PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 179, 437–441 (1985)

Catabolic Pathways of Purine Ribonucleotides and Deoxyribonucleotides in Lymphocytes (42120)

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Abstract. Deficiency of either one of the subsequent purine catabolic enzymes adenosine deaminase or purine nucleoside phosphorylase results in immunodeficiency disease in humans. However, the mechanism by which impairment of purine metabolism may cause immunodeficiency is unclear. In the present work we have studied the catabolism of purine ribonucleotides and deoxyribonucleotides in T lymphocytes to better understand the role of purine nucleoside phosphorylase and adenosine deaminase in the immune function. It was found that purine deoxyribonucleotides are degraded via catabolic pathways distinctly different from those used for purine ribonucleotide degradation. Thus both adenine and guanine ribonucleotides are deaminated to IMP whereas purine deoxyribonucleotides are exclusively dephosphorylated to the corresponding deoxyribonucleosides. These findings may explain the relatively higher degradation rates of purine deoxyribonucleotides in mammalian cells as compared to purine ribonucleotides. The catabolism of purine nucleotides is tightly linked to the active purine nucleoside cycles which consist of the phosphorolysis of purine nucleosides and deoxyribonucleosides to their corresponding bases, their salvage to monophosphates and back to the corresponding ribonucleosides. The above observations also imply that a possible role of the purine nucleoside cycles is to convert purine deoxyribonucleotides into their corresponding ribonucleotide derivatives. Deficiencies of purine nucleoside phosphorylase or of adenosine deaminase activities, enzymes which participate or lead to the purine nucleoside cycles, thus result in a selective impaired deoxyribonucleotide catabolism and immunodeficiency.

The discovery of inherited deficiencies of the purine degradation enzymes adenosine deaminase (ADA) purine nucleoside phosphorylase (PNP) allowed insight into purine catabolism in humans (1, 2). Observations in PNP deficient patients and their cultured cells revealed marked purine overproduction (fivefold the normal level) similar to that observed in hypoxanthine guanine phosphoribosyltransferase (HGPRTase) deficiency but, the urinary purines excreted are nucleosides and deoxyribonucleosides rather than uric acid (3).

Recent observations in PNP deficient lymphocytes gave evidence for the existence of active purine nucleoside cycles in mammalian cells (4). These cycles utilize nucleosides and deoxyribonucleosides produced by dephosphorylation of their respective nucleoside monophosphates which are subsequently salvaged back to the nucleoside monophosphate via the PNP and HGPRTase reactions. One of the questions addressed in the present work is the role of these cycles in the regulation of purine deoxyribonucleotide and ribonucleotide metabolism in lymphocytes.

Analysis of the relative amounts of purines produced by PNP-deficient patients also yielded interesting results concerning the catabolism of purine ribonucleotides and deoxyribonucleotides (3). It was found that the amounts of deoxyinosine and deoxyguanosine produced in PNP-deficient patients are approximately one-third of the amounts of the corresponding ribonucleosides inosine and guanosine. This observation is surprising since the intracellular levels of deoxyribonucleotides are between 100 to 10,000-fold lower than their respective ribonucleotides. It thus appears that the relative degradation rates of deoxyribonucleotides are much higher than that of ribonucleotides. The second question we addressed in the present study is whether deoxyribonucleotides and ribonucleotides have different catabolic pathways explaining the disparity observed in their catabolic rates.

Materials and Methods. *Materials.* [8-^{3}H] Adenine (24 Ci/mmole), [G-^{3}H] hypoxanthine (10 Ci/mmole), and [8-^{3}H] guanine (7.7 Ci/mmole) were purchased from Amersham (Arlington, Ill.). [8-^{3}H] Deoxyguanosine

0037-9727/85 $1.50

(5 Ci/mmole), [U-^{14}C] glycine (100 mCi/mmole), and [8-^{3}H] deoxyadenosine were purchased from ICN Pharamaceutics (Irvine, Calif.). Purine and pyrimidine nucleotides and bases were purchased from Sigma Chemicals, (St. Louis, Mo.).

Cells. Hypoxanthine guanine phosphoribosyltransferase-deficient human T lymphoblast cell line was generously supplied by H.-M. Dosch (The Hospital for Sick Children, Toronto, Ontario). Purine nucleoside phosphorylase-deficient mouse T lymphoma cell line was generously supplied by Dr. Ullman (University of Kentucky, Lexington, Ky.).

