Guanosine triphosphate catabolism in purine nucleoside phosphorylase deficient human B lymphoblastoid cells

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GTP catabolism induced by sodium azide or deoxyglucose was studied in purine nucleoside phosphorylase (PNP) deficient human B lymphoblastoid cells. In PNP deficient cells, as in control cells, guanylate was both dephosphorylated and deaminated but dephosphorylation was the major pathway. Only nucleosides were excreted during GTP catabolism by PNP deficient cells and the main product was guanosine. The level of nucleoside excretion was largely affected by intracellular orthophosphate (P_i) level. In contrast, normal cells excreted nucleosides only at low P_i level while at high P_i levels, purine bases (guanine and hypoxanthine) were exclusively excreted. PNP deficiency had no effect on the extent of GMP deamination.

GTP catabolism *Purine nucleoside phosphorylase deficiency* *Guanylate reductase*

1. INTRODUCTION

Deficiency of the purine nucleoside phosphorylase (PNP) in humans is associated with T-lymphocyte abnormalities and results in large urine excretion of purine nucleosides [1], the substrates of PNP. Both inosine and guanosine are found in the urine of PNP deficient patients while guanosine is the direct catabolic product of GTP catabolism, inosine may be produced either by ATP catabolism or as a catabolic product for GTP degradation. Guanylate formed during GTP catabolism can be dephosphorylated to guanosine by 5′-nucleotidase (EC 3.1.3.5) and nonspecific phosphatases (EC 3.1.3.1 and 3.1.3.2), or GMP can be deaminated to IMP by GMP reductase (EC 1.6.6.8) [2].

It was believed that in mammalian cells GMP is catabolized only via dephosphorylation [2] and reductive deamination of GMP is quantitatively important only in bacteria [3]. However, later studies demonstrated the presence of guanylate reductase activities in rabbit and human erythrocytes [4,5] in calf thymus [6]. Deamination of GMP occurred at low rates in erythrocytes incubated with or without glucose [3]. On the other hand, in Ehrlich ascites tumor cells incubated with deoxyglucose GMP was only dephosphorylated [7] and less than 1% of GMP was deaminated to IMP.

Our aim was to evaluate the catabolism of GTP and the importance of its alternative pathways, i.e. GMP dephosphorylation *vs* GMP deamination, in human B lymphoblastoid cells with PNP deficiency, where phosphorylisis of purine nucleosides is impaired.

2. MATERIALS AND METHODS

2.1. *Chemicals*

[8-^{3}H]Guanosine was purchased from ICN (Irvine, CA). Nonradioactive purine bases, nucleosides, nucleotides and 2-deoxy-D-glucose were purchased from Sigma (St. Louis, MO). Sodium azide was from Fisher (NJ).

Freon TF was purchased from DuPont (Maitland, Ont.), and Alamine 336 from Hankel (Kankakee, IL).

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RPMI-1640 medium was obtained from Grand Island Biologicals (Burlington, Ont.), PEI-cellulose TLC sheets from Fisher (Don Mills, Ont.), and cellulose-TLC from Eastman Kodak (Rochester, NY).

2.2. *Cells*

2.2.1. Mononuclear cell preparation, cell culture conditions

Mononuclear cells from peripheral blood were separated on Ficoll-Hypaque (specific gravity 1.077) as in [8]. Complete culture media consisted of RPMI-1640 with penicillin (100 units/ml), streptomycin (100 μg/ml), 2 mM glutamine and 15% heat-inactivated fetal bovine serum.

2.2.2. Virus stock, infection procedure

Epstein-Barr Virus (EBV) was obtained from EBV transformed marmoset lymphocytes, strain B95-8, and prepared as in [9]. EBV infection was performed by incubating PNP deficient patient's mononuclear cell preparation with EBV for 2 h at 37°C. The mononuclear cells were washed 3 times, then placed in long-term culture. Cultures were fed periodically as necessitated by expansion of the transformed cell population.

As the control cells spontaneously transformed EBNA negative IgM/λ producing human B-cell line was used.

2.2.3. Incubation

Cells (2×10^7) were incubated with 2 μCi [8-^{3}H]guanine (20 Ci/mmol) to synthesize radioactive GTP. After 20–60 min incubation unutilized precursor was washed out and the cells were resuspended in 0.15 μM NaCl and 2×10^6 cells were used for 0, 5, 10, 20, 30 min incubation with 5.5 mM deoxyglucose or 1 mM sodium azide to induce GTP catabolism. After incubation cells were collected by centrifugation and the radioactivity of purine nucleosides and bases was determined in the medium. The cell pellet was extracted with 50 μl of 0.4 M perchloric acid and extracts were neutralized with Alamine 336-Freon [10], and then used for ribonucleotide analysis.

