Phosphoribosylpyrophosphate Overproduction, a New Metabolic Abnormality in the Lesch Nyhan Syndrome

Abstract. *The activity of phosphoribosylpyrophosphate synthetase and the rate of phosphoribosylpyrophosphate accumulation are significantly increased in cultured lymphocytes of Lesch Nyhan patients deficient in hypoxanthine guanine phosphoribosyltransferase and in a clone of mutagen-induced, hypoxanthine guanine phosphoribosyltransferase deficient human lymphocytes. The increase in phosphoribosylpyrophosphate synthetase activity is the cause of the abnormally high cellular phosphoribosylpyrophosphate content and possibly of the purine overproduction described in this syndrome.*

Deficiency of hypoxanthine guanine phosphoribosyltransferase (HGPRT) associated with purine overproduction and elevated cellular concentration of phosphoribosylpyrophosphate (PP-rib-P) characterize the biochemical abnormalities in the Lesch Nyhan syndrome (*1, 2*). The disease is inherited in an X-linked manner and the clinical features in the affected male offspring are self-mutilation, choreoathetosis, spasticity, and mental retardation (*3*).

Previous studies have attributed the high cellular PP-rib-P content associated with HGPRT deficiency to a failure to utilize PP-rib-P for inosinic acid synthesis; although an increase in PP-rib-P synthetase activity as a cause of PP-rib-P accumulation was looked for, it had not been found (*2, 4, 5*).

I am presenting evidence that the PP-rib-P synthetase activity is significantly elevated in cultured cells of two patients with the Lesch Nyhan syndrome and in a clonal lymphocyte cell line selected for HGPRT deficiency. This newly detected biochemical abnormality in PP-rib-P metabolism of HGPRT deficient cells may play an important role in the genesis of purine overproduction in the Lesch Nyhan syndrome.

PP-rib-P has been proposed as the rate limiting substrate for the synthesis of phosphoribosyl-1-amine, the first intermediate of the purine pathway (*6*). The abnormally high PP-rib-P content of HGPRT deficient cells is viewed as a direct consequence of failure to use PP-rib-P by the salvage pathway, and therefore excessive amounts of PP-rib-P are available for de novo purine synthesis (*2*).

The elevated PP-rib-P concentration of HGPRT deficient lymphocytes, grown in complete RPMI 1640 medium containing glutamine (*5*), suggests however, that production of PP-rib-P may be accelerated in these cells, particularly since utilization of PP-rib-P for de novo purine biosynthesis is severalfold higher than in normal cells (*7, 8*).

For these reasons, PP-rib-P metabolism was examined in five cell lines; two had normal HGPRT activity, while the other three were severely HGPRT deficient. The PP-rib-P content, PP-rib-P synthetase activity, and accumulation were measured in cell-free extracts of cultured lymphocytes of two patients with the Lesch Nyhan syndrome (UM 10 and J-A), of a cell line selected for mutagen-induced 6-thioguanine resistance (T5.1) and of two normal individuals (UM 39 and NB 45) (*9*). The PP-rib-P content of the HGPRT deficient cell lines was 7 to 11 times higher than that of the control cells (Table 1).

The PP-rib-P synthetase activity was measured in a one-step assay, and PP-rib-P accumulation was determined in a two-step assay (Table 1). The synthetase catalyzes the following reaction:

$$\text{rib-5-P} + \text{ATP} \xrightarrow{Mg^{2+},\ P_i} \text{PP-rib-P} + \text{AMP}$$

where rib-5-P is ribose-5 phosphate, ATP is adenosine triphosphate, and AMP is adenosine monophosphate. The product of the reaction PP-rib-P can be measured by coupling this reaction to the synthesis of AMP from adenine by the method of Hershko *et al.* (*10*), or by coupling it to uridylate formation from orotic acid, based on the assay by Kornberg *et al.* (*11*). The former method can be carried out in two steps for the determination of PP-rib-P accumulation; first PP-rib-P is generated and, after deproteinization by heating, PP-rib-P is determined by the addition of the substrates and enzyme required for AMP synthesis. The two-step method measures PP-rib-P synthesis and degradation, and therefore does not measure PP-rib-P as soon as it is formed. In earlier studies of PP-rib-P synthetase activity in the Lesch Nyhan syndrome this method was used (*4, 5*). Since AMP is known to inhibit PP-rib-P synthetase (*12*), it could interfere with the accurate measurement of PP-rib-P synthetase activity in the one-step assay.

