TRANSPORT OF METHIONINE BY NORMAL AND TRANSFORMED RAT EMBRYO FIBROBLASTS*

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Methionine uptake was 2-2.3-fold higher in the transformed rat embryo fibroblasts as compared with the non-transformed cells. This was associated with a 2-fold greater $V_{max}$, with no detectable changes in $K_m$ values. Methionine starvation caused an increase in the rate of transport, the increase being higher in the transformed cells. Addition of methionine abolished the starvation effect, although to a smaller extent in the transformed cells. It is suggested that differences in methionine uptake might be due to a quicker depletion of intracellular methionine, and not to changes in the transport system of the transformed cells.

Studies on cellular regulation revealed that transport and uptake of nutrients are those functions of the cell cycle which are stimulated by mitogenic agents, oncogenic transformation or specific nutrient deprivation (Kalckar, 1976; Weber et al., 1976).

Numerous lines of fibroblasts show enhanced uptake of sugars, such as 2-deoxy-D-glucose, after transformation by oncogenic DNA or RNA viruses (Hatanaka et al., 1969; Hale et al., 1975). Opinions on the effect of transformation on amino acid transport are divergent. Foster & Pardee (1969) reported on a 2- to 3-fold increased uptake of $\alpha$-aminoisobutyrate and cycloleucine, with concomitantly unchanged $K_m$ and higher $V_{max}$ values in the polyoma-transformed mouse 3T3 fibroblasts. Similarly, Isselbacher (1972) found that the rate of uptake of arginine and glutamic acid in the BALB/3T3 and SV40-transformed BALB-3T3 cells were increased and even equal to that of $\alpha$-aminoisobutyrate and cycloleucine. In contrast, Hatanaka & Hanafusa (1970) noted no difference in the uptake of L-leucine by Rous sarcoma virus-transformed cells, consistently with the earlier observation of Hare (1967) who found no distinct changes in phenylalanine transport of the

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virus-transformed hamster cells. According to Patterson et al. (1976), transformation of human WI-38 cells by SV40 was not associated with any changes in the transport of α-aminoisobutyric acid.

The aim of the present study was to examine methionine transport in the spontaneously transformed rat embryo cells.

**MATERIALS AND METHODS**

**Cell cultures.** The experiments were performed on non-transformed and transformed rat embryo cells. The transformed cell line was established in our laboratory from a normal cell line following spontaneous transformation at the 20th passage. As revealed by karyotype analysis, the transformed cells are aneuploid with 51–88 chromosomes. The line was tested for the ability to produce tumours in syngeneic newborn Sprague-Dawley rats; 100% of the inoculated animals developed tumours at the site of inoculation within one month. Histologically all tumours were found to be sarcomas. The doubling time of both lines was about 42 h as calculated from growth curve.

Cells were routinely grown in monolayers at 37°C in the minimal Eagle's essential medium (MEM) supplemented with 10% heat-inactivated calf serum, penicillin (100 units/ml) and streptomycin sulphate (100 μg/ml). To study methionine uptake, cells were cultured for 2 days in 2 ml of the medium in Leighton’s bottles. The medium was inoculated with the cell suspension at a density of 3 x 10⁵ cells per bottle.

Cell number was estimated following removal from the surface with a small volume of trypsin solution (0.05%) and counted in a hemocytometer. The standard deviation of cell number per bottle ranged from about 5% to 10% of the mean.

**Protein.** This was estimated by the method of Lowry et al. (1951). The cell number was fairly well related to protein content when cell counts did not exceed 6 x 10⁵ per bottle.

**Measurement of methionine uptake.** Methionine transport was measured according to Foster & Pardee (1969) in the cells grown in the Leighton’s glass bottles. The cells were washed three times with 5 ml portions of phosphate-buffered saline (PBS, pH 7.2) prewarmed to the assay temperature, and were then incubated with 2 ml of PBS supplemented with 1% glucose for 1 h at 37°C to deplete them of intracellular amino acids. This was then replaced by the incubation medium which consisted of 2 ml of PBS containing 1% glucose and [14C]methionine (5 μCi/ml) at concentration ranging from 5 to 50 nM. Except where indicated, the transport was measured after 10 min incubation at 37°C. The medium was then decanted and the cultures were rinsed three times with PBS at 37°C and dried on filter paper at room temperature. Protosol (0.25 ml) was then added and after 2 h the contents of the bottles were transferred into scintillation vials containing 8 ml of scintillation solution (toluene, PPO, POPOP). Radioactivity was determined in a Beckman LS 3150P liquid scintillation spectrometer. The counting efficiency was 50%. The results were expressed as nanomoles of methionine accumulated by 1 mg of
cell protein. Each experimental point represents the mean uptake by the cultures in 3 or 4 bottles.