Measurements of nucleotide catabolism. Cells were preincubated for 2 hr with radioactive (1 μCi/ml) purine precursors in RPMI 1640 with 5% fetal calf serum (heat inactivated 30 min at 56°C), to allow sufficient labeling of the respective intracellular nucleotide pools (5). After washing out the radioactive precursor the cells were incubated in the presence or absence of catabolic inducer (5.5 m*M* deoxyglucose) and the intracellular and extracellular catabolic products analyzed as previously described (5, 6).

Preliminary experiments were conducted to select 2′-deoxycoformycin concentration of 50 μM which completely inhibited (>97%) adenosine deaminase activity in intact lymphocytes without any detectable inhibition of AMP deamination by adenylate deaminase in intact lymphocytes (6).

Results and Discussion. To study nucleotide catabolic pathways and the role of the purine nucleoside cycles in the degradation of purine deoxyribonucleotides and ribonucleosides we have analyzed the catabolism of ATP, deoxyATP, GTP, and deoxyGTP in T lymphoblastoid cells. For studies of ATP and deoxyATP catabolism we have used a human T lymphoblastoid cell line deficient in hypoxanthine guanine phosphoribosyltransferase activity (HGPRTase). The use of HGPRTase-deficient cells allowed exclusive labeling of deoxyATP pools by deoxyadenosine without labeling of intracellular ATP since no salvage of hypoxanthine is possible in HGPRTase-deficient cells. In order to achieve sufficient labeling of deoxyATP pools it was also necessary to prevent the deamination of deoxyadenosine using the potent ADA inhibitor 2′-deoxycoformycin. After labeling of deoxyATP pools with deoxyadenosine, nucleotide catabolism was induced by deoxyglucose in the presence of deoxycoformycin and the degradation of adenine deoxyribonucleosides was followed in cell extracts as well as the efflux of deoxynucleosides and bases into the extracellular medium (Table I). Deoxyglucose caused a rapid degradation of deoxyATP which was completed within 30 min, no accumulation of intracellular deoxyADP or deoxyAMP was observed (data not shown) and all the radioactivity appeared in the extracellular medium. Analysis of the radioactivity in nucleosides and bases in the medium revealed that over 95% of the radioactivity was found in deoxyadenosine. This observation indicates that in the absence of deoxycoformycin the catabolism of deoxyATP flows exclusively through the adenosine deaminase reaction and there is no deamination of deoxyAMP by adenylate deaminase.

The degradation of adenine ribonucleotides was also induced by deoxyglucose and the production of ribonucleosides and bases was followed in HGPRTase-deficient cells labeled with radioactive adenine and incubated in the presence or in the absence of deoxycoformycin (Table II). Again deoxyglucose induced a rapid breakdown of ATP without accumulation of ADP or AMP (results not shown) resulting in an efflux of the radioactive nucleosides and bases into the extracellular medium. In the absence of deoxycoformycin only inosine and hypoxanthine were formed

TABLE I. DEOXYATP CATABOLISM IN T LYMPHOBLASTS

Time (min)	Labeled deoxyATP catabolites (pmole/10^6 cells)		
	Hypoxanthine	Deoxyinosine	Deoxyadenosine
10	<1	<1	8.4
20	<1	<1	13.0
30	<1	<1	19.6

Note. Cells were preincubated with [8-^{3}H]deoxyadenosine in the presence of 50 μM deoxycoformycin allowing the formation of 36 pmole/10^6 cells radioactive deoxyATP. The radioactivity was washed out and the production of catabolic products analyzed. Results represent a single experiment; three other experiments gave similar results for additional details see Materials and Methods.

TABLE II. ATP CATABOLISM IN T LYMPHOBLASTS

Time (min)	Deoxycoformycin (50 μM)	Labeled ATP catabolites (pmole/10^6 cells) Hypoxanthine	Inosine	Adenosine
10	–	118	542	8
20	–	239	567	11
30	–	335	612	13
10	+	170	590	38
20	+	342	634	79
30	+	465	668	103

Note. Cells were preincubated with [8-^{3}H]adenine and in the presence or absence of 50 μM deoxycoformycin, ATP catabolism was induced with 5.5 mM deoxyglucose and catabolic products were analyzed in the extracellular medium. At the beginning of catabolic induction (time 0) the radioactive ATP concentration was 1360 (pmole/10^6 cells) in the absence of deoxycoformycin, and 1590 (pmole/10^6 cells) in the presence of deoxycoformycin. Results represent a single experiment; three other experiments gave similar results for additional details see Materials and Methods.

and no adenosine was found whereas in the presence of deoxycoformycin inosine and hypoxanthine were the major degradation products but small amounts of adenosine were also produced. These results indicate that adenine ribonucleotide degradation can flow mainly through the adenylate deaminase reaction and only a small amount of adenosine is deaminated by adenosine deaminase. These findings may explain the selective impairment of deoxyadenosine metabolism observed in adenosine deaminase-deficient patients (7–9).

