Biochimica et Biophysica Acta 821 (1985) 63–66
Elsevier

BBA 72828

Analysis of hexose transport in untransformed and sarcoma virus-transformed mouse 3T3 cells by photoaffinity binding of cytochalasin B

Kouichiro Kitagawa, Hoyoku Nishino and Akio Iwashima

Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamikyo-ku, Kyoto 602 (Japan)

(Received April 23rd, 1985)

Key words: Hexose transport; Photoaffinity labeling; Cytochalasin B; (Mouse 3T3 cell)

The effect of simian virus 40 transformation on the hexose transport system in mouse embryo fibroblast Swiss 3T3 cells was examined. The concentration of hexose transporters was estimated by measuring D-glucose-inhibitable cytochalasin B binding. The binding of cytochalasin B to the plasma membranes of simian virus 40-transformed mouse 3T3 cells (SV3T3 cells) was significantly greater than that of 3T3 cells. On the other hand, cytochalasin B binding to the microsomal membranes of SV3T3 cells was decreased, and the total amount of binding to plasma and microsomal membranes was not significantly changed in both cell lines. The electrophoretic analysis demonstrated that both hexose-transporter components of M_r 46000 and M_r 58000 affinity labeled were responsible for an increase in the hexose transport by viral transformation. These results suggested that the higher hexose-transport activity of transformed cells is caused by a redistribution of transporter from intracellular membranes to plasma membranes.

Introduction

An increase in the rate of hexose transport has been reported as one of the early biological events after transformation by an oncogenic virus [1–3]. Virally transformed 3T3 cells show a 2–3-fold increase in the hexose transport rate when compared to untransformed cells [4]. This has generally been explained by a specific increase in the number or availability of functional hexose-transport sites in plasma membranes [5–7]. However, it has also been proposed that an increased rate of phosphorylation [8] or alteration in the properties of transport sites by transformation [9] may contribute to the enhanced hexose transport.

In order to determine whether there is an increase in the number of hexose transporters after viral transformation, we used the photoaffinity labeling technique of hexose transporters by [^{3}H]cytochalasin B, which has been shown to be a highly effective inhibitor of hexose transport [10–12] and has been used successfully to calculate the number of hexose transporters [12,13].

Thus, in this study we measured the number of hexose transporters in microsomal and plasma membranes of untransformed and SV40-transformed cells. The results suggest that the increase in hexose transport induced by SV40 transformation is due to increased translocation of hexose transport components from microsomal to plasma membranes. These findings were similar to those obtained by the treatment of 3T3 cells with 12-*O*-tetradecanoylphorbol 13-acetate (TPA), a tumor promoter [14].

Materials and Methods

Chemicals. [^{3}H]AMP (50 Ci/mmol), [^{3}H]cytochalasin B (14.9 Ci/mmol) and [^{14}C]sucrose (673

Abbreviations: SV3T3, simian virus 40-transformed mouse 3T3; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; SDS, sodium dodecyl sulfate.

mCi/mmol) were purchased from New England Nuclear. 12-*O*-Tetradecanoylphorbol 13-acetate (TPA) was obtained from P-L Biochemical Inc., and cytochalasin B and E were from Nakarai Chemicals Co. Ltd. All other chemicals were obtained from commercial sources in either reagent grade or highest purity available.

Cell culture. Swiss 3T3 [15] and SV3T3 cells were prepared by plating $2 \cdot 10^5$ cells/dish in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum. Cells were grown in a plastic tissue plate in a humidified CO_2 incubator at 37°C. The following day the cultures were shifted to Dulbecco's modified Eagle medium containing 2% serum in order to diminish the effect of serum on the hexose-transport system [16]. After 3 days, cultures were used for experiments.

Preparation of plasma and microsomal membranes. Plasma and microsomal membranes were prepared from the incubated cells by differential centrifugation methods described previously [14]. 5'-Nucleotidase and NADH-cytochrome *c* reductase activities were measured by the methods by Avruch and Wallach [17] and Dallner et al. [18], respectively. Protein concentration was determined by the method of Markwell et al. [19].

