The Highest Levels of Purine Catabolic Enzymes in Mice Are Present in the Proximal Small Intestine*

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Recent studies on the tissue distribution and developmental regulation of adenosine deaminase (ADA) activity in mice show that very high ADA levels exist in the murine alimentary tract (tongue, esophagus, forestomach, proximal small intestine) and at the fetal-maternal interface. To understand the role of ADA in these tissues, we measured the levels of three other enzymes involved in purine catabolism, purine nucleoside phosphorylase (PNP), guanine deaminase (GDA), and xanthine dehydrogenase (XDH), to see how their levels correlated with ADA activity. Our results show that the highest level of PNP, GDA, and XDH is present in the proximal small intestine. Levels of these purine catabolic enzymes are much lower in the tongue, esophagus, forestomach, and fetal-maternal interface in marked contrast to ADA distribution. We also determined mRNA levels encoding PNP, XDH, and ADA in a variety of tissues. Tissue-specific differences in mRNA production, thus, ADA is part of a purine catabolic pathway leading to the production of uric acid that is present at the highest known level in the proximal small intestine. ADA may have additional roles in other tissues.

Adenosine deaminase (ADA, EC 3.5.4.4.)$ is an enzyme of considerable physiological importance. It catalyzes the deamination of adenosine and 2'-deoxyadenosine to their respective hypoxanthine nucleosides. Several 6-substituted purine derivatives including a number of clinically important antimetabolites also serve as substrates. Phosphorylated nucleosides are not substrates (1). ADA plays an important role in controlling the level of its substrates as indicated by the physiological consequences of elevated levels of adenosine and 2'-deoxyadenosine in individuals genetically deficient in this enzyme (2, 3). The physiological function of ADA is critical to control the effects of adenosine and 2'-deoxyadenosine in a variety of systems and includes immunological (2), neurological (4), and vascular (5) roles.

Because of the physiological importance of ADA it is not surprising that the enzyme is widely distributed in nature. The enzyme has been found in virtually all vertebrate tissues, and it has been observed that the level of ADA varies markedly among different tissues and within the same tissues from different species (6, 7). The most information is known concerning the cellular localization and developmental expression (8) of ADA in mice. In humans the highest levels of ADA have been reported in the thymus presumably reflecting the critical role of this enzyme in T-cell development (9-14). In adult mice the highest levels of ADA are present in the alimentary canal (15). The enzyme is most abundant in the keratinized squamous epithelium that lines the tongue, esophagus, and forestomach and the absorptive epithelium of the small intestine. High levels of ADA are not found in epithelial layers outside the alimentary canal, suggesting that the enzyme fulfills a function specific to the gastrointestinal epithelium. Exactly what this function may be is not known.

During prenatal development in mice, high levels of ADA are present at the fetal-maternal interface throughout the postimplantation phase of gestation (15-20). The enzyme is synthesized initially by the decidua (a maternal tissue that surrounds the early postimplantation embryo) and is one of the most abundant soluble proteins in this tissue. Between days 7 and 9 of gestation ADA levels undergo a developmentally regulated increase of more than 200-fold at the implantation site. Pharmacologic inhibition of ADA activity during this period results in resorption or malformation of nearly all of the embryos, suggesting that maternal ADA activity is critical to embryogenesis during this period (21, 22). The physiological role of ADA at the fetal-maternal interface is not understood.

In our effort to understand the function and regulation of ADA expression in the mucosal epithelium of the alimentary canal and at the fetal-maternal interface we determined the tissue distribution of three other enzymes of purine catabolism (Fig. 1), namely purine nucleoside phosphorylase (PNP), guanine deaminase (GDA), and xanthine dehydrogenase (XDH), and compared their levels to ADA. Our results indicate that each of these purine catabolic enzymes is found at its highest level in the proximal small intestine. We extended these findings by using PNP and XDH cDNA probes to show that the highest mRNA levels for these enzymes is found in the small intestine. The purine catabolic pathway leading to the synthesis of uric acid is present at the highest known levels in the proximal small intestine. However, in other tissues containing high levels of ADA, the levels of other enzymes of the catabolic pathway are lower suggesting a unique role for ADA in those tissues.