2.3. *Separation of purine nucleotides, nucleosides and bases*

Nucleotides were separated by thin-layer chromatography on PEI-cellulose in increased concentrations of formate buffer [11]. Separation of nucleosides and bases was carried out on Kodak cellulose thin-layer chromatograms in 5% Na_2HPO_4-water solvent. Spots were localized under ultraviolet lamp and their radioactivity was counted in a Beckman LS 2800 counter. Intracellular concentrations of guanine nucleotides measured by high-pressure liquid chromatography were taken into account in the calculations of results.

3. RESULTS AND DISCUSSION

These studies were conducted using PNP deficient (less than 0.3% of the activity in cell extract) human B lymphoblastoid cells.

To synthesize radioactive GTP, cells were incubated with radioactive guanine. After 1 h incubation, amounts of radioactive GTP formed were similar (3–4 pmol/10 cells) in both PNP deficient and normal cells indicating that the extents of GMP formation via salvage pathways from guanine and further phosphorylations were also similar. To induce GTP catabolism cells were incubated with sodium azide or deoxyglucose. These inductors work by different mechanisms and they increase or decrease intracellular P_i level, respectively [12]. In PNP deficient as well as normal cells, sodium azide and deoxyglucose caused rapid GTP breakdown.

In both PNP deficient and normal cells, sodium azide induced large accumulation of GMP and relatively small amounts of IMP were formed. The accumulation of large amounts of nucleoside monophosphates are a result of inhibition of dephosphorylation by high intracellular P_i level (fig.1A,2A). At low P_i level (cells treated with deoxyglucose), no GMP accumulation was observed because of increased dephosphorylation of GMP (fig.1C,2C).

Comparison of the excretion of purine bases and nucleosides by PNP deficient and normal cells reveals marked differences.

In normal cells, induction of GTP catabolism by sodium azide results in the production of large amounts of guanine and some hypoxanthine (fig.2B) but no nucleosides were found since at high P_i levels nucleoside phosphorolysis is completed. Induction of GTP degradation in normal

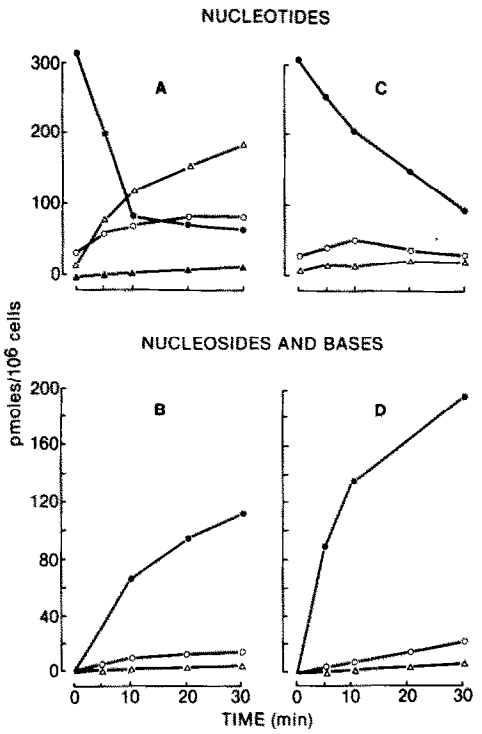

Fig. 1. GTP catabolism in human PNP deficient B lymphoblastoid cells. Cells (2×10^7) were incubated with 2 μCi [8-^{3}H]guanine. After 60 min incubation unused guanine was removed and 2×10^6 cells in 0.15 μM NaCl were incubated with 1 mM sodium azide (A,B) or 5.5 mM deoxyglucose (C,D). Nucleotides GTP (●), GDP (○), GMP (△) and IMP (▲) were analyzed in cell extracts, and nucleosides and bases guanosine (●), inosine (○) and guanine (△) in incubation medium.

