Selective Inhibition by Benzaldehyde of the Uptake of Nucleosides and Sugar into Simian Virus 40-transformed Cells

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ABSTRACT

The effects of benzaldehyde, which has been found in figs as a carcinostatic element, were studied on the uptake of nucleosides, 2-deoxy-D-glucose, and amino acids into simian virus 40-transformed rat fibroblast cells (SV40-transformed cells) and into the parent normal cells (normal cells). Benzaldehyde, at the concentrations of 25 to 100 μg/ml at which the selective growth inhibition against SV40-transformed cells was revealed, markedly inhibited the uptake of thymidine, other nucleosides, and 2-deoxy-D-glucose into SV40-transformed cells without any significant inhibition of the uptake of these compounds into normal cells. The uptake of amino acids into both transformed and normal cells was not inhibited by benzaldehyde. Selectively cytotoxic benzaldehyde-related compounds such as 4-nitrobenzaldehyde, 4-acetaminobenzaldehyde, thiophene-3-carboxaldehyde, etc., showed a similar inhibitory effect on thymidine uptake. The deprivation of glucose from the incubation medium strikingly diminished the inhibitory effect of benzaldehyde on the uptake of thymidine and 2-deoxy-D-glucose into SV40-transformed cells. The intracellular adenosine 5′-triphosphate level of SV40-transformed cells was reduced to less than one-half by treatment with benzaldehyde (50 μg/ml) in glucose-containing medium. This effect was not observed in glucose-free medium. Treatment with benzaldehyde caused no change of the intracellular adenosine 5′-triphosphate level of normal cells.

Based on the above results, the selective cytotoxicity of benzaldehyde was attributed to the reduction of intracellular adenosine 5′-triphosphate level of transformed cells, accompanied by the poor uptake of thymidine, glucose, etc., into SV40-transformed cells.

INTRODUCTION

Benzaldehyde, a periactin (18), has been reported to be clinically effective for the treatment of patients with various types of cancer by the p.o. administration of its inclusion compound with β-cyclodextrin (13, 30).

Miyakawa et al. (18) reported the selective inhibition of the growth of SV40-transformed2 cells in the presence of benzaldehyde (25 to 50 μg/ml) without any significant effect on the growth of normal cells and the preferential binding of benzaldehyde on membrane proteins. However, the mechanism of action of benzaldehyde has not been fully elucidated.

Several in vitro and in vivo studies have been reported on the carcinostatic aliphatic aldehydes, which share the common structural feature of a terminal aldehyde group conjugated with a double bond or a ketone. In tumor cells, the aliphatic aldehydes react with cellular thiol compounds (9, 16, 28), depress protein synthesis (8, 9, 21, 28), and cause ultrastructural alterations of plasma membranes, Golgi vesicles, mitochondria, and nucleus (1). However, our recent results on the preferential binding of benzaldehyde on the plasma membrane (18) and its effects on the membrane transport system of bacteria (26, 30) suggest a possible influence of benzaldehyde on the membrane transport activities of transformed cells.

In this communication, we studied the relationship between the cytotoxicity of benzaldehyde and its effect on the uptake of nutrients into SV40-transformed cells. The incorporation of thymidine, 2-deoxy-D-glucose, and amino acids into cells was specifically investigated because they are important for macromolecular synthesis and for energy supply. Furthermore, the activity of nucleoside incorporation may be important in controlling cell proliferation (19, 25). The nonmetabolizable sugar, 2-deoxy-D-glucose, was used because it shares the same transport system with D-glucose in cells and tissues thus far reported (10, 24). These compounds are incorporated into cells through various transport systems, i.e., the transport systems facilitated by the phosphorylation of nucleosides (17, 23) and 2-deoxy-D-glucose (6), and the active transport systems via sodium pump or proton pump for amino acids (7).

Therefore, we studied the effects of benzaldehyde on these various transport systems.