Table 1. Phosphoribosyl-1-pyrophosphate content, synthesis, and accumulation in normal and in Lesch Nyhan lymphocytes in culture and in a clone of mutagen-induced hypoxanthine guanine phosphoribosyltransferase deficient lymphocytes. The PP-rib-P content was determined on 5×10^6 cells grown in RPMI 1640 medium containing 2 m*M* glutamine and 20 percent fetal calf serum. Cells were harvested by centrifugation at 600*g*, washed three times in phosphate-buffered saline (*p*H 7.2), and suspended in 300 μl of 1 m*M* phosphate buffer containing 1 m*M* EDTA; the suspension was heated to 96°C for 60 seconds, chilled, and centrifuged to remove proteins. The supernatant was incubated for 20 minutes at 37°C in a total volume of 500 μl with 1 m*M* phosphate buffer, *p*H 7.2, 4 m*M* $MgCl_2$, 1.0 mg of yeast orotate phosphoribosyltransferase, and 0.2 μc of [^{14}C-carboxyl]orotic acid (specific activity, 42.4 c/mole). Under these conditions recovery of PP-rib-P was complete. PP-rib-P synthetase was determined in dialyzed cell-free extracts: After the cells were harvested, they were repeatedly (five times) frozen and thawed in 1 m*M* phosphate buffer (*p*H 7.2) containing 1 m*M* β-mercaptoethanol; the cell lysates were centrifuged for 60 minutes at 100,000*g*, and the cell-free supernatants were dialyzed for 60 minutes against three changes of 1 m*M* phosphate buffer (*p*H 7.2) containing 1 m*M* EDTA and 1 m*M* β-mercaptoethanol. At the end of dialysis no significant amount of PP-rib-P was detectable. The cell-free supernatants (10 to 100 μg) were incubated for 15 minutes at 37°C in a total volume of 500 μl, with 30 m*M* inorganic phosphate, 0.5 m*M* ATP, 0.35 m*M* ribose-5-phosphate, 4 m*M* $MgCl_2$, 1 m*M* EDTA, 50 m*M* tris-HCl (*p*H 7.4), 1.0 mg of yeast orotate phosphoribosyltransferase and 0.1 μc of [^{14}C-carboxyl]orotic acid (specific activity, 42.4 c/mole). The PP-rib-P accumulation was determined by a two-step assay in dialyzed cell extracts prepared as described above. Cell extracts were incubated for 20 minutes at 37°C in (total volume, 480 μl) 30 m*M* inorganic phosphate, 0.5 m*M* ATP, 0.35 m*M* ribose-5-phosphate, 4 m*M* $MgCl_2$, and 50 m*M* tris-HCl (*p*H 7.4), and 1 m*M* EDTA. The incubation mixture was heated to remove the protein; [^{14}C-carboxyl]orotic acid and orotate phosphoribosyltransferase were added; incubation was continued for 15 minutes at 37°C; and the PP-rib-P content was determined by $^{14}CO_2$ release. This phosphate concentration was optimal for these cell lines and this assay procedure. Incubations were carried out in scintillation vials, $^{14}CO_2$ was collected in Whatman 3MM filter discs (2.4 cm in diameter) inserted in the cap and wetted with 100 μl of NCS tissue solubilizer (Amersham/Searle). Ranges are given for synthetase activity and accumulation; the numbers in parentheses indicate the number of determinations made.