Cytochalasin B binding and photoaffinity cross-linking. The membrane protein suspension (1 mg) was mixed with 2 μM cytochalasin E containing 200 mM D-glucose or D-sorbitol. 5 min later, various concentrations of [^{3}H]cytochalasin B and [^{14}C]sucrose were added. The mixture was plated on ice in the dark for 30 min, and then irradiated at 0°C with a 300 W mercury lamp at a distance of 20 cm for 10 min. The samples were centrifuged and the pellets were suspended in distilled water. Corrections for trapped [^{3}H]cytochalasin B in the membrane pellets were determined by subtracting the amount of [^{14}C]sucrose associated with each sample.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Samples were boiled for 2 min in the presence of 1% SDS and 0.5% dithiothreitol. They were electrophoresed on a 1 mm thick 10% polyacrylamide slab gel as described by Laemmli [20]. The protein bands were cut out from the lanes, and the slices were incubated for 20 h at 37°C in 1 ml of Protosol and then radioactivity was assayed in 9 ml of scintillation fluid.

Results and Discussion

Since it has not been determined whether the increases in hexose-transport rate by viral transformation and TPA treatment are due to changes in the number of transport sites [5,21], in the properties of the transport sites [10,22] or in the rate of hexose metabolism [9], we investigated these possibilities.

In order to estimate the changes in the distribution of hexose transporter induced by SV40-transformation, the glucose-inhibitable cytochalasin B binding was assayed in plasma and microsomal membranes of which the purity and quantities were calculated on the basis of activities of 5'-nucleotidase and NADH-cytochrome *c* reductase. These two enzyme activities were not affected by SV40 transformation ($P < 0.05$) (data not shown). In 3T3 and SV3T3 cells, cytochalasin B inhibited stereospecific hexose transport and the K_i value was similar in both types of cells ($3.5 \cdot 10^{-7}$ M and $3.9 \cdot 10^{-7}$ M, respectively). These results suggested that there was no qualitative alteration of the transport system after SV40 transformation. In untransformed 3T3 cells, the amount of D-glucose-inhibitable cytochalasin B binding to the plasma membranes was increased in response to SV40 transformation or TPA treatment, in agreement with the results previously reported [14], whereas cytochalasin B binding to the microsomal membranes was decreased and there was no significant change in the amount of total cytochalasin B binding by these treatments ($P < 0.05$) (Table I). In contrast, in SV40-transformed 3T3 cells, cytochalasin B binding to the plasma and the microsomal membranes was scarcely affected by TPA treatment.

Scatchard analysis [23] of D-glucose-inhibitable cytochalasin B binding was performed to analyze the mechanism of stimulation of the hexose-transport system by SV40 transformation. The B_{max} value of cytochalasin B binding in the plasma membranes was increased from 27.5 to 58.6 pmol/mg protein ($P < 0.05$) without significant change in K_d (0.19 to 0.25 μM) (Fig. 1). These results suggest that the increase in the rate of hexose transport by SV40 transformation appears to be caused by a quantitative change in the hexose-transport system in plasma membranes,

TABLE I

ESTIMATED NUMBER OF HEXOSE TRANSPORTERS PER CELL IN 3T3 CELLS AND SV40 TRANSFORMED CELLS WITH OR WITHOUT TPA TREATMENT

3T3 and SV3T3 cells were incubated with 50 nM TPA or vehicle (ethanol) for 3 h at 37°C, and then membranes were prepared as described in the text. Data are expressed as means ± S.E. ($n = 3$). Calculated specific binding is based on the adjusted specific cytochalasin B binding activities and calculated membrane protein in the original homogenates. Measured specific cytochalasin B binding activities and membrane protein in original homogenates have been adjusted to those which would have been observed had the membrane fractions been free of cross-contamination. Adjustments were based on the enzyme marker specific activities and protein recoveries specified in the table, with the assumptions that (1) 5'-nucleotidase activity is localized specifically to the plasma membranes; (2) uncontaminated plasma membranes contain an indigenous NADH-cytochrome *c* reductase activity with a specific activity which is 20% that of uncontaminated microsomal membranes; and (3) contamination of the plasma and microsomal membrane fractions by other subcellular organelles is negligible.

