected chicken embryo fibroblasts cultured at the permissive temperature (37°C) had a 2-fold higher rate of 2-deoxy-D-glucose uptake than the same cells cultured at the non-permissive temperature (41°C). However, both the non-transformed and transformed cells had comparable rates of α-aminoisobutyric acid transport. Membrane vesicles, isolated from TS-68-infected chicken embryo fibroblasts cultured at 41°C or 37°C, displayed carrier-mediated, intravesicular uptake of D-glucose and α-aminoisobutyric acid. Membrane vesicles from TS-68-infected chicken embryo fibroblasts cultured at 37°C had an approx. 50% greater initial rate of stereospecific hexose uptake than the membrane vesicles from fibroblasts cultured at 41°C. The two types of membrane vesicle had similar uptake rates of α-aminoisobutyric acid. The results of hexose and amino acid uptake by the membrane vesicles correlated well with those observed with the whole cells. K_m values for stereospecific D-glucose uptake by the membrane vesicles from TS-68-infected chicken embryo fibroblasts cultured at 41 and 37°C were similar, but the V value was

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Abbreviations: CEF, chicken embryo fibroblasts; TS-68, temperature-sensitive mutant of the Rous sarcoma virus; Glc, glucose; Gal, galactose; 3-OMeGlc, 3-*O*-methyl-D-glucose; 2-dGlc, 2-deoxy-D-glucose; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

greater for the membrane vesicles from TS-68-infected cells cultured at 37° C. Cytochalasin B competitively inhibited stereospecific hexose uptake in both types of membrane vesicle. These findings suggest that the membrane vesicles retained many of the features of hexose and amino acid transport observed in whole cells, and that the increased rate of hexose transport seen in the virally-transformed chicken embryo fibroblasts was due to an increase in the number or availability of hexose carriers.

Introduction

Currently, there is considerable interest in the possibility that nutrient transport may play an important role in the regulation of cellular metabolism and growth, since the cell membrane acts both as a permeability barrier and as a transducer of biological information. Thus, for example, an increase in the rate of hexose uptake has been reported as one of the early biochemical events to occur when chicken embryo fibroblasts (CEF cells) are transformed by the Rous sarcoma virus [1]. Whole cell transport studies have not definitively resolved the controversy concerning the mechanism and kinetics of this altered hexose uptake. Some whole-cell studies have attributed the increased hexose uptake to a change in V [2—6], whereas one study reports that the altered uptake results from a K_m change [7]. In addition, some investigators [7—9] report that the transformed CEF cells have no change in the transport of amino acids. This is unusual in light of the increased amino acid uptake observed in other transformed fibroblasts [10]. Furthermore, since these studies have been conducted in whole cells, the analysis of the transport mechanism is complicated by factors such as intracellular metabolism and compartmentalization.

The recent development of techniques for the isolation of cell plasma membrane vesicles that retain transport activity has permitted a more detailed study of the possible mechanisms involved in the transport of nutrients across cell membranes. In this and other laboratories, membrane vesicles prepared from transformed and non-transformed cells have been successfully used for the analysis of transport of amino acids [11—16], phosphate [17,18] and nucleosides [19]. Recently, we reported the increased hexose transport in plasma membrane vesicles from Simian virus 40-transformed mouse fibroblasts by the analysis of initial uptake rates using modified assay techniques [21].

In this study, we have extended our earlier observations concerning hexose and amino acid transport mechanisms in mouse fibroblast membrane vesicles to CEF cells infected with the temperature-sensitive mutant of the Rous sarcoma virus, TS-68. This is a good system for studying the effects of transformation, since both types of CEF cell are derived from the same primary culture and both are infected with the TS-68 Rous sarcoma virus but grown at different temperatures. The results revealed that membrane vesicles isolated from CEF cells catalyze carrier-mediated transport of hexose and amino acid, and that the enhanced hexose transport activity observed with transformed CEF cells can also be demonstrated in membrane vesicles derived from them.