The effect of methionine starvation on the uptake of \( [^{14}\text{C}]\) methionine by transformed and non-transformed rat embryo cells was measured in the cells cultured for 48 h in the MEM containing 10% heat-inactivated calf serum. The cells were then kept in the methionine-free MEM containing 10% dialysed calf serum for 1 or 4 h. The cells remained viable (80 - 90%) in the methionine-free medium for at least 48 h as measured by trypan blue. The medium was then changed and the uptake of L-[\text{methyl}-^{14}\text{C}]methionine was determined under the same conditions as described above. Methionine uptake was also measured in the starved cells supplied subsequently with methionine by incubation in the PBS medium containing glucose and methionine, 150 mg/l, \textit{i.e.} the amount 10-fold greater than in the initial MEM. The uptake of L-[\text{methyl}-^{14}\text{C}]methionine was measured after 4 h.

\textit{Determination of} \( K_m \) \textit{and} \( V_{\text{max}} \). \textit{Kinetic values were calculated from Lineweaver-Burk plots.} \( K_m \) was expressed as nm; \( V_{\text{max}} \) as nanomoles of methionine \textit{per} 1 mg of protein \textit{per} 3 min. Concentration of methionine used was from 5 to 50 nm, and incubation conditions were as described in the legend to Table 1.

\textit{Reagents.} L-[\text{methyl}-^{14}\text{C}]\text{Methionine} (spec. act. 50 mCi/mmol) was obtained from the Radiochemical Centre (Amersham, England). A working solution was prepared by diluting the original stock in PBS to the concentration of 25 μCi/ml. L-Methionine, PPO, POPOP, Trypan Blue were from Sigma Chem. Co. (St. Louis, Mo., U.S.A.). Growth media and calf serum were supplied by Serum and Vaccines Factory (Lublin, Poland). Protosol was obtained from New England Nuclear (Boston, Mas., U.S.A.).

\section*{RESULTS}

The uptake of \( [^{14}\text{C}]\) methionine by the spontaneously transformed and non-transformed rat embryo cells was measured in the logarithmic phase of growth. Figure 1 shows typical rates of methionine uptake. As can be seen, the uptake was linear for 5 min in both cell lines. Most measurements were taken after 3 min incubation. \( [^{14}\text{C}]\text{Methionine} \) (10, 20 and 40 nm) was taken up at 2 - 2.3 faster rate by the transformed cells (Table 1).

Kinetics of methionine transport was analysed in the cells cultured in MEM containing 15 mg/l methionine. \( V_{\text{max}} \) value was consistently greater in the transformed (0.48) as compared with normal (0.22) cells (Fig. 2), but no difference in \( K_m \) values was observed (44 and 40 nm, respectively).

Table 2 illustrates methionine uptake by the cells preincubated for 1 or 4 h in the medium deprived of methionine. In both cell lines, starvation raised methionine uptake, the more so, the longer was preincubation in the methionine-free medium. In the transformed cells, the initial uptake rate increased by 67% after 1 h of starvation as compared with initial velocity and by 129% after 4 h of preincubation in the methionine-free medium. The corresponding values for the normal line were 43% and 93%. The difference is significant with \( p \leq 0.05 \).
Fig. 1. Time-course of [\(^{14}\text{C}\)]methionine uptake by transformed (○) and non-transformed (●) rat embryo cells. The concentration of methionine in the incubation medium was 20 nm. Experimental conditions were as described in the text. Each point represents the average of triplicate determinations.

Fig. 2. Lineweaver-Burk plots for methionine uptake by transformed (○) and non-transformed (●) rat embryo cells. Experimental conditions as in Table 1. Each point represents the average of triplicate determinations.

The rate of methionine uptake was determined also in the cells starved in the methionine-free medium for 4 h and then supplemented with methionine by incubation in the methionine - enriched medium (150 mg/l; 1 mm). Restoration of intracellular methionine level decreased significantly the uptake rate. From the results presented in Table 2 it is evident that this decrease was significantly \((p<0.05)\) smaller (40\%) in the transformed cells as compared with the normal cells (60\%).