MATERIALS AND METHODS

Cell Line and Cell Culture. The 3Y1-B clone 1-6 cell line (normal cell), derived from Fischer rat embryo fibroblast, and a derivative line, W-3Y-23, transformed by SV40, were kindly provided by Dr. Yamaguchi, Institute of Medical Science, University of Tokyo (27). Cells were grown in plastic Falcon Petri dishes at 37°C as described previously (18), using Eagle's minimum essential medium containing 10% newborn calf serum and antibiotics (100 units penicillin, 100 μg streptomycin, and 60 μg kanamycin per ml of medium). Fresh cells were thawed after every 40 passages. The viable cell numbers were counted in the presence of trypsin blue after trypsinization (0.25% trypsin; Grand Island Biological Company, Grand Island, N. Y.) for 15 to 20 min at room temperature.

Uptake Assay. The uptake of nucleosides, 2-deoxy-D-glucose, and amino acids was measured by either of the following 2 methods. One, the coverslip method, involved the use of a 15-mm-diameter coverslip set in a 16-mm-diameter well on a plastic tray for culturing cells and for uptake assay. Cells seeded on the coverslip at the density of 3 x 10⁴ were allowed to grow in 1 ml of medium for 2 days. After 2 washes with Hanks' BSS, pH 7.4, the cells were preincubated in 1 ml of Hanks' BSS for 30 min at 37°C in a CO₂ incubator to reduce
the intracellular pools of substrate. Incubation was initiated by replacing the incubation medium with 0.5 ml of Hanks' BSS containing the labeled compound (1 μCi/ml for 3H-labeled compound and 0.2 μCi/ml for 14C-labeled compound) and the desired amount of benzaldehyde or H2O (for controls). After the designated periods, incubation was stopped by chilling the plate on ice, followed by washing the cells 3 times with 1 ml of cold PBS, pH 7.3. The coverglasses were dried, and radioactivities incorporated into cells were determined with 10 ml of toluene scintillator solution in a Packard Model 3385 liquid scintillation system. Net counts incorporated into cells was obtained by subtracting counts incorporated into cells at 0°C from counts incorporated at 37°C. Determination of cell number was performed on cells cultured and treated identically and concurrently.

Another method, the scraping method, involved the use of 35-mm-diameter dishes. Cells seeded at the density of 10^5 cells per plate were cultured in 2 ml of medium for 2 days. These cells grown on the substratum were incubated in 1 ml of incubation buffer according to the procedure of the coverslip method, followed by scraping of cells with a rubber policeman into 1 ml of cold PBS. The scraped cells were transferred onto a Whatman GF/C glass microfiber filter with another 2 ml of cold PBS. The filters were dried, and radioactivities were determined as described.

The coverslip method was adequate for quick measurement of many samples, while the scraping method exceeded the coverslip method in measuring poorer incorporation. Either method was chosen according to the purpose of the experiment.

**Determination of Intracellular ATP Level.** Intracellular ATP level was determined with the method of Strehler and Tidder (29). Cells grown under the same conditions described for the scraping method were preincubated with 1 ml of Hanks' BSS at 37°C for 30 min in a CO2 incubator after 2 washings with 1 ml of Hanks' BSS. Further incubation of cells with 1 ml of Hanks' BSS in the presence or absence of benzaldehyde (50 μg/ml) was terminated by chilling the dishes on ice. After 3 washings with 2 ml of PBS, cells were scraped off in 1 ml of water. The ATP was extracted by boiling the cell suspension for 10 min, followed by centrifugation at 600 × g at 4°C for 10 min. The supernatant was used for the determination of intracellular ATP level. One hundred eighty μl of 50 mM Tris-HCl buffer (pH 7.4) containing 10 mM MgCl2 were mixed with 60 μl of the supernatant. The reaction was initiated by injecting 60 μl of firefly lantern extract (Sigma Chemical Co., St. Louis, Mo.) containing 50 μM arsenate and 20 μM MgCl2 into the reaction mixture. The maximum light emission was detected in the Photometer Pico ATP from Jobin Yvon, France. ATP (Sigma) was dissolved and used for the standard.