Materials and Methods

Cell culture. Body walls from 10-day-old chicken embryos were dissociated with 0.25% trypsin and the cells were seeded into plastic roller bottles (Corning, 490 cm^2) containing 50 ml of growth medium and $4.5 \cdot 10^7$ cells. The cell growth medium was Dulbecco's modified Eagle's medium containing 4.5 g/l glucose, 10% tryptose phosphate broth (Difco) and 2% irradiated fetal calf serum (Microbiological Associates). After 7 days as primary cultures at 41°C, the cells were removed from roller bottles using 0.06% trypsin and 0.5 mM EDTA in phosphate-buffered saline without Ca^{2+} and Mg^{2+}. The secondary cultures were seeded at $1.5 \cdot 10^7$ cells in the roller bottles using the same growth medium and incubated at 41°C for 24 h. Then, the temperature-sensitive mutant of Rous sarcoma virus, TS-68 ($5 \cdot 10^6$ focus-forming units) were added to each roller bottle and they were incubated for 4—5 days at the permissive (37°C) and non-permissive (41°C) temperatures. In some experiments, similarly, the cells were transferred into dishes (35 and 100 mm) and infected with TS-68, followed by incubation in humidified CO_2 incubators at 37 and 41°C, respectively.

Preparation of membrane vesicles. Mixed membrane vesicles were prepared according to a previous report [20]. Confluent cells were harvested by scraping with a rubber blade at a density of $1—2.5 \cdot 10^5$ cells/cm^2 and collected in culture medium. All subsequent operations were performed at 4°C. The cell suspension was centrifuged at $500 \times g$ for 10 min and then suspended in 0.25 M sorbitol/1 mM Tris-Hepes (pH 7.5)/0.5 mM $MgCl_2$ (buffer A) followed by centrifugation at $800 \times g$ for 10 min. The cell pellet was resuspended in the same buffer and centrifuged at $1000 \times g$ for 20 min. The packed cell pellet was resuspended in 20 vols. of buffer A, and the cells were gently suspended with five strokes of a loose-fitting Dounce homogenizer. The washed cell suspension was placed in a nitrogen cavitation bomb (Artisan Industries, Waltham, MA) at 680 lb/$inch^2$ for 10 min. After the homogenate was collected, K_2EDTA (pH 7.5) was added to a final concentration of 1 mM. The homogenate was centrifuged at $750 \times g$ for 15 min to remove nuclei and any intact cells. Then, the post-nuclear supernatant was centrifuged at $20\,000 \times g$ for 15 min to pellet the mitochondria. The supernatant was centrifuged at $100\,000 \times g$ for 60 min. The pellet was resuspended in 0.1 M sorbitol/1 mM Tris-Hepes (pH 7.5) (buffer S), and centrifuged again at $100\,000 \times g$ for 60 min. The pellet was suspended in buffer S to give a final protein concentration of 4—8 mg/ml by passage through a fine-gauge needle. This fraction was stored at 4°C and used as mixed membrane vesicles within 3 days. Compared with the homogenates, the membrane preparations were enriched 3—4-fold for 5'-nucleotidase activity.

Transport assay of whole cells. The uptake of 2-dGlc or α-aminoisobutyric acid was measured at 25 ± 1°C in cells attached to the culture dish (35 mm), as previously described [21] with some modifications. The cell incubation medium contained 137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (buffer B). For studies in Na^+-free medium, the NaCl and Na_2HPO_4 of buffer B were replaced with choline chloride and K_2HPO_4. After removing culture medium, each dish was washed twice with 2 ml of buffer B (incubation for 10 min). Buffer B (0.5 ml) containing

radioactively labeled substrate (2-d[^{14}C]Glc and α-amino[^{14}C]isobutyric acid, 1 Ci/mol) at the concentration of 1 mM was added to each dish and the cells were incubated for a specified period of time. At the end of the incubation period, medium was immediately removed by suction and the dish was rapidly rinsed four times with 2 ml of ice-cold buffer B. Then 0.5 ml of 70% ethanol was added and the aqueous ethanol allowed to remain for 1 h in contact with the denatured cells. The radioactivity of the ethanol extract was determined in 10 ml Instagel (Packard) by liquid scintillation counting. After removal of the ethanol, the dishes were dried and 1 ml of 0.1 N NaOH containing 0.1% sodium dodecyl sulfate was added to the denatured cells. This fraction was used for protein determination. In most uptake experiments, simple diffusion and nonspecific adsorption were monitored simultaneously by including L-[^{3}H]Glc (3 Ci/mol) in the assay medium, and corrections for them were made by subtracting the amount of L-Glc associated with each sample.