Cell lines	PP-rib-P content*	PP-rib-P synthetase activity†	PP-rib-P accumulation†
UM 39; normal	9.8	30 24–42 (3)	1.6 1.5–1.7 (2)
NB 45; normal	6.5	60 42–84 (2)	
UM 10; Lesch Nyhan	60.0	137 94–234 (3)	10.3 5.4–17.9 (3)
J-A; Lesch-Nyhan	72.0	105 (1)	3.0 (1)
T 5.1; HGPRT deficient	68.0	150 (3) 87–244	5.1 1.4–13.3 (4)

*Picomoles per 10^6 cells. †Nanomoles per milligram of protein per hour.

In my study, PP-rib-P synthetase activity was measured by determining $^{14}CO_2$ release from [^{14}C-carboxyl]-orotidylic acid. Orotidylate and uridylate synthesis were catalyzed by the addition of a partially purified extract of yeast orotate phosphoribosyl transferase (E.C. 2.4.2.10; orotidine-5′-phosphate: pyrophosphate phosphoribosyltransferase) and orotidine-5′-phosphate decarboxylase (E.C. 4.1.1.23; orotidine-5′-phosphate carboxy-lyase). This method is suitable for measuring PP-rib-P synthetase activity in a one-step assay as well as for the two-step determination of PP-rib-P accumulation. Assays are linear with protein concentration and with time. Accurate results can be obtained with incubation periods ranging from 5 to 30 minutes. Longer incubation periods were not tested. A similar assay procedure was used by others for the determination of PP-rib-P synthetase activity in hepatoma cells and in fibroblasts (*13, 14*). PP-rib-P content, PP-rib-P synthetase activity, and PP-rib-P accumulation were determined on enzyme extracts prepared from lymphocytes in the logarithmic phase of growth, and studies were carried out on freshly harvested cells (Table 1).

PP-rib-P synthetase activity was strikingly increased in all HGPRT deficient cells (Table 1). Enzyme activity in mutant cell lines exceeded that of the control cells three to tenfold.

PP-rib-P accumulation in HGPRT deficient cells was also significantly greater than in control cells, but only a small percentage (3 to 10 percent) of the PP-rib-P produced, as measured by PP-rib-P synthetase activity, accumulated in the cells. PP-rib-P accumulation is dependent not only on synthesis, but also on utilization and catabolism. A small but constant amount of PP-rib-P accumulation could be sufficient to explain the strikingly high cellular concentration of this substrate in HGPRT deficient cells.

Findings are in agreement with those reported by Martin *et al.* on increased PP-rib-P synthetase in clones of mutagenized HGPRT deficient rat hepatoma cells and in cultured fibroblasts of patients with HGPRT deficiency (*14*). Martin *et al.* determined PP-rib-P synthetase activity by a one-step assay, similar to the one used in this study, based on the formation and decarboxylation of orotidylate in the presence of excess orotidylate phosphoribosyltransferase and decarboxylase.

The relation between HGPRT deficiency and PP-rib-P overproduction could reflect a compensatory mechanism for the production of inosinic acid, since an increase in available PP-rib-P could increase the catalytic efficiency of the abnormal HGPRT enzyme. The mechanism of this interaction between the function of the salvage pathway and PP-rib-P synthesis is unknown. This interaction could be genetic, with the HGPRT gene having a regulatory function on PP-rib-P synthetase activity. At present, it is not known whether PP-rib-P overproduction is an obligatory consequence of HGPRT deficiency. In lymphoblasts selected for thioguanine resistance, HGPRT deficiency was linked to elevated cellular PP-rib-P content and purine overproduction (*7*). The study of natural mutations appearing in man will reveal whether increase in PP-rib-P production and in purine biosynthesis is obligatory for the survival of HGPRT deficient cells. The increase in PP-rib-P synthetase activity observed in Lesch Nyhan cells could result in the abnormally high cellular PP-rib-P content of these cells and represents an additional metabolic and genetic abnormality in this syndrome.