**Chemicals.** Benzaldehyde and related compounds were purchased from Tokyo Kasei Co. Ltd., Tokyo, Japan, and Aldrich Chemical Co. Inc., Milwaukee, Wis. Benzaldehyde, purified by passing through a dry silica gel column, was dissolved in distilled water at the concentration of 5 mg/ml and stored at −80°C under argon gas until use. Thiophene-3-carboxaldehyde was prepared from thiophene-3-carboxylic acid by reduction with lithium aluminum hydride, followed by oxidation with pyridinium chlorochromate (4). [3H]Thymidine was obtained from New England Nuclear, Boston, Mass. [3H]2-deoxy-D-glucose, other radioactive nucleosides and amino acids were purchased from the Radiochemical Centre, Amersham/Searle Corp., Arlington Heights, Ill. Eagle's minimum essential medium was obtained from the Nissui Pharmaceutical Co., Tokyo, Japan. Newborn calf serum was prepared by Mitsubishi-Kasei Institute of Life Sciences, Tokyo, Japan. Ouabain, ATP, and firefly lantern extract were obtained from Sigma. Other chemicals were analytical-grade products.

**RESULTS**

**Inhibition of Growth and of Thymidine Uptake.** Both normal and SV40-transformed cells seeded at the initial density of 10^5 cells per 35-mm-diameter dish were allowed to grow for 24 hr before treatments of cells with various concentrations of benzaldehyde as indicated in Chart 1A. Benzaldehyde, at concentrations between 10 and 100 μg/ml, inhibited the growth of only SV40-transformed cells during another 2 days of incubation without any significant inhibition of the growth of normal cells. Increasing the concentration of benzaldehyde higher than 400 μg/ml caused the inhibition of growth of normal cells. Benzaldehyde produced dramatic effects on sparsely populated cultures.
The linear uptake of \(^{3}H\)thymidine (1 µM, 1 µCi/ml) was observed on both normal and SV40-transformed cells for at least up to 75 min of incubation in either the absence or presence of benzaldehyde (50 µg/ml). The inhibition of thymidine uptake by benzaldehyde took place within the first 5 min of incubation. To examine the correlation between the inhibition of cell growth and the inhibition of thymidine uptake, cells were incubated for 30 min in Hank’s BSS containing \(^{3}H\)thymidine (1 µM, 1 µCi/ml) and various concentrations of benzaldehyde. Chart 1B shows that benzaldehyde revealed marked inhibition of the uptake of \(^{3}H\)thymidine only into SV40-transformed cells but not into normal cells at concentrations between 10 and 100 µg/ml at which the selective growth inhibition was observed (Chart 1A). Increase of the concentration to higher than 200 µg/ml caused inhibition of \(^{3}H\)thymidine uptake into normal cells. Neither morphological change nor decrease of total and viable cell numbers was observed during this uptake assay.

**Effect of Related Compounds with Benzaldehyde.** Zundel et al. (33) reported that several compounds of 35 derivatives or analogs of benzaldehyde tested revealed selective growth inhibition against SV40-transformed cells at concentrations between 25 and 100 µg/ml. Ten representative compounds, including selective and nonselective growth inhibitors and noncytotoxic compounds, were examined for their effects on \(^{3}H\)thymidine uptake into normal and SV40-transformed cells. Table 1 shows that all of the selective growth inhibitors such as 4-fluorobenzaldehyde, 3-fluorobenzaldehyde, 4-nitrobenzaldehyde, 3-bromobenzaldehyde, 3-hydroxybenzaldehyde, 4-acetaminobenzaldehyde, and thioephene-3-carboxaldehyde revealed the selective inhibitory effect on \(^{3}H\)thymidine uptake into SV40-transformed cells. Neither class nor position of the substituent on the benzene ring determined the activity of the compounds. For instance, 3-hydroxybenzaldehyde was a selective inhibitor, but 2-hydroxybenzaldehyde was not selective in both assay procedures. Strong cytotoxicity was observed with 2-hydroxybenzaldehyde but not with 2-carboxybenzaldehyde (33). As expected, noncytotoxic compounds, such as pyrrole-2-carboxaldehyde and benzoic acid, revealed no inhibitory effect on \(^{3}H\)thymidine uptake into the transformed cells. The inhibitory efficiency of \(^{3}H\)thymidine incorporation into SV40-transformed cells observed with the scraping method was generally greater than that observed with the coverslip method. However, a similar selective inhibitory effect was observed in the same dose range of benzaldehyde with either assay system.