Material under investigation		Total protein recovery (mg/cell) ($\times 10^9$)	Measurement of specific cytochalasin B binding in membrane fractions (fmol/mg protein)		Calculated specific cytochalasin B binding in the cells (pmol/cell) ($\times 10^9$)	
			Basal	TPA	Basal	TPA
Plasma membranes	3T3	19.5 ± 1.96	184 ± 14	450 ± 39	5.2 ± 1.2	14.3 ± 2.9
	SV3T3	21.6 ± 2.16	574 ± 46	534 ± 30	19.6 ± 4.1	17.8 ± 3.4
Microsomal membranes	3T3	13.1 ± 1.96	610 ± 76	330 ± 30	17.6 ± 4.2	10.5 ± 2.9
	SV3T3	14.2 ± 1.763	272 ± 32	262 ± 36	9.2 ± 3.0	8.2 ± 3.2

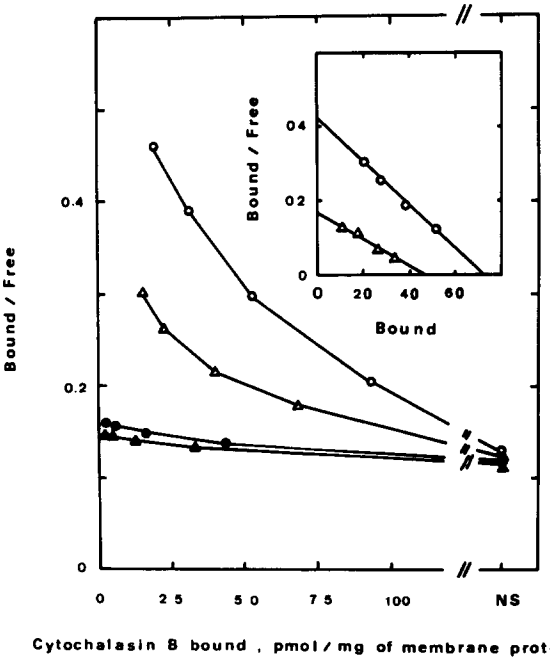

Fig. 1. Scatchard analysis of cytochalasin B binding to the plasma membranes of 3T3 and SV3T3 cells. The plasma membranes were prepared as described in the text. Plasma membrane proteins from 3T3 (△, ▲) and SV3T3 (○, ●) cells were incubated with 2 μM cytochalasin E containing 200 mM D-glucose (●, ▲) or D-sorbitol (○, △). 20 nM [^{3}H]cytochalasin B and unlabeled cytochalasin B were then added to give final concentrations of 40, 80, 150, 400 and 10000 nM. Inset: D-glucose-sensitive cytochalasin B binding. Measured specific cytochalasin B binding activities have been adjusted to those which would have been observed had the membrane fractions been free of cross-contamination. Adjustments were based on the enzyme marker specific activities and protein recoveries.

accompanied by translocation of functional hexose transporters from microsomal membranes to plasma membranes.

Furthermore, D-glucose-inhibitable cytochalasin B binding protein was analyzed by SDS-polyacrylamide gel electrophoresis. Both the M_r 46000 and the M_r 58000 proteins were photolabeled in plasma and microsomal membranes of transformed or untransformed cells (Fig. 2) and the two labeled proteins were found to be increased in the plasma membranes in response to SV40 transformation. On the other hand, in microsomal membranes, the M_r 46000 protein was decreased by transformation, without significant change in the M_r 58000 protein.

It is known that there exists an intracellular pool of various kind of membrane glycoproteins in cultured cells [24–26] and the hexose transporter seems to be a typical one among these proteins

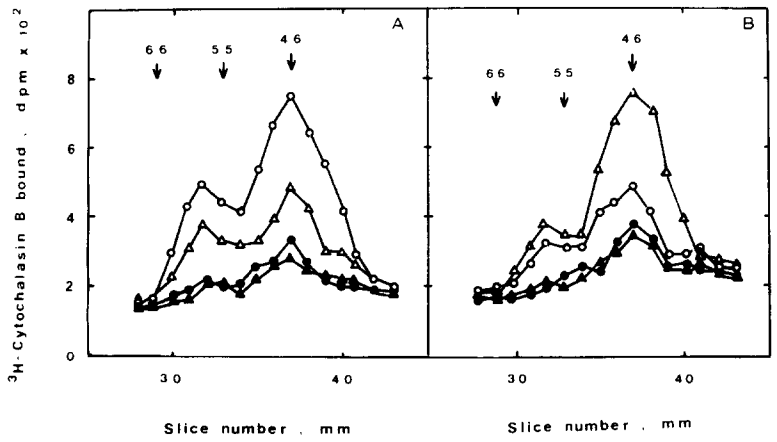

Fig. 2. Photoaffinity labeling of the plasma membrane (A) and the microsomal membranes (B) with cytochalasin B. Plasma and microsomal membrane protein (1 mg) from 3T3 (△, ▲) or SV3T3 (○, ●) cells was incubated with 1.0 μM [^{3}H]cytochalasin B in the presence of 200 mM D-glucose (▲, ●) or D-sorbitol (△, ○). After the binding reached equilibrium, samples were irradiated, washed and subjected to electrophoresis on 10% polyacrylamide gels.

