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SPECIFIC INCREASE IN THYMIDINE TRANSPORT AT A PERMISSIVE TEMPERATURE IN THE RAT KIDNEY CELLS INFECTED WITH *src*^{ts}-ROUS SARCOMA VIRUS

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Thymidine transport was found to be increased two-fold at a permissive temperature in the cells of a normal rat kidney (NRK) line which was infected with a temperature-sensitive Rous sarcoma virus. This increase in thymidine transport was independent of cell density and coincided with the changes in cellular morphology that result from this temperature shift. A double reciprocal plot of the data demonstrated two saturable components (K_m values of 60 μM and 250 μM at 39°, non-permissive temperature) of the uptake of thymidine, and the drop of temperature (at 33°, the permissive temperature for transformation) decreased K_m to one half, but did not change V_{max}. These results indicate that a qualitative alteration of thymidine transport took place in the presence of the active src gene product.

There is increasing evidence that the growth of animal cells may be regulated, at least in part, by the availability of essential nutrients (1). Therefore, there is much interest in membrane transport mechanisms in view of changes in transport activity that parallel the changes in cell growth properties. Although, alterations of the hexose (2,3) and amino acid (4) transport systems in cells transformed by viruses are generally accepted, no corresponding alteration in properties of the nucleoside transport system has yet been reported.

During a search for potential antitumor agents which are preferentially effective against tumor cells in which the src gene is expressed, we found an increase in thymidine transport in such cells. This increase appears to be responsible for the selective toxicity of some nucleoside antibiotics toward cells in which the src gene is expressed.

In this paper, we report the nature of alteration of thymidine transport. The toxicity studies of some antitumor agents related to this difference will be published elsewhere.

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MATERIALS AND METHODS

Cell culture The cells of a normal rat kidney (NRK) line infected with ts25, a T-class mutant of Rous sarcoma virus Prague strain, were grown in Dulbecco's modified Eagle medium (DME) supplemented with 10% heat-inactivated calf serum in 5% CO_2 and humidified air. When cultured at 33°, the permissive temperature for transformation, these cells showed the characteristic cell rounding, but at 39° (non-permissive temperature) they flattened out, resembling normal cell cultures (5). Untransformed NRK cells were also grown as above, but no difference in morphology was observed between the two temperatures.

Transport assay Uptake of isotopically labeled nucleosides by replicate monolayer cultures was measured at 20° using the cluster-tray method described by Gazzola et al. (6). Unless otherwise indicated, cells were grown at 33° in 24-well Falcon cluster trays seeded with 10^5 cells per well (2 cm^2) and incubated (18-20 h) at 33° or 39° in preparation for assays of nucleoside uptake. Cell numbers during the transport assay were about 1.5×10^5 per well for either temperature. After washing monolayers twice with Dulbecco's phosphate buffered saline containing 0.1% glucose (PBS-G), rates of cellular uptake of nucleoside were measured as follows. Monolayers were exposed during the first 30 seconds at 20° with 0.2 ml of PBS-G solution containing each labeled nucleoside and then were rapidly washed three times using 1.5 ml portions of ice cold PBS containing 5 μM of dipyridamole, a potent nucleoside transport inhibitor (7). After solubilization of cell sheets in 0.5N of KOH, liquid scintillation fluor was added and samples were counted for radioactivity in a liquid scintillation counter.

Materials NRK cells infected with ts-Rous sarcoma virus (tsNRK cells) (5) were provided by Dr. M. Yoshida, Cancer Institute, Tokyo. Untransformed NRK-52E line was obtained from American Type Culture Collection (Rockville, MD, USA). Radioactive compounds were obtained from Amersham International (Buckinghamshire, England). Cell culture materials were obtained from Gibco Laboratories (Grand Island, NY, USA).

RESULTS AND DISCUSSION

The cellular transport of a variety of labelled physiological nucleosides was measured, using tsNRK cells grown at permissive (33°) and non-permissive (39°) temperatures. After washing monolayers (in 24-well cluster trays) with PBS-G medium, transport was assayed at 20° by a rapid sampling method. The cellular uptake of permeant radioactivity was linear with time for the initial 30 seconds of exposure to permeant under the conditions given in Fig. 1. Thymidine uptake by cells grown at 33° was significantly faster than that by cells grown at 39°. As summarized in Table I, among the nucleosides tested, thymidine transport was enhanced more markedly by expression of the src gene (cultured at 33°) than was the transport of other nucleosides. Enhancement of deoxyuridine transport showed the next highest value. No difference in thymidine transport rate was observed between the two temperatures by untransformed NRK cells (data not shown).