**Substrate Specificity of the Effects of Benzaldehyde on Incorporation.** Table 2 shows the effect of benzaldehyde on the uptake of various compounds, including nucleosides, amino acids, and 2-deoxy-o-glucose. The cells were incubated with the labeled compounds (0.007 to 0.07 µM, 1 µCi/ml, for \(^3\)H compounds, and 0.5 to 5 µM, 0.2 µCi/ml, for \(^14\)C compounds) for 30 min either with or without benzaldehyde (50 µg/ml). Marked selective inhibition was observed on the uptake of nucleosides into SV40-transformed cells, especially of pyrimidine nucleosides. A similar inhibitory effect was found on \(^3\)Hdeoxy-o-glucose uptake. However, much less inhibition was observed on the uptake of amino acids into the transformed cells. Among these amino acids, lysine uptake was inhibited rather strongly because, at least partly, this amino acid readily forms Schiff base adduct with benzaldehyde.

Although SV40-transformed cells usually revealed approximately 2-fold greater rate of uptake of nucleosides and amino acids than did normal cells in the absence of benzaldehyde, such enhanced uptake of nucleosides into the transformed cells diminished in the presence of benzaldehyde (50 µg/ml), and the rate of uptake decreased to the level of normal cells. No appreciable difference was found between untreated normal and SV40-transformed cells in incorporating \(^3\)H-2-deoxy-o-

**Table 1**

<table>
<thead>
<tr>
<th>Derivatives of benzaldehyde</th>
<th>pmol/10^6 cells/30 min</th>
<th>% of normal</th>
<th>pmol/10^6 cells/30 min</th>
<th>% of normal</th>
<th>Growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>22.3 ± 0.1^d, 100%</td>
<td>100</td>
<td>29.0 ± 1.8, 100%</td>
<td>100</td>
<td>Selective</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>17.9 ± 0.4, 80%</td>
<td>3.5 ± 0.5, 12%</td>
<td>4.9 ± 0.5, 17%</td>
<td>4.0 ± 0.5, 18%</td>
<td></td>
</tr>
<tr>
<td>4-Fluorobenzaldehyde</td>
<td>18.5 ± 0.6, 83%</td>
<td>6.3 ± 0.6, 22%</td>
<td>3.5 ± 0.5, 12%</td>
<td>4.0 ± 0.5, 18%</td>
<td></td>
</tr>
<tr>
<td>3-Bromobenzaldehyde</td>
<td>17.9 ± 0.3, 80%</td>
<td>6.3 ± 0.6, 22%</td>
<td>3.5 ± 0.5, 12%</td>
<td>4.0 ± 0.5, 18%</td>
<td></td>
</tr>
<tr>
<td>Pyrrole-2-carboxaldehyde</td>
<td>17.7 ± 0.6, 79%</td>
<td>3.1 ± 0.4, 11%</td>
<td>2.7 ± 0.9, 9%</td>
<td>3.0 ± 0.5, 12%</td>
<td></td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>16.2 ± 0.6, 72%</td>
<td>27.9 ± 4.4, 96%</td>
<td>19.8 ± 1.4, 89%</td>
<td>31.3 ± 0.1, 108%</td>
<td></td>
</tr>
<tr>
<td>2-Deoxy-o-glucose</td>
<td>19.8 ± 1.4, 89%</td>
<td>31.3 ± 0.1, 108%</td>
<td>19.8 ± 1.4, 89%</td>
<td>31.3 ± 0.1, 108%</td>
<td></td>
</tr>
</tbody>
</table>

