Proposed Explanation for S-Adenosylhomocysteine Hydrolase Deficiency in Purine Nucleoside Phosphorylase and Hypoxanthine-Guanine Phosphoribosyltransferase-deficient Patients

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ABSTRACT We have examined the basis for the recently reported, but unexplained deficiency of S-adenosylhomocysteine hydrolase (AdoHcyase) in the erythrocytes of patients with genetic deficiencies of purine nucleoside phosphorylase and hypoxanthine-guanine phosphoribosyltransferase. We found that a hemolysate from a patient with purine nucleoside phosphorylase deficiency had only 7% of control AdoHcyase activity, confirming the original observation. Of the purine nucleosides known to accumulate in nucleoside phosphorylase-deficient patients, inosine alone caused the phosphate-dependent, irreversible inactivation of purified human placental AdoHcyase, and of AdoHcyase in intact erythrocytes and cultured lymphoblastoid cells. Hypoxanthine did not inactivate purified AdoHcyase, but potentiated the effect of inosine in intact hypoxanthine-guanine phosphoribosyltransferase-deficient human lymphoblastoid cells. This presumably resulted from the ability of hypoxanthine to shift the equilibrium of the nucleoside phosphorylase reaction, preventing inosine breakdown. This could account for the partial AdoHcyase deficiency reported in hypoxanthine-guanine phosphoribosyltransferase-deficient patients. We have also demonstrated the AdoHcyase-catalyzed synthesis of S-inosylhomocysteine from inosine and L-homocysteine, a reaction which may occur in nucleoside phosphorylase-deficient patients.

INTRODUCTION

Adenosine deaminase (ADA),1 purine nucleoside phosphorylase (PNP), and hypoxanthine-guanine phosphoribosyltransferase (HGPRT) catalyze sequential steps in the interconversion of purine nucleosides and reutilization of purine bases. Their respective deficiencies cause a severe combined immunodeficiency disease (ADA) (1), a selective defect in cell-mediated immunity (PNP) (2), and the Lesch-Nyhan syndrome (HGPRT) (3, 4), in which marked urate overproduction is accompanied by a severe neurologic disease. Recent evidence has suggested some involvement of the enzyme S-adenosylhomocysteine hydrolase (AdoHcyase) in each of these inborn errors of purine metabolism.

AdoHcyase catalyzes the reversible cleavage of S-adenosylhomocysteine (AdoHcy) to adenosine and L-homocysteine (5), a reaction which is essential to prevent the accumulation of AdoHcy, both a product and a potent inhibitor of S-adenosylmethionine-dependent transmethylations. We have identified AdoHcyase as the major high affinity adenosine-binding protein in the cytoplasm (6), and have shown that it may mediate toxic effects of both adenosine and deoxyadenosine, the nucleosides which accumulate in ADA deficiency. Adenosine, by displacing the equilibrium of the AdoHcyase reaction, causes AdoHcy accumulation and inhibition of nucleic acid methylation in ADA-inhibited cultured lymphoid cells (7, 8). Deoxyadenosine (and the antiviral agent adenosine arabinoside) causes the irreversible inactivation of AdoHcyase (9), such that erythrocytes of ADA-deficient children have <2% of normal AdoHcyase activity (10). In cultured human lymphoblastoid cells, this inactivation causes AdoHcy accumulation and contributes to the toxicity of deoxyadenosine (11). These mechanisms may contribute to

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1Abbreviations used in this paper: ADA, adenosine deaminase; AdoHcy, S-adenosylhomocysteine; AdoHcyase, S-adenosylhomocysteine hydrolase; Ara-A, adenosine arabinoside; Ara-Ix, arabinosylhypoxanthine; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; InoHcy, S-inosylhomocysteine; PNP, purine nucleoside phosphorylase.
the lymphopenia and immune dysfunction in ADA deficiency.

