Amino Acid and Sugar Transport in Cells Permissively Infected with Simian Virus 40

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SUMMARY

Transport of α-aminoisobutyric acid and 2-deoxy-D-glucose in African green monkey kidney cells was measured 8 to 100 hr following permissive simian virus 40 infection. No differences in transport were detected during the time-period studies, and no significant differences were seen between the apparent Michaelis-Menten constants of normal and virally infected cells. The absence of transport enhancement in permissive simian virus 40 infection suggests that the augmented transport of viral-transformed cell lines devolves upon altered host genome function.

INTRODUCTION

Although no single alteration of the neoplastic cell has explained its competitive success over normal cells, 1 hypothesis attracts increasing attention, namely, do malignant cells transport essential nutrients more effectively than normal cells?

Hatanaka (4) maintains that enhanced transport is associated with neoplastic transformation. It remains unknown, however, whether such enhancement occurs in permissive viral infections.

This paper is a study of the transport of a nonmetabolizable amino acid, AIB, and 2-deoxy-D-glucose in VERO infected with SV40. From measurements of AIB transport and 2-deoxy-D-glucose transport, we conclude that lytic viral infection is not associated with transport alterations such as those observed in the transformed state.

MATERIALS AND METHODS

Cell Cultures. VERO cells were maintained in modified McCoy's medium (Associated Biomedic Systems, Buffalo, N.Y.) with 10% fetal calf serum, using 38-mm screw-cap disposable roller bottle cultures (Bellco Glass, Inc., Vineyard, N. J.). For transport studies, the cells were subcultured on sterile glass coverslips, 11 x 22 mm (Arthur H. Thomas Co., Philadelphia, Pa.), previously secured to the bottoms of sterile, plastic 60- x 15-mm Petri dishes (Falcon Plastics, Oxnard, Calif.) using sterile silicone grease (Dow Corning Corp., Midland, Mich.). The roller bottle cultures were dissociated with 0.25% trypsin-EDTA (in modified Puck's Saline A), and the cells were resuspended in modified McCoy's medium and transferred to the Petri dishes containing the coverslips. The initial seeding densities were 2.5 to 13 x 10⁴ cells/ml. After 8 to 48 hr at 37° in a humid 5% CO₂/95% air atmosphere, the Petri dishes containing the coverslips were washed several times with sterile PBS (pH 7.4) (2). SV40, at a titer of 10⁶ plaque-forming units/ml, was suspended in serum-free medium, and 3.5 ml were layered over the subcultures. A multiplicity of infection of 100 was used in all experiments. After a 3-hr period of viral adsorption, 0.5 ml of 10% fetal calf serum was added to deactivate the virus. The cultures were then incubated under the previously described conditions, from 8 to 100 hr, before transport measurements. Control cultures were treated in a similar fashion, except that no virus was added to the serum-free medium. Several infected cultures were kept for 100 hr to monitor viral CPE. CPE was monitored by light microscopy of infected cultures. Cultures were examined every 24 hr for nuclear pyknosis, rounding up of cells, and the loss of adherence to the coverslip surface.

Determination of Cell Density. At the time of subculture 0.2 to 0.5 ml [¹⁴C]leucine (New England Nuclear, Boston, Mass.; specific activity, 280 mCi/mmole) was added to the cell suspension to label cell protein. Transport data are related to cell protein and cell number in terms of incorporated [¹⁴C]leucine cells per coverslip.

AIB Transport. Prior to the transport experiments, cultures were washed twice with 3.5 ml of PBS and then incubated with PBS containing 15 mm glucose for 1 hr. The PBS was then decanted and replaced by 3.5 ml of PBS containing [³H]AIB (ICN Nutritional Biochemicals Div., Cleveland, Ohio; specific activity, 2.5 mCi/mmole) at final AIB concentrations ranging from 2 to 20 x 10⁻⁴ M AIB. Uptake was then allowed to proceed at 37° for 20 min. Four replicate coverslips were incubated at each concentration.

At the end of the incubation period the isotope-containing medium was decanted, and the dish was washed 3 times with 10 ml of iced PBS to remove any unincorporated amino acid.
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acid. After the final washing, coverslips were transferred to glass scintillation vials containing 10 ml of Bray's solution (1) (New England Nuclear) and counted with a Packard Tri-Carb Model 3200 liquid scintillation spectrometer. Counting time was adjusted to obtain a minimum of 10⁴ counts per sample over background. Uptake of AIB was measured as a ratio of ³H/¹⁴C counts.