^a Percentage against the activity of nontreated cells.
^b Selective refers to growth inhibition revealed only against SV40-transformed cells by a compound at the concentration indicated. "Yes" and "No"; respectively, mean growth inhibition and no significant growth inhibition against both types of cells by a compound at the concentration indicated.
^c Mean ± S.E. of 4 independent experiments.
^d f, p<0.01 (Student’s t test).
^e p<0.05 (Student’s t test).
Effect of benzaldehyde on nucleosides, amino acids, and glucose uptake into normal and SV40-transformed cells

Effect of benzaldehyde (50 µg/ml) on the uptake of labeled compounds into normal and SV40-transformed cells was assayed by cover slip method. Cells were incubated with Hanks' BSS containing each labeled compound (1 µCi/ml for 3H-labeled compounds and 0.2 µCi/ml for 14C-labeled compounds) for 30 min.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Normal cells</th>
<th>SV40-transformed cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C]Thymine</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[14C]Uracil</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[14C]Adenine</td>
<td>108.6 ± 10.9</td>
<td>79.1 ± 3.4</td>
</tr>
<tr>
<td>[14C]Cytidine</td>
<td>91.0 ± 1.6</td>
<td>36.0 ± 1.6</td>
</tr>
<tr>
<td>[14C]Thymidine</td>
<td>73.2 ± 1.7</td>
<td>38.9 ± 1.7</td>
</tr>
<tr>
<td>[14C]Guanosine</td>
<td>81.1 ± 2.5</td>
<td>44.7 ± 1.4</td>
</tr>
<tr>
<td>[14C]Adenosine</td>
<td>90.0 ± 5.7</td>
<td>66.2 ± 8.2</td>
</tr>
<tr>
<td>[14C]Glycine</td>
<td>84.5 ± 3.3</td>
<td>53.7 ± 0.9</td>
</tr>
<tr>
<td>[14C]Glutamine</td>
<td>96.1 ± 2.3</td>
<td>83.6 ± 5.5</td>
</tr>
<tr>
<td>[14C]Asparagine</td>
<td>134.6 ± 2.1</td>
<td>97.0 ± 1.5</td>
</tr>
<tr>
<td>[14C]Glutamic acid</td>
<td>86.6 ± 7.8</td>
<td>102.7 ± 6.2</td>
</tr>
<tr>
<td>[3H]2-Deoxy-D-glucose</td>
<td>98.3 ± 5.1</td>
<td>72.9 ± 3.1</td>
</tr>
<tr>
<td>[3H]Uric acid</td>
<td>108.3 ± 10.3</td>
<td>78.0 ± 9.4</td>
</tr>
<tr>
<td>[3H]Cotransport</td>
<td>82.9 ± 7.0</td>
<td>91.3 ± 12.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percentage of uptake activity of benzaldehyde-treated cells against control</th>
<th>Normal cells</th>
<th>SV40-transformed cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3H]2-Deoxy-D-glucose</td>
<td>81.1 ± 1.0</td>
<td>53.3 ± 3.6</td>
</tr>
</tbody>
</table>

