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The rate of catabolism of dATP to deoxyadenosine during the growth of different cell lines in vitro

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A method has been developed for the determination of the rate of formation of deoxyadenosine from dATP in cultured cell lines. The lowest rate was found in the T-cell-derived Molt cell line while it was about 70-fold higher in Balb c/3T3 mouse fibroblasts. In the B-cell-derived Raji cells and in the murine sarcoma cell line SEWA it had intermediary values. It is concluded that in some cell types like the 3T3-cells the catabolism of dATP to deoxyadenosine may have a significant regulatory effect on the cellular content of dATP.

dATP; Catabolism; Deoxyadenosine; Cultured cell

1. INTRODUCTION

The de novo synthesis of the substrates for DNA replication, the four deoxyribonucleoside triphosphates (dNTPs), depends on ribonucleotide reductase, an enzyme the activity of which is highly regulated and subject to a complicated allosteric control [1]. The enzyme seems to play a major role in maintaining a fine tuned balance between the pools of the dNTPs during the S-phase of the cell cycle of mammalian cells [2]. This balance may, however, be impaired in growing cells if a deoxyribonucleoside is available, resulting in reduced replication of DNA. This was first observed for deoxyadenosine which in Ehrlich ascites tumor cells gives rise to the accumulation of large amounts of dATP [3]. This compound exerts a pronounced feed-back inhibition of ribonucleotide reductase and it leads consequently to the prevention of DNA replication due to lack of the other three dNTPs [2]. Degradation of DNA in vivo as it occurs during the formation of erythrocytes leads to the formation of deoxyribonucleoside monophosphates, which are further degraded. The catabolism of dAMP seems to exclusively lead to the formation of deoxyadenosine [4]. In normal organisms and cells adenosine deaminase prevents the accumulation of deoxyadenosine which is eventually converted to hypoxanthine and deoxyribose phosphates. However, individuals suffering from inherited adenosine deaminase deficiency contain in the blood deoxyadenosine which may be phosphorylated to dATP. It accumulates in relatively large amounts in especially T-lymphocytes and in other blood cells. Under these conditions the normal function of lymphocytes is curtailed. Adenosine deaminase deficiency is, thus, a potential lethal inherited disorder associated with combined immunodeficiency [5]. The phosphorylation of deoxyadenosine to dAMP is probably mainly catalyzed by deoxycytidine kinase which in addition has deoxyguanosine as a substrate [6]. Evidently, under conditions resembling partial or complete adenosine deaminase deficiency, the rate of phosphorylation of deoxyadenosine may exceed that of dephosphorylation of dAMP in cells like peripheral lymphocytes [7] as well as in cultured transformed lymphocytes [8] and in Ehrlich ascites tumor cells [9]. Estimates of the rates of degradation of high concentration of dATP induced by deoxyadenosine has previously been made in different cell lines after the removal of deoxyadenosine from the growth medium. In the present work we have determined the rate of degradation of dATP to deoxyadenosine in four different cell lines growing in normal media, i.e. in the absence of added deoxyadenosine.

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2. MATERIALS AND METHODS

Balb c/3T3 mouse fibroblast cells were grown in DME medium containing 10% newborn calf serum. For experiments, cells were treated as follows. Confluent cultures were trypsinized, diluted and transferred to 24-cm^2 flasks at a density of 3×10^5 cells in 5 ml; after 18 h approximately 47% of the cells were in the S-phase of the cell cycle. The transformed human thymocyte and lymphocyte cell lines, Raji and Molt, respectively and the murine sarcoma cell line SEWA [10] were grown as suspension cultures in RPMI medium containing 10% fetal calf serum. During the experiments the cell densities were about 10^6 cells per ml and close to 40% of the cells were in the S-phase of the cell cycle as determined by flow cytometry. Cells were prelabeled for 18 h with [2-^{3}H]adenine (0.2 μM, 42 Ci per mmol). After change of the medium coformycin was added at a concentration of 0.1 μM

Table I

Cell line	3T3	SEWA	Raji	Molt
Average content of dATP (pmol × $(10^6$ cells$)^{-1}$)	30 (34;26)	8.0 (±3.5)	21 (21,21)	46 (47,44)
Cells in S-phase (per cent)	47	39	38	39
Average specific radioactivity of dATP (cpm × $pmol^{-1}$)	192	413	380	202
Rate of formation of radioactive deoxyadenosine from dATP (10^3 cpm × $(10^6$ cells$)^{-1}$ × h^{-1})	72	10	20	1.1
Rate of formation of deoxyadenosine from dATP during the S-phase (pmol × $(10^6$ cells$)^{-1}$ × h^{-1})	800 (787,813)	62 (±19)	139 (165,113)	14 (8.3,19)
Fraction of dATP that is degraded to deoxyadenosine (per cent of the sum of dAMP incorporated into DNA plus the amount degraded to deoxyadenosine)*	56	11	18	2