Transport assay of membrane vesicles. In order to obtain data on the initial rates of uptake, hexose transport was assayed as described previously [20]. Membrane vesicles dispersed in buffer S were preincubated for 10 min (1.5—3 mg protein/ml). The medium of radioactive substrates was prepared with double labeling such as D-[^{14}C]Glc (100 μCi/ml) and L-[^{3}H]Glc (200 μCi/ml). Two 15-μl drops, one containing membrane vesicle suspension and the other containing labeled substrates in buffer S, were placed close to each other at the bottom of a clear plastic test tube (Falcon, 12 × 75 mm). The reaction was initiated by rapid mixing using a vortex mixer. At the stated time points, 1 ml of ice-cold stop solution (buffer S containing 0.1 mM phloretin and 0.1% (v/v) ethanol) was added to the reaction tube. The contents of the test-tube were immediately poured onto the Millipore nitrocellulose filter (0.45 μm, 2.5 cm diameter) and washed with 5 ml of ice-cold stop solution. Filtration and washing were finished within 10 s. Radioactivity of dried filters in Instagel (Packard) was determined by scintillation counting. In order to obtain reproducible results and also to determine stereospecific transport, corrections for simple diffusion and nonspecific trapping of hexose were made by subtracting the amount of L-Glc associated with each sample. Background activity was determined by the addition of 15 μl isotope mixture to 1 ml of ice-cold stop solution containing 15 μl of diluted membrane vesicles. The incubations were carried out at 25 ± 1°C except where specified otherwise.

α-Aminoisobutyric acid uptake was measured by either of two methods at 25 or 37°C. One method involved the use of a series of individual reaction tubes for each time point similar to hexose uptake. An isotope medium containing α-[^{3}H]aminoisobutyric acid (200 μCi/ml) with either 200 mM NaSCN or 200 mM choline chloride in buffer S was used, and the stop solution was ice-cold buffer S. A second assay method involved the use of a single reaction tube containing 200 μl of the reaction mixture, as described previously [13]. The reaction was initiated by the addition (20 μl) of α-[^{3}H]aminoisobutyric acid with either NaSCN or choline chloride to membrane vesicles (180 μl). Aliquots of 15 μl were withdrawn at the stated time points, diluted in 1 ml of ice-cold buffer S, filtered and washed with 5 ml of ice-cold buffer S.

Measurement of intravesicular volume. The intravesicular volume (μl/mg of protein) was calculated from the equilibrium uptake (pmol/mg of protein) of

1 mM D-Glc or 3-OMeGlc obtained at 20 min. Average values of intravesicular volumes were 1.53 μl/mg of protein (41°C) and 1.50 μl/mg of protein (37°C). These values were much smaller compared with membrane vesicles from mouse fibroblasts [20].

Analytical method. Protein was determined by the method of Lowry et al. [22]. Purity of mixed membrane vesicles was checked by measuring the activities of marker enzymes; 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) [23] for plasma membrane, NADH-cytochrome *c* reductase (NADH : (acceptor) oxidoreductase, EC 1.6.99.3) [24] for endoplasmic reticulum, and cytochrome oxidase (ferrocytochrome *c* : oxygen oxidoreductase, EC 1.9.3.1) [25] for mitochondria.

Materials. D-[U-^{14}C]Glucose (360 Ci/mol), L-[1-^{3}H]glucose (17.5 Ci/mmol), 3-*O*-methyl-D-[U-^{14}C]glucose (360 Ci/mol), 2-deoxy-D-[U-^{14}C]glucose (337 Ci/mol), D-[1-^{14}C]galactose (53.9 Ci/mol), α-amino[1-^{14}C]isobutyric acid (51.6 Ci/mol), α-[^{3}H]methyl-aminoisobutyric acid (10 Ci/mmol) and [^{3}H]-adenosine 5′-monophosphate (diammonium salt, 15 Ci/mmol) were purchased from New England Nuclear. Cytochalasin B, cytochrome *c* and NADH were obtained from Sigma Chemical Co. and phloretin was from ICN Pharmaceuticals.