* Percentage of uptake activity of benzaldehyde-treated cells against control cells.
* Effect of benzaldehyde on the intracellular ATP level. It is reported that the omission of glucose from the incubation medium reduces the intracellular ATP level (15). Moreover, the substitution of glucose with 2-deoxy-D-glucose in the preincubation medium enhances this effect (32). Under these conditions, the effect of benzaldehyde on the thymidine (Chart 2) and 2-deoxy-D-glucose (Chart 3) uptake into the normal and SV40-transformed cells was examined. The appreciable inhibition of thymidine uptake by benzaldehyde (50 µg/ml) was exclusively observed with transformed cells incubated in the presence of 5.6 mM glucose (Charts 1 and 2; Tables 1 and 2). This effect of benzaldehyde was no longer observed when the cells were incubated in the absence of glucose (Chart 2). In the glucose-depleted medium, the rate of thymidine uptake into the transformed cells was approximately one-half and was as slow as that observed under the inhibition by benzaldehyde in the presence of glucose. The cells starved from glucose recovered their response to benzaldehyde through incubation with 0.5 ml Hanks' BSS containing 5 mM glucose (data not shown). A similar effect of glucose was observed on the uptake of [3H]-2-deoxy-D-glucose into the transformed cells treated with benzaldehyde (Chart 3). The presence of glucose, an energy source, was indispensable for the expression of the effect of benzaldehyde on the transformed cells. These results were obtained consistently in 6 independent experiments. The lower uptake efficiency revealed in the glucose-containing medium was due to the dilution effect of glucose on the 2-deoxy-D-glucose because they are incorporated through the same membrane transport system (11, 24).

Effect of benzaldehyde on the intracellular ATP level. Since nucleosides (17, 23) and glucose (6) are transported by the phosphorylation process, the inhibition of their incorporation into SV40-transformed cells by benzaldehyde was supposed to be due to the lowered level of ATP in cells. Chart 4 indicates that treatment with benzaldehyde (50 µg/ml) in the presence of glucose greatly reduced the intracellular ATP level of the transformed cells without any significant reduction of ATP level of the normal cells. This reduction of ATP level with benzaldehyde was not observed in the cells incubated in the absence of glucose, 4.1 ± 0.1 (S.E.) and 4.4 ± 0.4 pmol/10^6 cells in untreated and treated transformed cells, respectively.

DISCUSSION
The effect of benzaldehyde on the uptake of nutrients into
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of glucose, and their intracellular ATP levels were measured by the method of Strehler and Totter (29). Values represent mean of 3 independent experiments. Bars, S.E.

Chart 4. Effect of benzaldehyde on the intracellular ATP level of normal and SV40-transformed cells. Following the procedure described in “Materials and Methods,” 3 x 10^5 cells (normal cell) and 4 x 10^5 cells (SV40-transformed cell) were treated with (B) and without (C) benzaldehyde (50 μg/ml) in the presence of glucose, and their intracellular ATP levels were measured by the method of Strehler and Totter (29). Values represent mean of 3 independent experiments. Bars, S.E.

normal and SV40-transformed rat fibroblast cells was studied. Benzaldehyde (18) and related compounds (33), which were found to inhibit selectively the growth of SV40-transformed cells, inhibited the uptake of thymidine, other nucleosides, and 2-deoxy-β-glucose only into the transformed cells. Furthermore, their effective doses to show these inhibitory effects were the same in both cases (Chart 1; Table 1). This correlation suggests the possibility that the malnourishment caused by the poor uptake of these compounds into the transformed cells can elucidate the selective growth inhibition of the transformed cells. The poor uptake was not due to cell death, because viable cell number was consistent during this uptake assay.

Several investigators have reported that the carcinostatic substances, methylhydrazones (2) and nitrobenzothioinosine (3, 20, 22), are strong inhibitors of nucleoside uptake into cultured cells and that methotrexate is an inhibitor of thymidine uptake into Ehrlich ascites carcinoma cells (12). Nitrobenzothioinosine binds to the carrier protein of nucleosides and alters the rate of transport (31). Methotrexate may inhibit hexokinase activity (12). However, an analogous mechanism could not be directly applied to the inhibitory activity of benzaldehyde because of their markedly different structures.

It has been reported that benzaldehyde inhibits the activity of Na+–K+–ATPase (EC 3.6.1.3) of mouse brain to the extent of 50% at 5 x 10^{-4} M (5), the concentration that we routinely use. However, we observed that 10^{-3} M ouabain, which is enough to inhibit Na+–K+–ATPase, did not inhibit thymidine uptake into the cells that we used. Further, depletion of sodium ion in the incubation medium by substitution with choline ion revealed no effect on thymidine uptake or on the action of benzaldehyde (data not shown). These results indicate that the thymidine uptake system inhibited by benzaldehyde does not involve Na+–K+–ATPase.