### Table 1

**Uptake of [\(^{14}\text{C}\)]methionine by transformed and non-transformed rat embryo cells**

Experiments were performed on cells in the logarithmic phase of growth. The cells were washed with phosphate - buffered saline, then incubated for 1 h with 2 ml of the same buffer containing 1\% glucose. Isotope was added to the final concentration indicated, and cells were incubated at 37°C for 3 min. Each experiment was carried out in triplicate.

<table>
<thead>
<tr>
<th>L-[methyl-(^{14}\text{C})]- Methionine concentration (nm)</th>
<th>Methionine uptake (nmol/min per mg protein) by cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>transformed</td>
</tr>
<tr>
<td>10</td>
<td>0.022</td>
</tr>
<tr>
<td>20</td>
<td>0.048</td>
</tr>
<tr>
<td>40</td>
<td>0.075</td>
</tr>
</tbody>
</table>
Table 2

*Methionine uptake by the methionine-starved and methionine-supplemented non-transformed and transformed rat embryo cells*

Cells of both lines were incubated in the methionine-deprived medium for the time indicated and transferred for 4 h to the medium containing 1 mm-methionine. The relative differences are given in parentheses. Each value represents the mean of at least six determinations ± S.E.M. For details see Materials and Methods.

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Methionine uptake (nmol/3 min per mg protein) by cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>transformed</td>
</tr>
<tr>
<td>Met-deprived medium</td>
<td></td>
</tr>
<tr>
<td>0 h</td>
<td>0.142±0.028</td>
</tr>
<tr>
<td>1 h</td>
<td>0.238±0.024</td>
</tr>
<tr>
<td>4 h</td>
<td>0.325±0.065</td>
</tr>
<tr>
<td>Met-enriched medium</td>
<td></td>
</tr>
<tr>
<td>4 h</td>
<td>0.198±0.038</td>
</tr>
</tbody>
</table>

* Differences refer to the values at 0 h.
*b* Differences refer to the value after 4 h incubation in the Met-deprived medium.

**DISCUSSION**

The results presented show differences in the initial rate of methionine uptake between transformed and non-transformed rat embryo cells: 2.3-fold greater rate of uptake and increased $V_{\text{max}}$ values were found in the transformed cells as compared with parental line. This is consistent with the results of Foster & Pardee (1969) obtained with $\alpha$-aminoisobutyrate and cycloleucine. The increased $V_{\text{max}}$ value may indicate, as suggested by Isselbacher (1972), an increase in the number of membrane sites involved in the transport system, while lack of difference in $K_m$ values excludes qualitative alterations of these sites.

In 1976 Weber et al. suggested that changes in the transport activity may be due to the cellular regulatory mechanisms, such as regulation by the endogenous amino acid pool (Gazzola et al., 1972). The results presented in this study are in agreement with this supposition. Methionine transport was regulated in both lines by concentration of intracellular methionine; however, enhancement of the transport by the starved cells was greater, and the same amount of methionine supplied to the starved cells decreased to a lesser extent methionine uptake by the transformed cells than by normal cells. One can presume, therefore, that the extent of methionine depletion within the cell could account for differences in the rate of uptake. These differences were not due to differences in the growth rate since both cell lines were multiplying at a similar rate (doubling time about 42 h).

According to Holley (1972), intracellular concentration of specific nutrients is controlled by the transport systems of the surface membranes. These systems can be altered during process of transformation (Isselbacher, 1972; Foster & Pardee, 1969). However, the results of the present studies indicate that spontaneous transformation of the rat embryo cells most probably did not involve transport systems.
REFERENCES


TRANSPORT METIONINY W PRAWIDŁOWYCH I STRANSFORMOWANYCH ZARODKOWYCH FIBROBLASTACH SZCZURZYCH

Streszczenie

Pobieranie metioniny przez transformowane zarodkowe fibroblasty szczurze było dwukrotnie większe niż przez prawidłowe zarodkowe fibroblasty szczurze. Zwiększone pobieranie było związane z dwukrotnie większą wartością $V_{max}$; wartości stałej Michaelisa były jednakowe dla obu rodzajów komórek. Brak metioniny w pożywce w większym stopniu zwiększał transport metioniny w komórkach transformowanych. Po dodaniu metioniny zmniejszenie szybkości transportu w komórkach transformowanych występowało w mniejszym natężeniu. Wydaje się, że różnice w pobieraniu metioniny są raczej wynikiem szybkiej występującej niedoboru wewnątrzkomórkowej metioniny niż są spowodowane zmianami w systemie transportowym komórek transformowanych.

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