Results

Evidence for carrier-mediated transport of hexose and amino acid by membrane vesicles

As we had previously shown with mouse fibroblast membrane vesicles [20], the hexose and amino acid uptakes by membrane vesicles from TS-68-infected CEF cells were osmotically sensitive. The amount of substrate accumulated in the vesicles was inversely proportional to the sorbitol concentration of the medium. Extrapolation to infinite sorbitol concentration passed through the origin for both D-Glc and α-aminoisobutyric acid (data not shown).

The time course of α-aminoisobutyric acid uptake is shown in Fig. 1. The simultaneous addition of α-aminoisobutyric acid and NaSCN to the vesicles produced a transient accumulation (overshoot) of α-aminoisobutyric acid above the maximal accumulation attained when the vesicles were in the presence of choline chloride.

As shown in Fig. 2, the transport of D-Glc by the membrane vesicles from TS-68-infected CEF cells cultured at 41°C was time-dependent, stereospecific and reached equilibrium within 10 min. Stereospecific uptake of D-Glc as measured by the difference between D-[^{14}C]Glc and L-[^{3}H]Glc uptake, appeared to be linear for 5 s, reached a maximum value at 5—10 min, and then declined as L-Glc levels slowly reached those of D-Glc after several hours. In contrast, stereospecific uptake of D-Gal was much slower compared with D-Glc. Cytochalasin B has been shown to be a potent inhibitor of hexose transport in a wide variety of cell types, but has no discernible effect on hexose phosphorylation [26,27]. Cytochalasin B strongly inhibits stereospecific D-Glc and D-Gal uptake by membrane vesicles from CEF cells.

The substrate specificities of the hexose and amino acid-transporting systems were examined by competitive inhibition studies involving either D-Glc or α-aminoisobutyric acid uptake in the presence of unlabeled substrates (Table I).

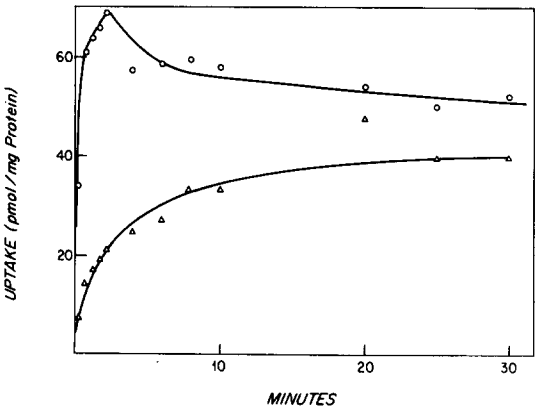

Fig. 1. Time course of α-aminoisobutyric acid uptake by membrane vesicles from TS-68-infected CEF cells cultured at 41°C. The vesicles (180 μl, 500 μg of protein) were preincubated at 37°C for 10 min in buffer S, and the reaction was initiated by the addition (20 μl) of 1 mM α[^{3}H]aminoisobutyric acid (1000 Ci/mol) in the presence of 1 M NaSCN or 1 M choline chloride. Then aliquots of 15 μl were removed at the stated time points. Final concentrations were as follows: α-aminoisobutyric acid, 0.1 mM; NaSCN (○), 100 mM; choline chloride (△), 100 mM.

Of these substrates 2-dGlc had the strongest inhibitory effect on the stereospecific D-Glc uptake, followed by 3-OMeGlc, D-Glc and D-Gal, respectively, and L-Glc had no effect. α-Methylaminoisobutyric acid, α-aminoisobutyric acid and alanine (A system) exerted a pronounced inhibitory effect on α-aminoisobutyric acid uptake; this was in contrast to the minimal effect observed with leucine (L system).

The above results indicate that the nutrient carriers of D-Glc and α-aminoisobutyric acid in the membrane vesicles function in a specifically selective manner.