[³H]inulin (1.25 mCi/ml) was substituted for the substrate in 1 culture plate of each experimental group. The retained radioactivity after washing of the coverslips provided an estimate of trapped extracellular fluid. Less than 5% of the ³H radioactivity remained on the coverslips after washing.

2-Deoxy-D-glucose Transport. The methods used for assay of AIB transport were also used to determine uptake of [³H]-2-deoxy-D-glucose (New England Nuclear; specific activity, 7.2 Ci/mmole) at final sugar concentrations ranging from 1 to 30 × 10⁻⁴ M.

RESULTS

Leucine Incorporation. [¹⁴C]Leucine uptake proceeded linearly over 4 to 100 hr at cell concentrations ranging between 1 and 45 × 10⁴ cells/ml. This linear relationship allows us to express amino acid uptake and sugar transport in terms of [¹⁴C]leucine incorporation.

Transport at Varying Cell Densities. Cells were deposited onto coverslips at initial densities of 2.5 to 13 × 10⁴ cells/sq cm. Thus, experiments were done on cells sparsely distributed on coverslips as well as cells approaching confluence. Virus infection did not affect transport over the entire range of densities examined.

Viral CPE. Several cultures from each of the experimental groups were examined for viral CPE at 24-hr intervals. Vacuolization was seen in 30% of the cells at 24 hr, rounding and pyknosis were evident in 50% by 48 hr, and by 96 hr almost 100% of the cells had rounded up and detached from the coverslips.

Transport Experiments. AIB transport was measured at 8, 24, 52, and 100 hr after viral adsorption. No differences in initial uptake between control and virus-infected cells could be detected at substrate concentrations ranging from 2 to 20 × 10⁻⁴ M. Transport proceeded linearly over the 20-min incubation and no saturation of transport was observed in either infected or control cells. A plot of the reciprocal initial uptake velocities, against the reciprocal substrate concentrations, 1/S, i.e., the Lineweaver-Burk plot, yielded a 2-component curve for AIB transport at all times transport was examined (Chart 1).

In cells transformed by DNA viruses, transport stimulation is characterized by increases in Vₘₐₓ. As pointed out by Isselbacher (5), uptake does not conform with simple Michaelis-Menten kinetics, and Weber (8) reports both saturable and nonsaturable modes of uptake in normal and virally transformed chicken embryo fibroblasts. Plotting such data on the Lineweaver-Burk double reciprocal plot results in a 2-component curve indicating 2 transport systems of differing substrate affinity. By extrapolation of the high 1/S values, approximate Kₘ’s can be obtained for high-affinity transport in normal and virally infected cells. As shown in Chart 1, we find a 2-component Lineweaver-Burk plot for AIB transport, suggesting the participation of multiple uptake modalities.

Using a program for a nonlinear regression least-squares power series (Wang 500 computer, Program Library Number 2029), a best-fit curve was obtained for the data points (Chart 2). The apparent Michaelis-Menten binding constant, Kₘ, and the maximal rate of uptake, Vₘₐₓ, were obtained for the high-affinity component of AIB uptake by extrapolation of the high 1/S values. Table 1 shows the results of these analyses at various times after viral infection.

![Chart 1. Lineweaver-Burk plots for AIB transport in VERO cells. Approximately, 36 hr before use the cells were trypsinized, suspended with [¹⁴C]leucine, and seeded onto sterile glass coverslips. At 24, 52, and 100 hr before transport of AIB measured, one-half of these cultures were infected with SV40 in serum-free medium to which 10% fetal calf serum was added 3 hr later. At the time of use, the cells were washed, incubated for 1 hr in PBS plus 15 mM glucose, and [³H]AIB in PBS (pH 7.4), was then added. After incubation at 37° for 20 min (uptake was linear for 30 min), the coverslips were washed in 10 ml of iced PBS and transferred to glass scintillation vials for counting. AIB uptake per cell is expressed as a ratio of [³H]AIB cpm/[¹⁴C]leucine cpm/20 min. Each point represents the average of at least 3 replicate specimens. O, control; V, viral infected.](image-url)
Table 1