EXPERIMENTAL PROCEDURES

Materials

Phenylmethylsulfonyl fluoride, xanthine oxidase, EDTA, leupeptin, allopurinol, guanine, xanthine, inosine, and adenosine were purchased...
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**Fig. 1. Purine catabolism in the small intestine.** Conversion of uric acid to allantoin occurs in the liver. Humans do not possess uricase and hence excrete uric acid as the end product of purine catabolism.

From Sigma. Dithiothreitol and NAD⁺ were obtained from Boehringer Mannheim, potassium oxonate from Aldrich Corp., and potassium phosphate from Mallinkrodt. All other chemicals were of reagent grade.

Female BALB/c mice (10-17 weeks of age) used throughout the experiments were supplied by Harlan Sprague-Dawley.

**Preparation of Tissue Samples for Xanthine Dehydrogenase, Adenosine Deaminase, Purine Nucleoside Phosphorylase, and Guanine Deaminase Assay**

Tissues of the embryo-decidual unit (day 9) and the placenta (day 13) were provided by Lyhna Hong and Dr. John Winston (Department of Biochemistry, Baylor College of Medicine). Adult female BALB/c mice were sacrificed and the organs were quickly removed and placed in ice-cold 50 mM potassium phosphate, pH 7.5, buffer. Gastrointestinal luminal contents were removed prior to homogenization by rinsing in the buffer above. The stomach of the adult mouse, which is divided into a nonglandular forestomach and a more muscular glandular stomach, was bisected along the junction and each portion assayed separately using the protocol described below. Tissue extracts were prepared by homogenization (using a Junke & Kunkel KG Ultra-Turrax TP 18-10 homogenizer) in ice-cold 50 mM potassium phosphate, pH 7.5, buffer. Cell debris was removed by centrifugation at 3,000 x g for 10 min (Sorvall RC-5B, DuPont GSA rotor). Other insoluble material was removed by high speed centrifugation at 350,000 x g for 7 min (TI-100 ultracentrifuge, Beckman TLA-100.2 rotor) and assayed, along with the supernatant, for activity. Activity for all the enzymes under investigation was found in the supernatant. Enzyme levels were measured immediately using the specific assays outlined below. Specific inhibitors of enzymes were used as controls where appropriate.

**Determination of XDH-specific Activity**

There are several forms of xanthine dehydrogenase (23). The distinctions are made based on the enzyme's use of either NAD⁺ or O₂ as oxidizing agents (type D versus type O, respectively) and on whether the type O form can be converted to the type D form (reversible type O versus irreversible type O). Type D is thought to be the physiological state of the enzyme. The XDH of rat liver supernatant actually appears as a NAD⁺-dependent dehydrogenase and is converted into an oxidase by various treatments (24). It has also been observed that the enzyme from mouse duodenum (25) and from various rat organs (26) can be obtained as a dehydrogenase, if the activity of proteolytic enzymes and the oxidation of thiols is prevented during the preparation of the organ extracts. Therefore, to measure the entire XDH activity present in the tissue, two assays were used, one specific for type D and reversible type O and the other for irreversible type O. kₘₐₓ (steady state) for type D is 19 s⁻¹ and for type O 18 s⁻¹ (27). The similarity of these values allows for ratios of type D versus type O to be calculated directly from the specific activity.