The experimental figures are the average of 4 experiments with SEWA cells (S.E.M. is given in parentheses) and of 2 experiments with each of the other cells (the individual results are given in parentheses).
*These figures are based on a cellular DNA content of 6 pg and a molar adenine content in DNA of 28% [15]. Thus, 10^6 G_1-phase cells contain 5.8 nmol of dAMP and the average rate of incorporation of dAMP into DNA during the S-phase of the cell cycle (assuming it to be 9 h) is accordingly 640 pmol per h per 10^6 cells.

or more and incubation was continued for 6 h. dATP was extracted from cells by a modification of the method of North et al. [11]. At least 10^6 cells were extracted for 30 min with 60% methanol (4°C), the extract was centrifuged and the supernatant evaporated to dryness. The residue was treated with 2% perchloric acid (4°C) for 30 min, neutralized and centrifuged. The resulting supernatant was evaporated to dryness and the residue used for enzymatic determination of dATP as described [11] using the large fragment of DNA polymerase I and the alternating copolymer poly(dA-dT) as primer template. The specific radioactivity of dATP in the extract was obtained from the total radioactivity (as determined in the presence of unlabeled dTTP) and from the total amount (as determined with [^{3}H]dTTP (10 Ci/mmol). Analyses of parallel experiments performed with cells that had not been prelabeled with [^{3}H]adenine resulted in the same figure for the amount of dATP. The ^{3}H-labeled deoxyadenosine that accumulated in the medium after medium change was determined after its isolation on a reversed-phase column. Isocratic elution (0.15 M potassium phosphate, pH 4.0; 1% methanol, 1% acetonitrile, 0.2% tetrahydrofuran) was employed.

3. RESULTS AND DISCUSSION

The design of the experiments were as follows. The cellular adenine ribonucleotides and deoxyribonucleotides were labeled by pretreatment of cultured cells with [2-^{3}H]adenine. After change of the growth medium, the incubation was continued in medium containing coformycin at a concentration that prevented deamination of any 2′-deoxyadenosine that might be formed. At the beginning of the experiment and after incubation of the cells for 3 and 6 h, low molecular compounds were extracted from the cells and the content and the radioactivity of dATP was determined. Any deoxyadenosine accumulated during the experiment was isolated from the growth medium and its radioactivity was determined. The amount of deoxyadenosine formed from dATP during the experiment was determined from its radioactivity and from the average specific radioactivity of dATP.

The experimental results appear from Table I which shows that the average content of dATP ranged from 6.5 to 45 pmol per 10^6 cells, with the lowest content in the SEWA cells and the highest in 3T3 cells. Since nucleotide reductase is active only during the S-phase of the cell cycle it seems justified to assume that dATP is present in the cells in normal growth medium only during this period. On this assumption and from the fraction of cells in the S-phase, the dATP content and the rate of formation of deoxyadenosine has been calculated during this phase of the cell cycle. It appears that in the T-cell-derived Molt cells the rate of formation of deoxyadenosine is about 60-fold lower than in the 3T3 fibroblasts while in the SEWA and B-cell derived Raji cells the rates were about 13- and 6-fold lower, respectively. The experiments showed a constant rate of 2′-deoxyadenosine formation in the experimental period and the specific radioactivity of dATP did not change in a systematic way (data not shown). In the presence of hydroxyurea (0.5 mM), no labeled deoxyadenosine was formed in experiments with 3T3 cells (data not shown). Since hydroxyurea is a specific inhibitor of nucleosidediphosphate reductase, this confirms that the deoxyadenosine as measured in the present work stems from dATP formed by de novo synthesis catalyzed by the reductase reaction. With SEWA cells it was found that the amount of labeled deoxyadenosine that accumulated in the medium varied less than 9% when the concentration of coformycin was 0.1, 1.0 and 25 μM. This is in agreement with the assumption that dAMP is degraded exclusively to deoxyadenosine and not to dIMP [4]. The activity of adenosine deaminase is known

to be inhibited completely by 0.1 μM coformycin while that of AMP deaminase is inhibited at 25–50 μM coformycin [12]. In the present experiments cofomycin was found to have no effect on the cellular dATP content. The results show, finally, that for the 3T3 fibroblasts the amount of dATP which is degraded to deoxyadenosine is similar to the amount that is incorporated into DNA as dAMP. This is in sharp contrast to the finding for especially Molt cells where only a few per cent of dATP is catabolized while in the two other cell lines intermediate figures were obtained. These findings may indicate that in some cell types like fibroblasts the rate of catabolism of dATP to deoxyadenosine may have a significant regulatory effect on the cellular content of dATP. In other types of cells like the transformed lymphocyte, Molt, this regulation may almost exclusively come about through an allosteric feed-back regulation of the ribonucleotide reductase. For 3T6 fibroblasts in the S-phase of the cell cycle, Nicander and Reichard [13] and Bianchi et al. [14] have similarly found that the synthesis of pyrimidine deoxyribonucleotides was larger than their demand for DNA synthesis and that the cells degraded the surplus material to deoxyribonucleosides.

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