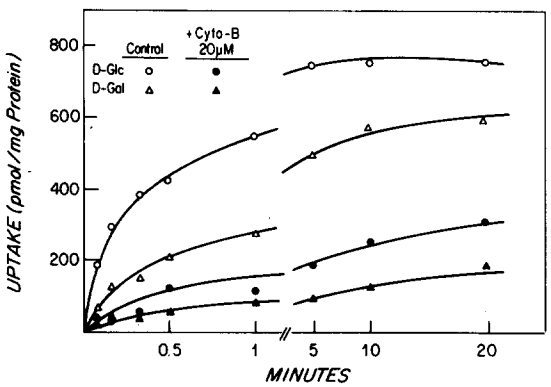

Fig. 2. Time course of stereospecific D-Glc and D-Gal uptake by membrane vesicles from TS-68-infected CEF cells cultured at 41°C. Membrane vesicles were preincubated for 10 min in buffer S with or without 40 μM cytochalasin B, dissolved in 1% dimethylsulfoxide. The vesicles (15 μl, 15 μg of protein) were incubated with isotope mixture (15 μl) containing either D-[^{14}C]Glc (50 Ci/mol) and L-[^{3}H]Glc (100 Ci/mol) or D-[^{14}C]Gal (50 Ci/mol) and L-[^{3}H]Glc (100 Ci/mol) (final concentration, 1 mM). Stereospecific D-Glc and D-Gal uptakes were calculated as described in the text. Each point represents the mean value of two experiments. ○, D-Glc, control; ●, 20 μM cytochalasin B; △, D-Gal, control; ▲, 20 μM cytochalasin B.

TABLE I

COMPETITIVE INHIBITION FROM UNLABELED SUBSTRATE OF D-Glc AND α-AMINOISOBUTYRIC ACID UPTAKE BY MEMBRANE VESICLES FROM TS-68-INFECTED CEF CELLS CULTURED AT 41°C

The vesicles (15 μl, 30 μg of protein) were incubated with the isotope (15 μl, either 2 mM D-[^{14}C]Glc (50 Ci/mol) and 2 mM L-[^{3}H]Glc (100 Ci/mol) or 0.2 mM α-[^{3}H]aminoisobutyric acid (1000 Ci/mol) and 200 mM NaSCN) containing additional unlabeled hexose (10 mM) or amino acid (1 mM) in buffer S. Stereospecific D-Glc uptake and Na^{+}-dependent α-aminoisobutyric acid uptake at 5 s were determined as described in the text. Each value represents the mean ± S.E. of four to five experiments.

Unlabeled substrate added	Uptake	
	pmol/mg protein in 5 s	% of control
D-Glucose uptake		
control	100 ± 4	100
D-glucose	56 ± 9	55
2-deoxy-D-glucose	50 ± 2	50
3-*O*-methyl-D-glucose	53 ± 5	53
D-galactose	82 ± 3	82
L-glucose	102 ± 6	102
α-Aminoisobutyric acid uptake		
control	20 ± 1	100
α-aminoisobutyric acid	14 ± 1	72
α-methylaminoisobutyric acid	12 ± 1	61
alanine	10 ± 1	53
leucine	18 ± 1	90

Hexose and amino acid transport by whole cells and by membrane vesicles of TS-68-infected CEF cells

In order to confirm the difference of transport activity in the intact whole cells of non-transformed and transformed cells, the uptake of 2-dGlc and α-aminoisobutyric acid was studied using monolayer cells in plastic culture dishes. The initial rate of 2-dGlc uptake (Fig. 3A) was increased approx. 2-fold in TS-68-infected CEF cells cultured at 37°C, but α-aminoisobutyric acid (Fig. 3B) was not increased. The results of hexose uptake are comparable with those reports by Weber [2] and Kletzien and Perdue [4]. Although the results of α-aminoisobutyric acid uptake basically agreed with previous findings that there was no increase in α-aminoisobutyric acid transport by Rous sarcoma virus-transformed CEF cells [7—9], our results actually showed a slight decrease in α-aminoisobutyric acid uptake.

The initial rate of stereospecific D-Glc uptake (Fig. 4A) by membrane vesicles from TS-68-infected CEF cells cultured at 37°C was approx. 60% greater compared with the membrane vesicles from fibroblasts cultured at 41°C. This was similarly observed for 2-dGlc uptake by whole cells. 3-OMeGlc, a non-metabolizable glucose analogue, was used to verify that metabolic function was not responsible for this transport assay system of hexose. Consequently, D-Glc and 3-OMeGlc showed the same equilibrium value of uptake. On the other hand, Na^{+}-dependent α-aminoisobutyric acid uptake showed no enhancement or a slight decrease in uptake by the membrane vesicles from TS-68-infected CEF cells cultured at 37°C (Fig. 4B).