Recently, Kaminska and Fox (12) reported that PNP-deficient patients had 12–19% of normal erythrocyte AdoHcyase activity, and Lesch-Nyhan patients, 50–60%, though the researchers were unable to define the cause of this deficiency. They reported that none of the purines that accumulate in PNP- and HGPRT-deficient patients affected AdoHcyase activity, and suggested that their AdoHcyase deficiency might result from some mechanism other than the type of inactivation caused by deoxyadenosine. We have reexamined this question, both because of our interest in the unusual properties of AdoHcyase and because understanding the cause of its deficiency is necessary to assess its possible role in producing the immunologic or neurologic abnormalities in these patients.

METHODS

Materials. Radioactive chemicals were obtained from Amersham Corp., Arlington Heights, Ill.; nonradioactive compounds from Sigma Chemical Co., St. Louis, Mo., or P-L Biochemicals, Inc., Milwaukee, Wisc.; cellulose thin-layer plates from Eastman Kodak, Rochester, N. Y. The nonspecific ADA from Aspergillus oryzae was purified (13) from Szyme-R (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.), and was a gift from Dr. N. Kredich. AdoHcyase was purified from human placenta by a procedure to be described in detail elsewhere.2 The enzyme was homogeneous by the criteria of sodium dodecylsulfate-acylamide gel electrophoresis and analytical ultracentrifugation, and contained 1 mol tightly bound NAD+/mol of subunit (48,000 mol wt), as described for beef liver AdoHcyase (14).

Cell growth and preparation of extracts. Clone 35-1, an HGPRT-deficient mutant (15) of the WI-L2 human splenic lymphoblastoid cell line, was grown as described (16), except that 10% horse serum was used instead of fetal calf serum. For measuring AdoHcyase in extracts, the cells were harvested by brief centrifugation at 4°C, and washed once with phosphate-buffered NaCl; the cell pellet was frozen and thawed three times in 0.1 ml of Tris-HCl, pH 7.4, 1 mM Na4EDTA. After centrifugation for 2 min in a Beckman microfuge (Beckman Instruments Inc., Fullerton, Calif.), 0.1 ml of supernate was passed over Sephadex G-25 as described (11). Hemolysates prepared as described (10) were treated similarly, and the excluded fractions from the G-25 columns were assayed for AdoHcyase.

Assays. AdoHcyase was assayed in the direction of AdoHcy synthesis from [3H]adenosine and L-homocysteine, using a thin-layer chromatographic method (6, 9). In the assay of S-inosylhomocysteine (InoHcy) synthesis by purified placental AdoHcyase, [8-3H]inosine (15 cpm/pmole) replaced [3H]adenosine, and nonradioactive InoHcy (prepared by quantitative deamination of AdoHcy by nonspecific A. oryzae ADA) was used as a thin-layer marker instead of AdoHcy. The product of the reaction was also shown to coelute with InoHcy by high-pressure liquid chromatography on a C18 uBondapak reversed-phase column (Waters Associates, Inc., Milford, Mass.), eluted with 50 mM sodium acetate, pH 3.9, containing 3.0% methanol, flow rate 2 ml/min. Absorbance was monitored at 280 and 254 nm with a Waters model 440 detector. In this system, InoHcy eluted at 7.2 min, inosine at 8.1 min, AdoHcy at 14.4 min, and adenosine at 20.4 min.

RESULTS

A hemolysate from a PNP-deficient patient was kindly provided to us by Dr. S. K. Wadman, Utrecht Universiteitskinderkliniek, Utrecht, The Netherlands. It had normal levels of ADA and adenine phosphoribosyltransferase activities, but its AdoHcyase activity, 0.27 nmol/h/mg protein, was only 6–7% of our control population erythrocyte activity (10). This confirmed the result reported by Kaminska and Fox (12), who found values of ~15% of controls. In studying possible explanations for this deficiency, they evaluated the effects of inosine, deoxyinosine, guanosine, deoxyguanosine (PNP substrates), and of hypoxanthine and guanine (PNP products, HGPRT substrates) on AdoHcyase activity in hemolysates of normal individuals. To prevent breakdown of the nucleosides by PNP present in these hemolysates, they chose to conduct these studies in phosphate-free buffer. Under these assay conditions, they found no effect of any of the above mentioned compounds (0.5 mM, 20- and 60-min incubations) on AdoHcyase, but did observe inactivation by deoxyadenosine, used as a “positive control.”