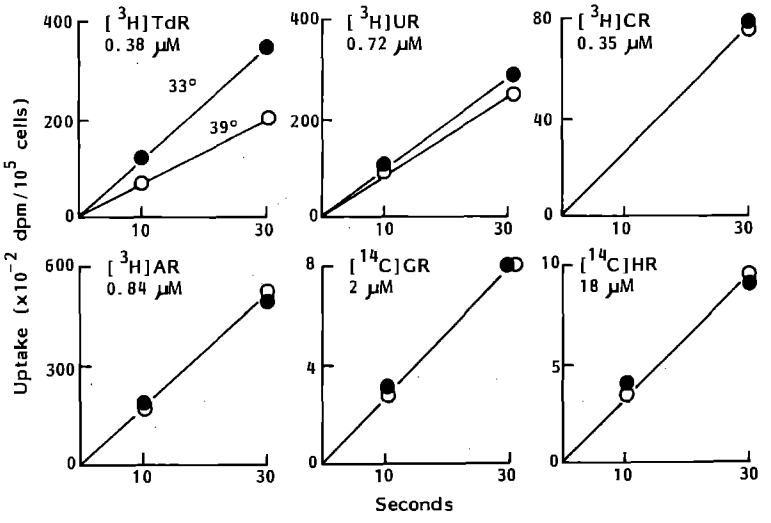

Figure 1. Rate of uptake of various nucleosides by tsNRK cells grown at permissive and non-permissive temperatures. TsNRK cells grown either at 33° (●) or 39° (○) at 1.5×10^5 cells per each of 2 cm^2 well of 24-multi tray were washed and measured for the initial rate of transport of various nucleosides at the concentrations indicated in each graph at 20° by the rapid sampling technique as described in Materials and Methods. Isotopically labelled nucleosides (TdR, thymidine; UR, uridine; CR, cytidine; AR, adenosine; GR, guanosine and HR, inosine) were used undiluted so that the concentrations of radioactivity were 10 μCi/ml. Radioactivity associated with the cells was estimated as described in Materials and Methods.

It has been reported that several physiological modifications take place in nucleoside uptake depending on the physiological state of the cells (1,3,8). For example, the cell density is thought to be inversely related to the rate of uptake in the case of normal cells, but not in the case of transformed cells (1,3). Therefore, we examined whether the alteration of thymidine

TABLE I.
Uptake Rate of Nucleosides by tsNRK Cells at Permissive and Non-permissive Temperatures

Nucleoside	Conc. (μM)	Uptake (pmole/10^6/min) 33°	Uptake (pmole/10^6/min) 39°	Ratio 33°/39°
Thymidine	0.19	2.2	1.1	2.0
Deoxyuridine	0.67	4.3	3.1	1.4
Uridine	0.72	6.1	5.3	1.2
Deoxycytidine	0.45	1.6	1.4	1.1
Cytidine	0.35	1.7	1.7	1.0
Adenosine	0.42	8	7.3	1.1
Guanosine	1.96	9.3	10	0.9
Inosine	18.2	100	106	0.9

Cells were incubated for 30 seconds at 20° as described in the legend to Fig. 1.

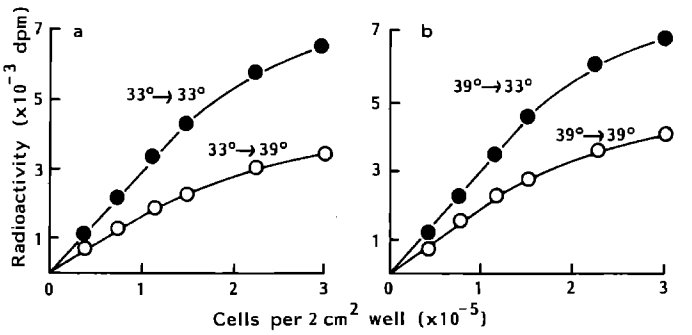

Figure 2. Transport of thymidine at different cell densities with or without temperature shift. tsNRK cells which were grown successively either at 33° (a) or 39° (b) were either shifted to the other temperature or left at the original temperature overnight to the different cell densities shown on the abscissa. The initial rate of thymidine (0.54 μM, 2 μCi/ml) transport during the first 30 seconds at 20° was measured as described in Materials and Methods.

transport was related to the cell density. As shown in Fig. 2, the increase of thymidine uptake in cells grown at 33° was found to be independent of the cell densities.