[27]. In the present study, it was suggested that there was an intracellular pool of hexose transporter in both transformed and untransformed 3T3 cells and that viral transformation increased the number of hexose transporters in the plasma membrane, accompanied by a decrease in the number of transporters in microsomal membranes. It is therefore concluded that such a redistribution of transporter from intracellular membranes to the plasma membranes may be a cause of the higher hexose-transport activity of transformed cells.

Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, and the Ministry of Health and Welfare of Japan.

References

1 Hatanaka, M. (1974) Biochim. Biophys. Acta 355, 77–104
2 Salter, D.W. and Weber, M.J. (1979) J. Biol. Chem. 254, 3554–3561
3 Singh, M., Singh, V.N., August, J.T. and Horecker, B.L. (1978) J. Cell. Physiol. 97, 285–292
4 Inui, K., Moller, D.E., Tillotson, L.G. and Isselbacher, K.J. (1979) Proc. Natl. Acad. Sci. USA 76, 3972–3976
5 Weber, M.J. (1973) J. Biol. Chem. 248, 2978–2983
6 Kletzien, R.F. and Perdue, J.F. (1974) J. Biol. Chem. 249, 3375–3382
7 Dolberg, D.S., Bassham, J.A. and Bissell, M.J. (1975) Exp. Cell. Res. 96, 129–137
8 Colby, C. and Romano, A.H. (1975) J. Cell. Physiol. 85, 15–24
9 Hatanaka, M., Augl, C. and Gilden, R.V. (1970) J. Biol. Chem. 245, 714–717
10 Plagemann, P.G.W. and Richey, D.P. (1974) Biochim. Biophys. Acta 344, 263–305
11 Zoccoli, M.A., Baldwin, S.A. and Lienhart, G.E. (1978) J. Biol. Chem. 253, 6923–6930
12 Kletzien, R.F. and Perdue, J.F. (1973) J. Biol. Chem. 248, 711–719
13 Cushmann, S.W. and Wardzala, L.J. (1980) J. Biol. Chem. 255, 4758–4762
14 Kitagawa, K., Nishino, H. and Iwashima, A. (1985) Biochem. Biophys. Res. Commun. 128, 1303–1309
15 Todaro, G.J. and Green, H. (1963) J. Cell Biol. 17, 299–313
16 Dubrow, R., Pardee, A.B. and Pollack, R. (1978) J. Cell. Physiol. 95, 203–212
17 Avruch, J. and Wallach, D.F.H. (1971) Biochim. Biophys. Acta 233, 334–347
18 Dallner, G., Siekevitz, P. and Palade, G.E. (1966) J. Cell Biol. 30, 97–117
19 Markwell, M.A.K., Hass, S.M., Bieber, L.L. and Tolbert, N.E. (1978) Anal. Biochem. 87, 206–210
20 Laemmli, U.K. (1970) Nature (London) 227, 680–685
21 Lee, L.S. and Weinstein, I.B. (1979) J. Cell. Physiol. 99, 451–460
22 Yamanishi, K., Nishino, H. and Iwashima, A. (1983) Experientia 39, 1036–1037
23 Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51, 660–672
24 Shanahan, M.F. and Czech, M.P. (1977) J. Biol. Chem. 252, 8341–8343
25 Kasahara, M. and Hinkle, P.L. (1977) J. Biol. Chem. 252, 7384–7390
26 Doyle, D., Baumann, H., England, B., Friedman, E., Hou, E. and Tweto, J. (1978) J. Biol. Chem. 253, 965–973
27 Olden, K., Pratt, R.M., Jaworski, C. and Yamada, K.M. (1979) Proc. Natl. Acad. Sci. USA 76, 791–795