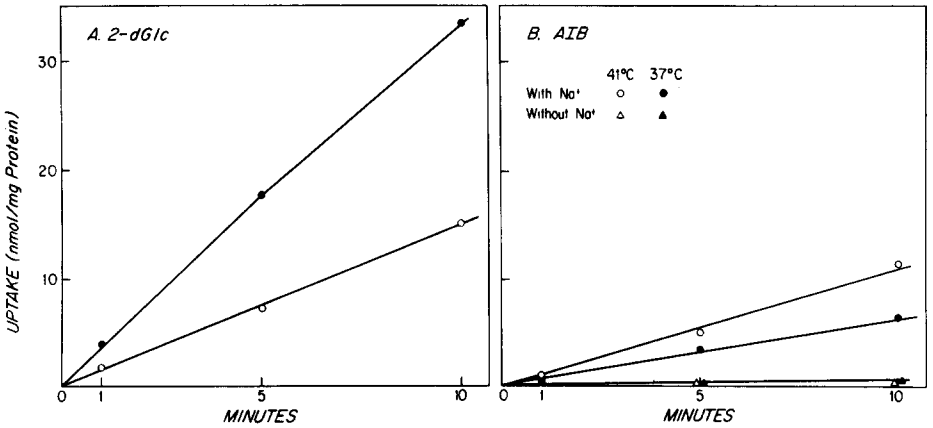

Fig. 3. Time course of 2-dGlc (A) and α-aminoisobutyric acid (AIB) (B) uptake by whole cells of TS-68-infected CEF cells cultured at 41 and 37°C. Confluent cells in culture dishes were washed twice with buffer B. The uptake was initiated by the addition (0.5 ml) of radioactively labeled substrate medium at the concentration of 1 mM. The conditions were as follows: (A) 2-[^{14}C]dGlc (1 Ci/mol) and L-[^{3}H]Glc (3 Ci/mol) in buffer B, cultured at 41°C (○) and 37°C (●); (B) α-amino[^{14}C]isobutyric acid (1 Ci/mol) and L-[^{3}H]Glc (3 Ci/mol) in buffer B (○,●) or Na^{+}-free buffer B (△,▲), cultured at 41°C (○,△) and 37°C (●,▲).

For comparison purposes Table II summarizes the results of hexose and amino acid transport by whole cells and by membrane vesicles of TS-68-infected CEF cells cultured at 41 and 37°C.

Furthermore, in order to confirm the differences in hexose transport by membrane vesicles, we studied the effect of countertransport on stereospecific D-Glc uptake (Fig. 5). Vesicles preloaded with high concentration of unlabeled D-Glc showed enhancement of stereospecific D-[^{14}C]Glc accumulation by countertransport in both types of membrane vesicle, compared with controls. These results suggest that the enhancement in the initial rate of hexose uptake by membrane vesicles from transformed cells is due to the increase in carrier-mediated hexose transport into vesicles.

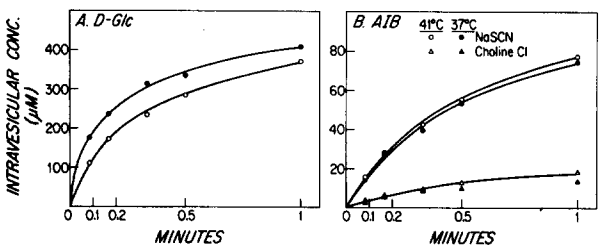

Fig. 4. Time course of D-Glc (A) and α-aminoisobutyric acid (AIB) (B) uptake by membrane vesicles from TS-68-infected CEF cells cultured at 41 and 37°C. (A) The membrane vesicles, cultured at 41°C (○) and 37°C (●), were incubated with substrate medium (15 μl) containing D-[^{14}C]Glc (50 Ci/mol) and L-[^{3}H]-Glc (100 Ci/mol) (final concentration, 1 mM). (B) The membrane vesicles (15 μl), cultured at 41°C (○,△) and 37°C (●,▲), were incubated with substrate medium (15 μl) containing α-[^{3}H]aminoisobutyric acid (1000 Ci/mol) with NaSCN (○,●) or choline chloride (△,▲) (final concentration, 0.1 mM α-aminoisobutyric acid, 100 mM NaSCN, 100 mM choline chloride). The intravesicular volumes were 1.4 μl/mg of protein (41°C) and 1.6 μl/mg of protein (37°C). Each point represents the mean value of two experiments.

