REDUCED LEVELS OF ADENOSINE DEAMINASE IN CHICK EMBRYO FIBROBLASTS TRANSFORMED BY ROUS SARCOMA VIRUS

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SUMMARY: Adenosine deaminase activities in chick embryo fibroblasts were substantially reduced after infection and transformation by Rous sarcoma virus. Concomitant with the reduction in adenosine deaminase activities, the incorporation of exogenous adenosine into RNA species of the virus transformed cells was moderately increased. The significance between reduction in adenosine deaminase activity and malignant transformation by Rous sarcoma virus remains to be elucidated.

INTRODUCTION

Malignant transformation of a variety of cells by oncogenic viruses is accompanied by morphological alterations and by a number of biochemical changes. Notable among these biochemical changes in chick embryo fibroblasts transformed by Rous sarcoma virus (RSV) are increased accumulation of water (1), decrease in intracellular cyclic AMP concentrations (2), lower adenylate cyclase activities (3,4), increased hexose uptake and hyaluronic acid synthesis (5,6), increased protease activity (7), and decreased alkaline phosphatase activities (8). We report here that adenosine deaminase

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(EC 3.5.4.4) activity in these RSV infected cells is substantially decreased.

MATERIALS AND METHODS

Chick embryo fibroblasts and similar cells transformed by different strains of RSV, were cultured in Eagle's minimal medium supplemented by 5% fetal bovine serum (5,6). Two days after replating, at which time growth rates and hence cell density were approximately the same, the fibroblasts were rinsed twice with Dulbecco's phosphate buffered saline, scraped off by a rubber policeman and suspended in 20 mM potassium phosphate, 250 mM sucrose, 0.1 mM dithiothreitol, pH 7.4, 4°C. The pooled cells were homogenized at 4°C by a ground glass homogenizer, and subsequently transferred to a glass centrifuge tube, frozen in liquid N_2 and thawed. The supernatant, after centrifugation at 15,000 X g for 10 min, was used to assay adenosine deaminase activity by a method adapted from Gustin and Kemp (9). The assay medium in a final volume of 0.5 ml contained 20 mM potassium phosphate, 1 mM EDTA, and 0.5 mM [8-^{14}C]adenosine, pH 7.4, 37°C. After 10 min of incubation, 50 µl of 5 M formic acid was added to stop the reaction. The amount of [8-^{14}C]inosine formed was determined by a SP-Sephadex column (9). Specific activity is expressed as µmoles of inosine formed per milligram of protein per hr; each value is the average of 3 to 4 determinations based on a linear relationship between enzyme activity and protein concentrations.

The incorporation of adenosine and uridine into RNA species of infected and normal chick embryo fibroblasts were determined after 10 hrs of incubation with [2,8-^{3}H]adenosine (10 µCi/ml; 30.4 Ci/mmol) and [U-^{14}C]uridine (1 µCi/ml; 463.6 mCi/mmol), the RNA was extracted with 1% sodium dodocyl sulfate-phenol, precipitated by ethanol, sedimented in a 15-30% sucrose gradient, and radioactivity determined. RNA sedimenting between 20S - 26S was adsorbed to oligo(dt) to select the polyadenosine containing species.

RESULTS AND DISCUSSION

Table 1 shows the activity of adenosine deaminase in chick embryo fibroblasts and in similar cells after transformation by two RSV strains. The activity of adenosine deaminase in the cells infected by the Bryan high-titer strain of Rous sarcoma virus (RSV-BH), or the Schmidt-Ruppin strain (RSV-SR) decreased to 11 and 7% of that of the non-infected cells, respectively. It is noteworthy that under similar experimental conditions the levels of acid phosphatase remain essentially unchanged (A. V. Bader, J. Kondratick and J. P.

Table 1. Adenosine deaminase activity in chick embryo fiborblasts infected with strains or mutants of Rous sarcoma virus (RSV). Specific activity is expressed as μmoles of inosine formed per milligram of protein per hr. Actinomycin D or cycloheximide were added at 2 μg/ml of culture medium.