Fig. 2 also shows the reversibility of the alteration of thymidine transport by shifting temperatures. In the experiment of Fig. 2a, cells grown at 33° were either shifted to 39° or left at 33° for one night before the transport assay. Fig. 2b shows the reverse experiment, that is, cells grown at 39° were shifted to 33° or left at 39°. In either case, the thymidine transport rate was significantly higher in cells grown at permissive temperature. The reverse changes of thymidine transport was associated with the morphological changes of the cells, suggesting that the increased rate of thymidine transport in transformed cells seems to be due to the presence of the active src gene product.

To determine whether the increase in the transport efficiency was due to the increase in the number of functional transport sites in the membrane or due to the increase in the affinity of the transport systems for substrate, a kinetic analysis of thymidine transport by cells grown at the permissive and non-permissive temperature was conducted. The double reciprocal plots so obtained indicated that two entry mechanisms were present in the tsNRK cells. One operated more efficiently at low concentrations and the other at high

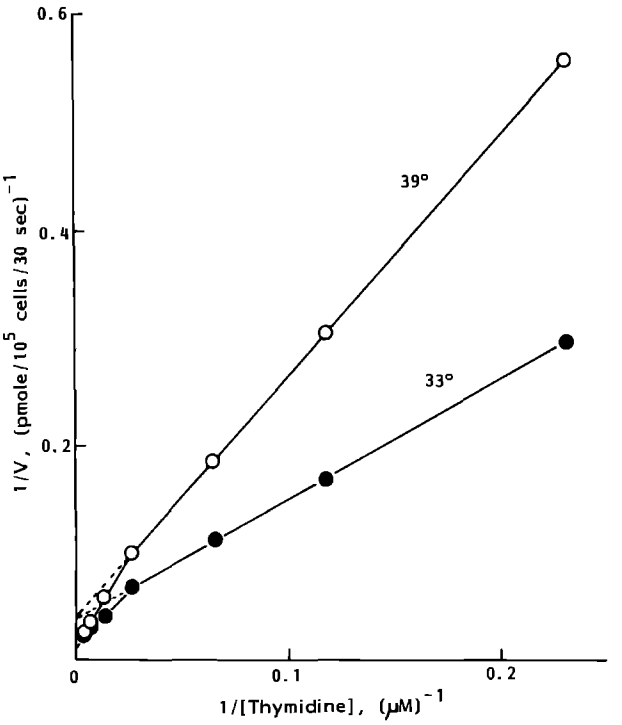

Figure 3. Kinetics in thymidine transport. Cell monolayers (1.5×10^5 tsNRK cells per each of 2 cm^2 well of 24-multi tray) which were grown at 33° (●) or 39° (○) were incubated with ^{3}H-thymidine in concentrations from 4.3 to 320 μM for 30 seconds at 20°. The cells were washed and radioactivity counted as described in Materials and Methods.

concentrations, as shown in Fig. 3. Apparent K_m values were about 30 and 170 μM for cells grown at 33°, while 60 and 250 μM for cells grown at 39°. Apparent V_{max} values for cells grown either at permissive or non-permissive temperature were the same (500 and 1600 pmole/10^6/min for high and low affinity systems, respectively), indicating a qualitative change in the thymidine transporter takes place in the membrane depending on the temperature.

This specific increase and the dual kinetics in thymidine transport seem to be relevant with the observation of Stauss et al. (9) who suggested that the expression of thymidine, but not adenosine, transport system is correlated with the presence of the murine leukemia virus genome in mouse lymphocytes. Other cells such as isolated rat hepatocytes have two thymidine transport systems, the high affinity one having a narrower substrate range than the low affinity one (10).

Recently, Koren et al. (11) reported the significant increase in the number of specific binding sites of nitrobenzylthioinosine (NBMPR), a specific potent inhibitor of nucleoside transport in cells of the murine sarcoma virus-transformed clone (NilSV). This also suggests that an alteration in the nucleoside transport mechanism was associated with retrovirus infection.

Thymidine kinase activities in these cells were about the same between the two temperatures (data not shown). Moreover, cytotoxic agents which are activated by thymidine kinase, such as 5-bromo-2'-deoxyuridine and 5-iodo-2'-deoxyuridine (12) were equally toxic to tsNRK cells grown at 33° or 39° (data not shown), suggesting that activities of thymidine kinase were not changed by the temperature shift and, therefore, were not responsible for the reported alteration of thymidine transport.

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