**Measurement of Type D and Reversible Type O XDH activity**—This activity was assayed under saturating substrate conditions at 25 °C in a reaction mixture containing 0.1 mM xanthine and 0.05 mM NAD⁺ in 50 mM K-Oxo buffer. The total reaction volume was 1 ml containing 50-100 μl of homogenate. The increase in absorbance at 340 nm resulting from the formation of NADH from NAD⁺ was continuously monitored in a Cary 118 spectrophotometer, and the rate of its production was calculated at linearity. Reactions containing no xanthine, NAD⁺, or cell extract were run for base-line and control determinations. At pH 7.5, the spectral difference between NADH and NAD⁺ at 340 nm is 6.22 OD/mL.
Recent isolation of a mouse XDH cDNA clone (35) and/or mouse ADA (36) cDNA probes. All sequences were amplified by the guanidine HCl procedure

The increase in absorbance at 290 nm resulting from the formation of uric acid from xanthine was continuously monitored in a Cary 11 spectrophotometer, and the rate of its production was calculated at linearity. Reactions containing no xanthine or cell extract were run for base-line and control determinations. At pH 7.5, the spectral difference between uric acid and xanthine at 292 nm is 10.4 OD/mg cm (30). One unit of XDH activity was defined as the amount of enzyme that produced 1 nmol of uric acid/min.

**Determination of GDA-specific Activity**

GDA activity was measured at saturating substrate conditions at 25 °C in a reaction mixture containing 1.5 mM isoine, 0.015 unit of xanthine oxidase, and 4–20 μl of homogenate in 50 mM K-oxo buffer. The increase in absorbance at 292 nm resulting from the formation of uric acid from isoine was monitored. At pH 7.5, the spectral difference between isoine and uric acid is 12.5 OD/mm cm (31). One unit of PNP activity was defined as the amount of enzyme that produced 1 nmol of uric acid/min.

**Determination of ADA-specific Activity**

Limited assays for ADA were also performed, particularly to quantify levels of ADA horizontally down the small intestine. The assay mixture contained 0.3 mM adenine and 5–20 μl of homogenate in K-oxo buffer. The increase in absorbance at 235 nm resulting from the formation of isoine from adenine was monitored. At pH 7.5, the spectral difference between adenine and isoine is 2.5 OD/mM cm. One unit of ADA activity was defined as the amount of enzyme that produced 1 nmol of isoine/min.

**Probes for Northern Hybridization Analysis**

0.8-kb mouse ADA cDNA, 1.1-kb mouse PNP cDNA (33), and 2.9-kb rat XDH cDNA (34) fragments were used as probes for Northern blots (see below). The PNP plasmid (pEMPD) was a generous gift from the laboratory of Dr. Scott McFerr, University of Minnesota, Minneapolis. A plasmid containing partial sequences of rat XDH (pRXD16) was kindly provided by Dr. Takeishi Nishino, Yokohama City University School of Medicine, Yokohama, Japan. Rat cDNA was used because of the unavailability of mouse ADA cDNA at the time of Northern blot analysis. Recent isolation of a mouse XDH cDNA clone (35) reveals a 92% homology with rat sequence.

**Isolation and Blot Hybridization Analysis of RNA**

Total cellular RNA from BALB/c mice was isolated by a modified guanidine HCl procedure (15). The small intestine was cut in 6-cm portions, covering over 90% of the small intestine. The integrity of the RNA preparations was checked by running the samples on formaldehyde-agarose gels followed by ethidium bromide staining. These samples were run on 1.5% agarose gels containing formaldehyde, transferred to nylon membranes (GeneScreen, New England Nuclear), and hybridized with 32P-labeled mouse PNP, rat XDH, and/or mouse ADA (36) cDNA probes. All sequences were amplified and purified according to the method of Birnboim and Doly (37). Probes were labeled as previously described (38). Prehybridization and hybridization solutions were as described elsewhere (15). Filters were washed at 65 °C as described elsewhere (15).