Cells infected with	Adenosine deaminase activity (specific activity)
None 37°	4.20
RSV-BH 37°	0.48
RSV-SR 37°	0.31
RSV-BH-Ta 41°	1.32
RSV-BH-Ta 37°	0.45
RSV-SR-T5 41°	2.86
RSV-SR-T5 37°	0.57
RSV-BH-Ta 41°→37° (6 hrs)	1.44
RSV-BH-Ta 41°→37° (actinomycin D, 6 hrs)	1.62
RSV-BH-Ta 41°→37° (cycloheximide, 6 hrs)	1.32
RSV-BH-Ta 37°→41° (6 hrs)	0.55
RSV-BH-Ta 37°→41° (actinomycin D, 6 hrs)	0.39
RSV-BH-Ta 37°→41° (cycloheximide, 6 hrs)	0.36

Bader, in preparation). Dialyzing the enzyme extracts against large volumes of buffer did not change the specific activities of adenosine deaminase appreciably. Also, adding the enzyme extract from RSV-BH infected cells to the enzyme extract from normal cells had no noticeable effect on the expected specific activity of adenosine deaminase from the normal cells. Hence, a virally induced production of dialyzable or non-dialyzable inhibitor of adenosine deaminase in the infected cells seems improbable.

Two mutants of RSV, RSV-BH-Ta and RSV-SR-T5, induce temperature-dependent transformation in infected cells. Cells grown at 41°C are morphologically non-transformed, while at 37°C cells are morphologically transformed and exhibit the

associated biochemical changes. Adenosine deaminase activity in mutant-infected cells cultured at 41°C was markedly reduced compared to non-infected cells. Nevertheless, these activities are significantly higher than when the same cells were grown at 37°C, at which temperature adenosine deaminase levels similar to those of wild-type transformed cells were found (Table 1).

When cells infected with RSV-BH-Ta are switched from 41°C to 37°C, morphological changes characteristic of transformation are usually observable within 30 min, and increases in hexose uptake and hyaluronic synthesis are unequivocal within 4 to 6 hours (5,6). These changes are reversible; cells shifted from 37°C to 41°C lose their transformed morphology within a few hours. By contrast, after shifting from 41°C to 37°C, the activity of adenosine deaminase in the RSV-BH-Ta infected cells remained essentially unaltered after 6 hours. Inhibition of RNA synthesis by actinomycin D, or of protein synthesis by cycloheximide had no effect on adenosine deaminase activity in these cells. A small increase in the activity of adenosine deaminase was observed 6 hours after shifting RSV-BH-TA infected cells from 37°C to 41°C. This increase was not observed in the presence of actinomycin D or cycloheximide. Apparently, changes in adenosine deaminase activities are relatively slow when compared to some other reported features of transformation (5,6), and may not be responsible for the morphological changes which occur following temperature shifts of mutant infected cells.

Multiple forms of adenosine deaminase have been reported (10,11,12,13,14) and alterations in the adenosine deaminase isozyme patterns of transformed cells were observed (10), but

Table 2. Incorporation of adenosine and uridine into RNA of infected and normal chick embryo fibroblasts.

	Ratio [^{3}H]adenosine/[^{14}C]uridine	
RNA type	Normal	RSV-BH infected
Total	7.0	8.3
Ribosomal 28S	6.7	7.7
Ribosomal 18S	7.6	9.1
Low Mol. Wt. 4-7S	7.0	8.5
Oligo(dt) selected	9.0	11.7

it is not known whether changes observed here involve all of the isozymes of adenosine deaminase or only some of them.

Concomitant with the reduction in adenosine deaminase activity, there was an increase in the incorporation of [^{3}H]adenosine into all of the RNA species from the RSV-BH infected cells (Table 2). The increase in adenosine incorporation could be attributed to reduced degradation of adenosine as a result of the decrease in the level of adenosine deaminase. However, the possibility that the increased rate of adenosine incorporation was due to other factors, such as an alteration in the rate of uptake of adenosine or a change in the intracellular phosphorylation capacity of adenosine, has not been eliminated. It should be noted here that at the levels used, there was no competition between the uptake of adenosine and uridine in the present investigation.

Adenosine deaminase has received much attention recently with the discovery of a human combined immunodeficiency disease that is accompanied by either an absence of or much

reduced levels of adenosine deaminase (15,16,17). It was also found that the levels of adenosine deaminase in lymphocytes from patients with untreated chronic lymphocytic leukemia was consistenly lower than in lymphocytes from normal subjects (18,19). Adenosine deaminase plays a key role in the normal metabolism of adenine nucleotides and the production of hypoxanthine for use in the purine salvage pathway (20,21). How reduced levels of adenosine deaminase affect other metabolic parameters in the cells transformed by Rous sarcoma virus remains to be elucidated.

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