In our published studies of AdoHcyase inactivation we have used phosphate-containing buffers (9–11). Furthermore, we have found that the rate of inactivation of homogeneous placental AdoHcyase by several purines is phosphate dependent (17). Fig. 1 compares the rates of inactivation of homogeneous human placental AdoHcyase, in the presence and absence of 10 mM phosphate, by 0.05 mM adenine arabinoside (Ara-A), 0.2 mM adenosylhypoxanthine (Ara-Hx), and by 0.2 mM inosine, demonstrating the phosphate dependence of the rates of inactivation by Ara-A and inosine. No inactivation was observed with 0.2 mM inosine in the absence of phosphate, and Ara-Hx did not inactivate even in the presence of phosphate. In similar kinetic experiments performed in the presence of phosphate, we observed no appreciable inactivation with 0.25 mM hypoxanthine, guanine, deoxyinosine, guanosine, or deoxyguanosine (Table I). The rate of inactivation by inosine is pseudo-first order and saturable (Fig. 2). From these studies we determined a $K_i$ (18) for inosine as an inactivator of 5.85 mM, with a $V_{max}$ of 0.16 min$^{-1}$. In experiments not shown, we also found that prolonged dialysis (up to 68 h) did not restore activity to inosine-treated AdoHcyase (dialyzed, untreated enzyme lost no activity), and that low concentrations of adenosine blocked inactivation of the enzyme by inosine. Thus the inactivation by inosine is irreversible and is active site directed, as we previously showed with deoxyadenosine and adenine arabinoside.
We have also found that inosine can replace adenosine as a substrate for human AdoHcyase, combining with l-homocysteine to form inosylhomocysteine (InoHcy) (Fig. 3). However, this is a very inefficient reaction, with a $K_m$ of 9.3 mM, compared with a $K_m$ for adenosine of 1 µM (6). More detailed studies of the mechanism of inactivation by inosine and effects of InoHcy on cells will be presented elsewhere.

In other experiments (not shown), we found that exposure to 0.25 mM inosine for 2 h caused a 50% reduction of AdoHcyase activity in intact normal erythrocytes. More precise studies of inactivation kinetics in intact erythrocytes were not attempted because of the inability to control the phosphorolytic cleavage of inosine to hypoxanthine and ribose-1-PO$_4$ by PNP. The equilibrium for the PNP reaction is known to favor nucleoside formation (19), but in the intact cell the reaction is "pulled" in the direction of cleavage by removal of hypoxanthine, suggesting an explanation for the 50–60% (12) deficiency of AdoHcyase in the erythrocytes of HGPRT-deficient patients: higher steady-state concentrations of guanine or hypoxanthine (particularly the latter in the allopurinol-treated pa-

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pH 7.0, mixture of cental time, dicated of (V) vs. (B) described (Table (V)).

The inactivation of cultures between patient) could increase the steady-state concentration of intracellular inosine and cause a slow but progressive inactivation of AdoHcyase activity in long-lived erythrocytes. We have tested this hypothesis in an experiment employing cultured HGPRT-deficient human lymphoblastoid cells (Table II). Hypoxanthine alone had little if any effect on intracellular AdoHcyase activity, but clearly potentiated the effect of inosine. The increase in AdoHcyase activity in the inosine-treated cultures between 6 and 24 h of incubation probably results from synthesis of new enzyme, which cannot occur in mature erythrocytes.

DISCUSSION

We have confirmed the finding of a significant (80–90%) decrease in erythrocyte AdoHcyase in PNP deficiency reported by Kaminska and Fox (12), and we are able to offer a reasonable explanation for its occurrence.