For studies of GTP and deoxyGTP catabolism we have used a T lymphoblast cell line deficient in purine nucleoside phosphorylase activity which enabled the exclusive labeling of intracellular deoxyGTP pool by radioactive deoxyguanosine without any flow of radioactivity into the GTP pool. The results of deoxyGTP catabolism are summarized in Table III. During 4-hr incubation in medium the radioactivity in deoxyGTP and deoxyGDP pools decreased without a significant change in the radioactivity in deoxyGMP (results not shown). The final breakdown products of deoxyGTP are found exclusively in the extracellular medium. Analysis of the radioactivity associated with

TABLE III. DEOXYGTP CATABOLISM IN T LYMPHOMA CELLS

Time (hr)	Labeled deoxyGTP Catabolites (pmole/10^6 cells) Deoxyinosine	Deoxyguanosine
1	<0.1	4.3
2	<0.1	7.5
4	<0.1	9.4

Note. Purine nucleoside phosphorylase-deficient cells were labeled with [8-^{3}H]deoxyguanosine to label deoxyGTP pools (15.5 pmole/10^6 cells at time 0). Cells were than incubated in growth medium and the labeled extracellular metabolites were analyzed after various times. Results represent a single experiment; three other experiments gave similar results. See Materials and Methods for additional details.

nucleosides and bases revealed that all the radioactivity was found in deoxyguanosine. These results indicate that all the deoxyGMP formed during deoxyGTP catabolism is dephosphorylated directly to deoxyguanosine.

The results of guanine ribonucleotide catabolism are presented in Table IV. Again there is a decrease in the radioactivity associated with GTP with little change in the radioactivity of GDP and GMP pools (data not shown). Analysis of radioactive degradation products in the extracellular medium revealed the production of equal amounts of guanosine and inosine from guanine nucleotide degradation. This finding suggested that half of the GMP formed during guanine nucleotide degradation is dephosphorylated first and half undergoes a reductive deami-

TABLE IV. GTP CATABOLISM IN MOUSE T LYMPHOMA CELLS

Time (hr)	Labeled GTP Catabolites (pmole/10^6 cells) Inosine	Guanosine
1	53	47
2	92	90
4	137	142

Note. Purine nucleoside phosphorylase-deficient cells were labeled with [8-^{3}H]guanine to label the GTP pool, (394 pmole/10^6 cells at time 0), the radioactive guanine was washed out and the extracellular catabolites produced were analyzed. Results are from a single experiment; three additional experiments gave similar results. See Materials and Methods for additional details.

nation yielding IMP which is then dephosphorylated to inosine.

These results clearly demonstrate the existence of different catabolic pathways for degradation of purine ribonucleotides than those for purine deoxyribonucleosides possibly explaining the disparity observed in their catabolic rates (3). Thus, both adenine and guanine deoxyribonucleotides are dephosphorylated all the way to the nucleoside level, while the corresponding ribonucleotides can be first deaminated to IMP which is then dephosphorylated to inosine (Figs. 1 and 2). These findings may explain the widely different catabolic rates of purine ribonucleotides and deoxyribonucleotides revealed in PNP-deficient patients (3). Deoxyribonucleotides are dephosphorylated to the corresponding deoxyribonucleoside, and their salvage requires the ADA and PNP reactions, whereas purine ribonucleotides are deaminated to IMP (Figs. 1 and 2) which may be directly incorporated back to the nucleotide triphosphate pools independent of the PNP and ADA reactions. These findings reveal a possible role for the purine nucleoside cycles namely the conversion of purine deoxyribonucleotides to their corresponding ribonucleotide derivatives (4). Thus, guanine deoxyribonucleotides are converted to guanine ribonucleotides through the deoxyguanosine cycle while adenine deoxyribonucleotides are converted first to deoxyinosine via the adenosine deaminase reaction. The deoxyinosine produced this way is cycled to yield IMP which may be subsequently converted to adenine and guanine ribonucleotides by the purine nucleoside cycles (Figs. 1 and 2). It thus appears