**RESULTS AND DISCUSSION**

**Highest Levels of Purine Catabolic Enzymes Are Present in the Gastrointestinal Tract and Specifically in the Small Intestine**—In an effort to better understand the function and regulation of adenosine deaminase expression in the gastrointestinal tract we determined the tissue distribution of three other enzymes of purine catabolism. While levels of these three enzymes had been measured in several species including mice (10, 39–48), none of these studies measured levels in the tongue or esophagus, nor did they separately measure activity in the forestomach and glandular stomach. In addition, no previous attempt has been made to determine if these four purine catabolic enzymes show a coordinate pattern of tissue specific expression. Our results (Fig. 2) confirm earlier findings (15) and show that the highest levels of ADA are found in tissues of the alimentary tract and the female reproductive tract (discussed below). Within the alimentary tract the highest levels of ADA are present in the tongue and the proximal small intestine, with high levels also appearing in the forestomach and esophagus. The highest levels of PNP, GDA, and XDH were observed in the small intestine. For each enzyme the highest specific activity was present in the duodenum with values declining in the jejunum and ileum. The values for colon were uniformly low. Relatively low levels of PNP, GDA, and XDH were found in the forestomach, esophagus, and tongue, tissues having among the highest levels of ADA activity. These tissues show activity levels for these enzymes between 6 and 20 times lower than levels in the proximal small intestine. In addition, although levels of all four enzymes are higher in the forestomach than in the more muscular glandular stomach, the wide margin of difference seen in ADA levels in these tissues is not matched. Relatively low levels of purine catabolic enzymes were found outside the alimentary tract. The results indicate that a purine catabolic pathway including ADA, PNP, GDA, and XDH is present at the highest known levels in the proximal small intestine where these enzymes are coordinatedly expressed.

**Northern Blot Analysis Indicates That Regulation of Gene Expression of Purine Catabolic Enzymes Is at the Level of mRNA Production**—To compare mRNA message levels with their corresponding protein levels particularly in the upper alimentary tract and the decidua, we prepared RNA samples for hybridization analysis. Mature ADA and XDH mRNAs were hybridized on a single blot to directly compare message levels (Fig. 2a). Specificity of the probes is not affected when multiple probes are used on the same blot.2 The highest levels of ADA mRNA are seen in the proximal small intestine, tongue, esophagus, and forestomach. The decidua also shows a high level of ADA transcripts while the glandular stomach and the thymus indicate lower levels. mRNA levels are not detectable in the remaining tissues. XDH mRNA was found at high levels only in the proximal intestine with all remaining tissues including the other upper alimentary tract tissues showing much lower message levels. XDH mRNA levels in the tongue, glandular stomach, heart, and skeletal muscle were too low to be detected.

Fig. 3b shows a northern blot of PNP transcripts using a 1.1-kb 32P-labeled mouse-PNP cDNA. While appreciable message levels are present in the liver, the highest mRNA levels are in the proximal small intestine with all other tissues showing very low or undetectable levels. Our data show that mRNA levels of PNP, ADA, and XDH correlate well with enzyme activity in all tissues.

**Coordinate Expression of These Enzymes in the Small Intestine May Be to Produce Uric Acid**—The enzymes assayed form part of a metabolic pathway that catalyzes the conversion of uric acid.

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2 K. A. Mohammedi, O. M. Guichert, R. E. Kellemes, and P. B. Rudolph, unpublished data.
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FIG. 2. Tissue distribution of ADA, PNP, GDA, and XDH in various tissues of mice. Tissues were prepared from adult (10-17 weeks of age) female BALB/c mice. SI 1-6 refers to 6-cm portions of the small intestine with SI 1 the most proximal end and SI 6 the most distal. Activity at two stages of gestation at the fetal-maternal interface were also measured. EDP9, embryo-decidual unit (day 9 of gestation); P13, placenta (day 13).

guanosine, inosine, adenosine and their respective 2'-deoxy nucleosides to uric acid. The existence of this pathway in the proximal small intestine is consistent with previous metabolic studies demonstrating that most purines in labeled nucleic acids fed to mice are rapidly broken down to uric acid without gaining access to the systemic circulation (2). Uric acid is a purine catabolite with no obvious dietary role produced in high amounts in both humans and mice. What then is the function of the uric acid produced in the gut? The role of uric acid, particularly as an antioxidant, has been the subject of considerable research (49). Uric acid has been shown to inhibit lino-