In Vivo Inactivation of S-Adenosylhomocysteine Hydrolase by Inosine

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<th>Table II</th>
<th>Inactivation of AdoHcyase in HGPRT-deficient Lymphoblasts</th>
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<td>Purine (0.5 mM)</td>
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<tr>
<td>Hypoxanthine</td>
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<tr>
<td>Inosine</td>
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<tr>
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30-ml cultures (4.5 × 10⁶/ml) of HGPRT-deficient human lymphoblastoid cells were grown as described in Methods, with no purines (control) or with 0.5 mM hypoxanthine and/or inosine, for 6 or 24 h, at which time cells were harvested and washed, and lysates were prepared for assays of AdoHcyase activity as described in Methods. Cell densities increased by 2.05–2.2-fold in all cultures in 24 h. Results are expressed as percent activity in the control lysate after 6 and 24 h.

We have shown that there is a clearly demonstrable inactivation of purified human placental AdoHcyase by inosine in the presence of phosphate, and that inosine inactivates intracellular AdoHcyase in cultured human lymphoblasts and intact erythrocytes. Although inosine is a weak inactivator (Kᵢ = 5.9 mM compared with 66 μM for deoxyadenosine and 24 μM for adenine arabinoside [9]), the concentration of plasma inosine in PNP-deficient patients has been reported in the 40–100 μM range (20, 21), whereas deoxyadenosine in the plasma of ADA-deficient children is usually <2 μM (10, 22). Slow, irreversible inactivation by inosine in long-lived erythrocytes, which are incapable of new enzyme synthesis, seems the most likely explanation for the observed deficiency of AdoHcyase. The ability of hypoxanthine to displace the equilibrium of the PNP reaction toward inosine formation can explain its potentiation of the effect of inosine on AdoHcyase in HGPRT-deficient human lymphoblasts (Table II), and probably accounts for the lower erythrocyte AdoHcyase deficiency that has been found in Lesch-Nyhan patients.

Could the observed deficiencies of AdoHcyase contribute to the clinical manifestations of HGPRT or PNP deficiency? Abnormalities in neurotransmitter methylation might conceivably play some role in producing the motor disturbance or self-mutilation that occurs in the Lesch-Nyhan syndrome. However, since more extensive AdoHcyase inactivation in both ADA and PNP deficiency does not result in any neurologic disease, this seems unlikely. More consideration needs to be given to the possibility that interaction of intracellular AdoHcyase with inosine contributes to the lymphopenia and defect in cell-mediated immunity in PNP deficiency. Both lymphopenia and AdoHcyase inactivation do occur in ADA deficiency, and selec-
tive lymphopenia has also recently been reported to occur in Ara-A-treated patients (23), in whose blood cells AdoHcyase inactivation has been observed (24, 25, and our unpublished studies). However, inosine is not likely to produce as complete inactivation of AdoHcyase in cells capable of protein synthesis as either deoxyadenosine or Ara-A.

We have shown that inosine, unlike deoxyadenosine or Ara-A, can also act as a nucleoside substrate in the formation of a thioether bond with L-homocysteine. Though the $K_m$ for inosine is nearly four orders of magnitude higher than that of adenine, there has been a report of the detection of trace amounts of InoHcy in mouse lymphoblasts exposed to 150 $\mu$M inosine (26). Although some accumulation of InoHcy in cells of PNP-deficient patients may occur, InoHcy is probably much less effective than AdoHcy as an inhibitor of methylation reactions. Inosine is virtually nontoxic to PNP-deficient mouse lymphoblasts (27), in marked contrast to the AdoHcy-mediated toxicity of adenine to ADA-inhibited mouse (7) and human (8) lymphoblasts. L-Homocysteine greatly potentiates adenosine toxicity by augmenting AdoHcy synthesis catalyzed by AdoHcyase. In preliminary experiments we have found that 1 mM inosine did not inhibit growth of WI-L2 lymphoblasts, alone or in the presence of 1 mM L-homocysteine.

Although additional studies in PNP-deficient lymphoid cells would be useful, it presently seems unlikely that inosine produces sufficient AdoHcyase inactivation to cause significant accumulation of AdoHcy, or that its conversion to InoHcy by AdoHcyase is sufficiently toxic to contribute to the selective T cell defect in PNP deficiency. The more severe defects in both T and B cell development in ADA deficiency may, however, be attributable in part to the far more potent interactions of both adenosine and deoxyadenosine with AdoHcyase, a hypothesis under investigation.

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