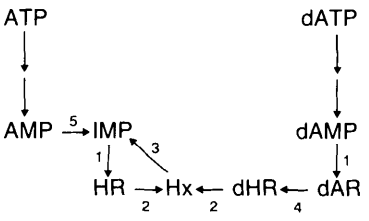

FIG. 1. Catabolic pathways of ATP and deoxyATP and the inosine cycle in T lymphocytes. Arrows represent the following enzymatic reactions. (1) 5'-Nucleotidase, (2) purine nucleoside phosphorylase, (3) hypoxanthine guanine phosphoribosyltransferase, (4) adenosine deaminase, and (5) adenylate deaminase. See text for additional details.

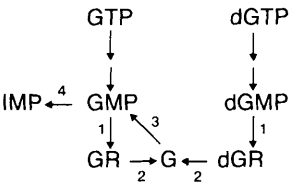

FIG. 2. Catabolic pathways of GTP and deoxyGTP and the guanosine cycle in T lymphocytes. Arrows represent the following enzymatic reactions. (1) 5'-Nucleotidase, (2) purine nucleoside phosphorylase, (3) hypoxanthine guanine phosphoribosyltransferase, and (4) guanylate deaminase.

that the purine nucleoside cycles are essential for the regulation of purine deoxyribonucleotide levels. Disruption of these cycles as in the case of ADA and PNP deficiencies leads to the observed abnormalities of deoxyribonucleosides triphosphate metabolism (7–10).

We thank Ellen Thompson and Chungyee Leung for expert technical assistance. This research was supported by grants from the Medical Research Council of Canada and the National Cancer Institute of Canada. A.C. is a recipient of National Cancer Institute of Canada Scholarship.

1. Giblett ER, Anderson JE, Cohen F, Pollara B, Meuwissen HJ. Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. Lancet **2**:1067–1069, 1972.
2. Giblett ER, Ammann AJ, Wara DW, Sandman R, Diamond LK. Nucleoside-phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. Lancet **1**:1010–1013, 1975.
3. Cohen A, Doyle D, Martin DW Jr., Ammann AJ. Abnormal purine metabolism and purine overproduction in a patient deficient in purine nucleoside phosphorylase. N Engl J Med **295**:1449–1454, 1976.
4. Barankiewicz J, Gelfand EW, Issekutz A, Cohen A. Evidence for active purine nucleoside cycles in human mononuclear cells and cultured fibroblasts. J Biol Chem **257**:11,597–11,600, 1982.
5. Cohen A, Barankiewicz Z, Lederman HM, Gelfand EW. Purine metabolism in human T lymphocytes: Role of the purine nucleoside cycle. Canad J Biochem Cell Biol **62**:577–583, 1984.
6. Barankiewicz J, and Cohen A. Evidence for distinct catabolic pathways of adenine ribonucleotides and deoxyribonucleotides. J Biol Chem **259**:15,178–15,181.
7. Cohen A, Hirschhorn R, Horowitz SD, Rubinstein A, Polmar SH, Hong R, Martin DW Jr. Deoxyaden-

osine triphosphate as a potentially toxic metabolite in adenosine deaminase deficiency. Proc Natl Acad Sci USA **75**:472–476, 1978.

8. Coleman MS, Donofrio J, Hutton JJ, Hahn L, Daoud A, Lampkin B, Dyminski J. Identification and quantitation of adenine deoxynucleotides in erythrocytes of a patient with adenosine deaminase deficiency and severe combined immunodeficiency. J Biol Chem **253**:1619–1626, 1978.
9. Donofrio J, Coleman MS, Hutton JJ. Overproduction of adenine deoxynucleosides and deoxynucleotides in adenosine deaminase deficiency with severe combined immunodeficiency disease. J Clin Invest **62**: 882–887, 1978.
10. Cohen A, Gudas LJ, Ulman B, Martin DW Jr. Nucleotide metabolism in cultured T cells and in cells of patients deficient in adenosine deaminase and purine nucleoside phosphorylase. In: Enzyme Defects and Immune Dysfunction, pp101–114. Amsterdam, Elsevier, Ciba Foundation 1979.

Received January 28, 1985. P.S.E.B.M. 1985, Vol. 179. Accepted March 6, 1985.