Martz et al. (17) reported that intracellular phosphorylation of thymidine was the rate-determining step of thymidine incorporation into Novikoff rat hepatoma cells, which was studied by using ATP-depleted cells or a thymidine kinase-negative subline. Incubation of cells in glucose-free medium decreases the adenylate energy charge [(ATP + ½ ADP)/(ATP + ADP + AMP)] (15). We observed a similar decrease of intracellular ATP level and a reduced rate of thymidine uptake into the cells (Chart 2B) when cells were incubated in glucose-depleted medium. These results indicate that, in the cells that we used, the phosphorylation process requiring ATP takes an important part in the nucleoside uptake system as reported in other types of cells (17, 23). Benzaldehyde treatment of the transformed cell in the presence of 5 mM glucose also caused a marked decrease of intracellular ATP level and a reduced rate of thymidine uptake (Charts 2A and 4) which means that the selective inhibition of thymidine uptake into SV40-transformed cells by benzaldehyde is due to the lowered intracellular ATP level by benzaldehyde. The inhibitory effect of benzaldehyde on thymidine uptake in the presence of glucose disappeared in the absence of glucose (Chart 2B), keeping a half-maximal level of thymidine uptake even under the treatment with benzaldehyde. This result suggests that benzaldehyde does not inhibit the penetration of thymidine through plasma membrane which includes no phosphorylating process. Since a similar marked decrease of uridine uptake has been reported also in the ATP-depleted Novikoff rat hepatoma cells and since all nucleosides are considered to be transported by the same system (23), the inhibition of uptake of other nucleosides into the transformed cells by benzaldehyde is considered to occur through the same mechanism as observed in the case of thymidine uptake.

Graff et al. (6) reported that the long-term rate of 2-deoxy-β-glucose uptake into phosphorylating cells reflects the rate of its phosphorylation rather than that of its transport. Our assay conditions were similar to theirs. As observed in the case of thymidine uptake, benzaldehyde inhibited 2-deoxy-β-glucose uptake into the transformed cells only when they were incubated in the presence of glucose (Chart 3). There is a possibility that the inhibition of 2-deoxy-β-glucose uptake into the transformed cells is also due to the decrease of intracellular ATP level caused by some mechanisms such as activation of ATPase or leaking out of ATP from cells, etc. However, this decrease of ATP was not observed with glucose-free Hanks’ BSS, meaning that it is not the first and general event caused by treatment with benzaldehyde. On the contrary, there is another possibility that the inhibition of β-glucose uptake into the transformed cells causes the reduction of intracellular ATP level. Our preliminary experiment showed that 3-O-methylglucose transport into the transformed cells was also inhibited by benzaldehyde only in the presence of 5.6 mM glucose, strongly suggesting this possibility (data not given). However, the latter requires that benzaldehyde selectively and directly inhibit the glucose transport system only in the presence of glucose. Krupka (14) reported that 1-fluoro-2,4-dinitrobenzene inhibited sugar transfer through the erythrocyte membrane when the most readily transported sugars were present. He proposed 2 conformational states of carrier, one being an intermediate in transport that rapidly reacts with 1-fluoro-2,4-dinitrobenzene. It is conceivable that a chemical group of the protein included in the glucose transport system of the transformed cells becomes more exposed or more reactive with benzaldehyde in the presence of glucose as proposed by Krupka.

The present study demonstrated that benzaldehyde selectively inhibits the uptake of nucleosides and 2-deoxy-β-glucose into SV40-transformed cells and that it reduces the intracellular
ATP level of the transformed cells. Although the picture of the mechanism of inhibition is still incomplete, the fact that benzaldehyde treatment lowers the intracellular ATP level of SV40-transformed cells selectively may be one important result to understand the carcinostatic activity of benzaldehyde.

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REFERENCES


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