TABLE II

HEXOSE AND AMINO ACID TRANSPORT BY WHOLE CELLS AND BY MEMBRANE VESICLES OF TS-68-INFECTED CEF CELLS CULTURED AT 41 AND 37°C

Stereospecific hexose uptake and Na^+-dependent α-aminoisobutyric acid uptake by whole cells or membrane vesicles were determined as described for Figs. 3 and 4. The data are normalized as the relative ratio of initial uptake rate in the cells cultured at 37 to 41°C. Each value represents the mean ± S.E. for separate preparations (number in parentheses), with duplicate or triplicate determinations.

	Relative transport activity (cultured at 37°C/ cultured at 41°C)
Whole cells	
2-deoxy-D-glucose	2.12 ± 0.18 (5)
α-aminoisobutyric acid	0.60 ± 0.10 (4)
Membrane vesicles	
D-glucose	1.47 ± 0.10 (7)
D-galactose	1.66 ± 0.11 (3)
α-aminoisobutyric acid	0.86 ± 0.05 (6)

Kinetic parameters were obtained and calculated by plotting the initial rates by means of a number of linear transformation methods and similar results were obtained. As shown in Fig. 6A, Lineweaver-Burk plots of the initial rates of stereospecific D-Glc uptake at substrate concentrations between 0.5 and 20 mM revealed that K_m (7.1 mM) for D-Glc transport was the same for membrane vesicles from TS-68-infected CEF cells at 41°C and 37°C. However, V values for stereospecific D-Glc uptake were 9.6 (nmol/mg protein per min) for the fibroblasts cultured at 41°C and 17.1 (nmol/mg protein per min) for the fibroblasts cultured at 37°C, suggesting an increase in the number or availability of hexose carriers in transformed cells. The inhibition of stereospecific

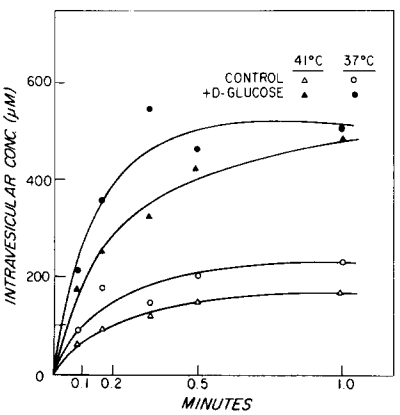

Fig. 5. Countertransport effects on stereospecific D-Glc uptake. Vesicles were preincubated with (▲,●) or without (△,○) 10 mM unlabeled D-Glc for 10 min, and then the aliquots (15 μl) (△ and ▲, 41°C; ○ and ●, 37°C) were incubated with the isotope mixture (150 μl) containing 1 mM D-[^{14}C]Glc (10 Ci/mol) and 1 mM L-[^{3}H]Glc (20 Ci/mol) during the indicated periods. The intravesicular volumes were 1.5 μl/mg of protein (41°C) and 1.3 μl/mg of protein (37°C). Each point represents the mean value of two experiments.