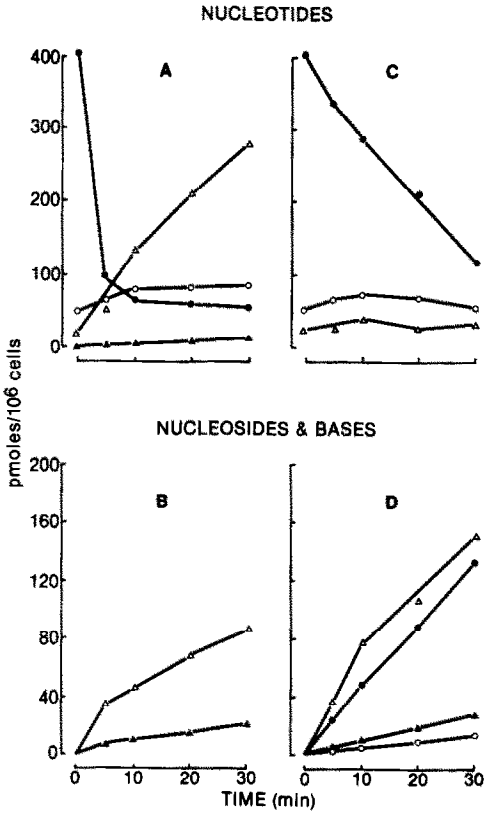

Fig. 2. GTP catabolism in human B lymphoblastoid control cells. Nucleotides GTP (●), GDP (○), GMP (△) and IMP (▲) were analyzed in cell extracts, and nucleosides and bases guanosine (●), guanine (△), inosine (○) and hypoxanthine (▲) in incubation medium. For details see fig.1.

cells by deoxyglucose results in excretion of large amounts of guanosine and some of inosine, in addition to guanine and hypoxanthine (fig.2D). This indicates that at low P_i level nucleoside phosphorolysis by PNP is impaired and guanosine cleavage is not completed. In contrast, in PNP deficient cells guanosine formed in the presence of either sodium azide or deoxyglucose was not metabolized and therefore excreted to the medium in large amounts (fig.1B,D). In addition to guanosine, inosine was also excreted by PNP deficient cells. Total purine excretion by both PNP deficient and normal cells was significantly increased at low P_i levels as a result of enhanced GMP dephosphorylation. Similar results were obtained for PNP deficient mouse T lymphoblastoid cells (not shown).

The accumulation of IMP and the secretion of inosine and hypoxanthine as degradation products of GTP catabolism indicate that reductive deamination of GMP is active in both PNP deficient and in normal lymphoblastoid cells, and represents 10–15% of the total GTP catabolised. In contrast, AMP deamination represents the major pathway of ATP catabolism whereas AMP dephosphorylation participates only to small degrees [13,14].

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REFERENCES

[1] Cohen, A., Doyle, D., Martin, D.W. jr and Amman, A.J. (1976) New Engl. J. Med. 295, 1449–1454.
[2] Henderson, J.F. and Paterson, A.R.P. (1973) in: Nucleotide Metabolism, p. 145, Academic Press, New York.
[3] Brox, L.W. and Hampton, A. (1968) Biochemistry 7, 398–401.
[4] Henderson, J.F., Zombor, G. and Burridge, P.W. (1978) Can. J. Biochem. 56, 474–478.
[5] MacKenzie, J.J. and Sorensen, L.B. (1973) Biochim. Biophys. Acta 327, 282–294.
[6] Stephens, R.W. and Wittaker, V.K. (1973) Biochem. Biophys. Res. Commun. 53, 975–981.
[7] Lomax, C.A., Bagara, A.S. and Henderson, J.F. (1975) Can. J. Biochem. 53, 231–241.
[8] Cohen, A., Lee, J.W.W., Dosch, H.-M. and Gelfand, E.W. (1980) J. Immunol. 125, 1578–1582.
[9] Stein, L.D., Ledgley, C.J. and Sigal, N.H. (1983) J. Immunol. 130, 1640–1645.
[10] Khym, J.X. (1975) Clin. Chem. 21, 1245–1251.
[11] Henderson, J.F., Fraser, J.H. and McCoy, E.E. (1974) Clin. Biochem. 7, 339–358.
[12] Matsumoto, S.S., Raivio, K.O., Willis, R.C. and Seegmiller, J.E. (1980) Adv. Exp. Med. Biol. 122, 277–282.
[13] Smith, C.M., Rovamo, L.M. and Raivio, K.A. (1977) Can. J. Biochem. 55, 1237–1240.
[14] Van den Berghe, G., Bontemps, F. and Hers, H.-G. (1980) Biochem. J. 188, 913–920.