Kinetic parameters of permissive viral infection

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Substrate</th>
<th>Time of infection (hr)</th>
<th>Vmax</th>
<th>Km (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AIB</td>
<td>24</td>
<td>7.6</td>
<td>1 x 10^-8</td>
</tr>
<tr>
<td>2</td>
<td>AIB</td>
<td>52</td>
<td>24.4</td>
<td>2 x 10^-8</td>
</tr>
<tr>
<td>3</td>
<td>AIB</td>
<td>100</td>
<td>91</td>
<td>2.9 x 10^-8</td>
</tr>
<tr>
<td>4</td>
<td>2-Deoxy-D-glucose</td>
<td>8</td>
<td>67</td>
<td>1.5 x 10^-7</td>
</tr>
<tr>
<td>5</td>
<td>2-Deoxy-D-glucose</td>
<td>72</td>
<td>33</td>
<td>1.8 x 10^-7</td>
</tr>
</tbody>
</table>

DISCUSSION

Numerous lines of fibroblasts exhibit enhanced uptake of sugars, such as 2-deoxy-D-glucose, after transformation by oncogenic DNA or RNA viruses (4, 5). Opinion is divided as to the effect of transformation on amino acid transport. Foster and Pardee (3) report a 2-fold increase in AIB and cycloleucine accumulation in polyoma-transformed mouse 3T3 fibroblasts, but they attribute this stimulation to a loss of density dependence in the neoplastic cells rather than neoplastic changes. Isselbacher (5), however, found a 2.5 to 3.5 greater Vmax in polyoma-transformed baby hamster kidney fibroblasts and SV40-transformed BALB/c 3T3 mouse fibroblasts compared to the value measured in nonconfluent parental cells and attributed these changes to neoplastic transformation.

Examining the transport of AIB and 2-deoxy-D-glucose permissive infection of VERO cells by SV40 virus, we find no changes in either transport at 8 to 100 hr after viral adsorption. This suggests that previously reported transport stimulation in transformed cells (3, 4–6) is due to changes in host phenotype and is not a direct expression of the SV40 genome. Recent evidence by Romano and Colby (7) on 2-deoxy-D-glucose uptake in mouse 3T3 cells transformed by SV40 also suggests that the increased uptake is not due to the simple expression of viral genome.

The use of 2-deoxy-D-glucose as pointed out by Romano and Colby (7) is complicated by the fact that this sugar can be phosphorylated. This can lead to misinterpretation of the data since phosphorylation of the sugar yields an anionic product that will not exchange with extracellular 2-deoxy-D-glucose. This clearly is not an issue in our studies since we observed no enhancement of 2-deoxy-D-glucose uptake in virally infected VERO cells.
Fitting the experimental points for initial AIB uptake by means of a nonlinear regression least-squares power series program, and extrapolating the values at the low substrate concentrations (Chart 2), we obtained $K_m$ of 1 to $2.9 \times 10^{-4}$ M for control cells and $K_m$ of 1 to $2.5 \times 10^{-4}$ M after viral infection (Table 1). Inspection of the experimental data for AIB transport revealed no significant differences between the extrapolated $V_{\text{max}}$ values of the high affinity system in infected versus control cells. In all cases, the high-substrate region of the Lineweaver-Burk plots curved toward the origin.

In the case of 2-deoxy-D-glucose transport, the Lineweaver-Burk plots yielded straight lines, indicating a single mode for transport in these cells. The $K_m$ for control was 1.5 to $1.8 \times 10^{-4}$ for controls and 1.5 to $2.0 \times 10^{-4}$ for virally infected cells; again there was no significant change in the $V_{\text{max}}$.

Despite the uncertainties in the kinetic parameters for AIB transport, our data clearly show that permissive infection of VERO cells with SV40 virus does not appreciably increase or decrease transport of AIB or 2-deoxy-D-glucose between 8 hr after virus infection and cell death. This suggests no general impairment of sugar or amino acid transport prior to cell lysis. Our results also imply that the enhancement of sugar and amino acid transport seen after oncogenic transformation with SV40 virus is a function of the host genome and the transformed state, rather than a change programmed by the transforming virus itself. In our continuing investigations, we will direct our attention from the viral genome to the function of the transformed genetic material of the cell.

REFERENCES

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