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References and Notes

1. J. E. Seegmiller, F. M. Rosenbloom, W. N. Kelley, *Science* **155**, 1682 (1967).
2. F. M. Rosenbloom *et al.*, *J. Biol. Chem.* **243**, 1166 (1968).
3. M. Lesch and W. L. Nyhan, *Am. J. Med.* **36**, 561 (1964).
4. M. A. Becker, L. J. Meyer, A. W. Wood, J. E. Seegmiller, *Science* **179**, 1123 (1973).
5. A. W. Wood, M. A. Becker, J. E. Seegmiller, *Biochem. Genet.* **9**, 261 (1973).
6. O. Sperling, J. B. Wyngaarden, C. F. Starmer, *J. Clin. Invest.* **52**, 2468 (1973).
7. G. Nuki, J. E. Lever, J. E. Seegmiller, in *Purine Metabolism in Man*, O. Sperling, A. De Vries, J. B. Wyngaarden, Eds. (Plenum, New York, 1974), p. 255.
8. G. H. Reem, in *ibid.*, p. 245.
9. K. Sato, R. S. Slesinski, J. W. Littlefield, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1244 (1972).
10. A. Hershko, A. Razin, J. Mager, *Biochim. Biophys. Acta* **184**, 64 (1969).
11. A. Kornberg, I. Liebermann, E. S. Simms, *J. Biol. Chem.* **215**, 389 (1955).
12. N. Sciaky, A. Razin, B. Gazit, J. Mager, in *Purine Metabolism in Man*, O. Sperling, A. De Vries, J. B. Wyngaarden, Eds. (Plenum, New York, 1974), p. 87.
13. C. D. Green and D. W. Martin, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3698 (1973).
14. D. W. Martin, Jr., L. H. Graf, Jr., J. A. McRoberts, T. M. Harrison, *Clin. Res.* **23** (abstr.), 263 (1975).
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5-Iododeoxyuridine Potentiation of the Replication In Vitro of Several Unrelated RNA and DNA Viruses

Abstract. *Enhancement of the replication of unrelated viruses (three RNA viruses and one DNA virus), representative of four major virus groups, occurs in human, rodent, or avian cells treated in vitro with 5-iododeoxyuridine (IdU). The results suggest that the potentiation of viral replication by IdU is a widespread phenomenon.*

The normally inefficient replication of cytomegalovirus (CMV) is potentiated in cells treated with 5′-iododeoxyuridine (IdU) prior to the addition of virus (*1*). Adenovirus replication also is enhanced in semipermissive cells treated with IdU before the addition of virus (*2*). We now report the unexpected finding that IdU potentiates the synthesis of a number of unrelated RNA and DNA viruses [vesicular stomatitis, sindbis, mouse encephalitis (GD-7), and vaccinia]. These viruses replicate in apparent independence of nuclear control (*3*). Thus, IdU incorporation into host cell DNA may induce an alteration (or alterations) of general significance to viral replication.

In our experiments cells were grown in the presence of IdU for 48 hours, medium containing IdU was removed, and virus was added. Suspensions of 4×10^4 to 5×10^4 cells [mouse L, BHK-21, primary chicken embryo or human foreskin fibroblasts (HR203) (*4*)] in 0.1 ml of Eagle's medium supplemented with 10 percent fetal bovine serum (FBS) and antibiotics (*5*) were added to microtiter culture plates (plastic) (*6*). The IdU (*7*), in a final concentration of 10 μg/ml, was added to portions of the cell suspensions prior to their addition to culture plates. After 40 to 48 hours, culture supernatants were decanted. Virus dilutions were added to quadruplicate cultures of IdU-treated and untreated (control) cells and allowed to adsorb for 1 hour; the dilutions and medium were decanted, the cells were rinsed three times, and 0.2 ml of medium was added. Cultures were observed for the development of a viral cytopathic effect (CPE), or were frozen (−70°C) and thawed for titration of viral hemagglutinin (HA) (*8, 9*) or infectious virus. Titration of cultures for virus yields was performed in duplicate. Tissue culture infective doses, 50 percent effective ($TCID_{50}$), were determined by accepted techniques.

The effect of IdU treatment of cells on HA yield was determined for GD-7 virus in mouse L cells, and for Sindbis virus in HR203 fibroblasts (Fig. 1). The cells were infected at varying multiplicities of infection (MOI), and HA was determined 48 hours later. Consistently greater quantities of GD-7 virus HA were produced at all MOI's in IdU-treated cultures (Fig. 1A) as