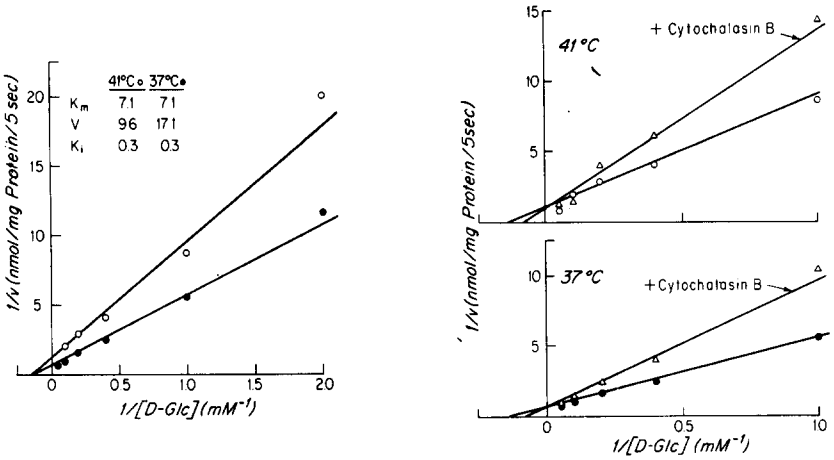

Fig. 6. Lineweaver-Burk plots of initial rates of stereospecific D-Glc uptake by membrane vesicles from TS-68-infected CEF cells cultured at 41 and 37°C. Sterespecific uptake for 5 s at concentrations between 0.5 and 20 mM was determined in the absence (A) and the presence (B) of cytochalasin B (0.2 μM), dissolved in 0.01% dimethylsulfoxide, as described for Fig. 2. (A) ○, 41°C; ●, 37°C. (B) ○ and ●, control; △, cytochalasin B. The intravesicular volumes were 1.5 μl/mg of protein (41°C) and 1.8 μl/mg of protein (37°C). Each point represents the mean value of three experiments.

D-Glc uptake by cytochalasin B was also evaluated by kinetic methods. Fig. 6B shows that this inhibition was of the competitive type with an apparent dissociation constant (K_i) of 0.3 μM for both types of membrane vesicle.

Discussion

Functional membrane vesicles are quite useful in investigating the uptake of amino acids and hexoses because any possible cytoplasmic and metabolic effects on transport are minimized. The present results are noteworthy in that stereospecific hexose transport and Na^+-dependent α-aminoisobutyric acid transport by membrane vesicles, isolated from TS-68-infected CEF cells cultured at 41 and 37°C, reflected similar changes in uptake as seen with intact whole cells. These findings therefore emphasize the utility of the membrane vesicle technique in studying transport processes.

The nutrient uptake by the membrane vesicles fulfilled the criteria of osmotic sensitivity, stereospecificity and concentration-dependency for carrier-mediated transport. Furthermore, D-Glc uptake was shown to be specifically inhibited by cytochalasin B and stimulated by countertransport. The α-aminoisobutyric acid uptake demonstrated Na^+-stimulated active transport by the 'overshoot' of α-aminoisobutyric acid accumulation.

In order to elucidate the regulation mechanism of hexose and amino acid transport changes following the virus transformation in CEF cells, D-Glc and α-aminoisobutyric acid transport activities were compared using whole cells and membrane vesicles. As reported in previous papers [7—9], the rate of Na^+-dependent α-aminoisobutyric acid uptake by whole cells and by membrane vesicles was not increased with transformation, but was slightly decreased.

When D-Glc, 3-OMeGlc or D-Gal was used as substrate for transport activity, the membrane vesicles from the fibroblasts cultured at 37°C were found to retain the increased activity of stereospecific uptake compared with the membrane vesicles from the fibroblasts cultured at 41°C, and thus reflected the increase in 2-dGlc uptake observed with transformed whole cells.

An increase in the rate of hexose uptake is one of the early biochemical events in transformation of CEF cells by Rous sarcoma virus. Weber [2] and Kletzien and Perdue [4] reported that the apparent V for hexose transport was increased in transformed cells, consistent with the concept that the increased hexose transport rate is due to an increased number of hexose carriers. Furthermore, Salter and Weber [28] have used cytochalasin B binding as a method to determine if the increased hexose transport rate in transformed CEF cells is due to an increased number of hexose carriers. They found that the difference in glucose-specific cytochalasin B binding between non-transformed and transformed cells correlate closely with the difference in the hexose transport rate, suggesting that the increased hexose transport rate is due to an increased number of hexose carriers. The present results with the membrane vesicles from TS-68-infected CEF cells cultured at 41 and 37°C also indicate that the alteration in nutrient transport following transformation is primarily an increased rate of hexose uptake. That the V of hexose uptake increased with transformation, was consistent with our previous findings concerning hexose transport by transformed mouse fibroblasts [20]. It also agreed with the recent finding of Zala and Perdue using membrane vesicles from CEF cells [29,30]. However, the extent of increased hexose uptake was greater in whole cells than in membrane vesicles. Recently, Lee and Lipmann [31] have isolated a glucose-binding protein loosely bound to normal and Rous sarcoma virus-transformed CEF cells which stimulates cellular glucose uptake. Thus, a partial removal of such a dissociable protein during membrane preparations may explain transport differences between intact cells and membrane vesicles.

In conclusion, the membrane vesicles from TS-68-infected CEF cells retained the features of hexose and amino acid transport observed in whole cells. The increased rate of hexose transport seen in the virally-transformed CEF cells probably is the result of an increase in the number or availability of hexose carriers.

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