FIG. 3. a, simultaneous Northern blot analysis of XDH and ADA transcripts in the indicated tissues of adult BALB/c female mice. Twenty micrograms of total cellular RNA were applied per lane and analyzed following formaldehyde-agarose gel electrophoresis, transferred to nyl-
on membranes, and hybridized with the appropriate cDNA probe. The 1.7-kb ADA mature mRNA was hybridized with a 0.8-kb [32P]cDNA probe for murine ADA. The 4.9-kb XDH transcript was hybridized with a 2.9-kb [32P]-labeled rat cDNA probe. The location of both XDH and ADA mature mRNA is indicated as are 28 and 18 S rRNA. Earlier findings of ADA message levels in the upper alimentary tract were confirmed (19). The distribution of message levels in the tissues for both enzymes compares well with enzyme activity levels. b, Northern blot analysis of PNP transcripts hybridized with a 1.1-kb [32P]cDNA probe for mouse PNP. The location of the 1.3-kb PNP mature mRNA is indicated as are 28 and 18 S rRNA. Blot shows that the highest amount of PNP mRNA is in the proximal small intestine. All other tissues show much lower message levels.

leic autooxidation in water and act as a water soluble antioxi-
dant in vivo (50). It is present in the soluble fraction of human nasal secretions suggesting that it plays a major role in protection of human airway surfaces (51). Its function in the gut may be to trap peroxyl radicals formed as a result of the diges-
tion of foodstuffs.

Most uric acid (52-54) and minimal amounts of hypoxan-
tine and guanine (55) pass, against a concentration gradient, from the lumen of the small intestine through the epithelial layer containing these enzymes to the serosa. In most mamma-
lian species uric acid is further metabolized to allantoin in the liver by the liver enzyme uricase. Primates lack uricase and as a result urate achieves very high levels in the circulation where it may also serve a useful function (14). Sevinian et al. (56) have shown that uric acid stabilizes ascorbate in human serum where uric acid, vitamin C, and vitamin E together contribute 27-47% of the total peroxyl radical-trapping capacity of human plasma, the remaining antioxidant activity being attributed largely to plasma proteins, particularly albumin (57). This last role of uric acid appears to be somewhat redundant because XDH deficient individuals do not have any major problems
consequent to the lack of uric acid production, perhaps due to the presence of dietary levels of ascorbic acid. It has been proposed that the uric acid may have replaced ascorbic acid as an antioxidant in primates. The inability to synthesize ascorbic acid may be the consequence of a mutation in higher primates which occurred simultaneously with the loss of uricase (58). In humans the potential benefits of high levels of serum urate presumably outweigh the known hazards of hyperuricemia and gout.

ADA may have additional roles in the murine upper alimentary tract where, of the four enzymes studied here, only ADA is equally high in the mucosal epithelium of the proximal alimentary canal, including the tongue, esophagus, and forestomach. One possible role for ADA in these tissues is simply to initiate the purine catabolic pathway by metabolizing adenosine and deoxyadenosine to products that can be further metabolized by other enzymes of the pathway. It is also possible that ADA has alternative, or additional, roles in gastrointestinal physiology. In reflecting on the role of ADA it is useful to consider the physiological impact of its substrates. Adenosine exerts a broad range of biological effects by interaction with adenosine receptors on the surface of specific cells (59). These receptors in turn influence the intracellular synthesis of cyclic AMP. ADA may function to insure that dietary sources of adenosine do not exert unwanted physiological effects. ADA could also play an immunological role: lymphoid tissue heavily populates the murine gastrointestinal mucosa. Treatment of mice with deoxycoformycin, a potent inhibitor of ADA, leads to the accumulation of deoxyadenosine. This nucleoside produces lymphoid toxicity (60, 61) presumably by serving as a precursor to deATP which is a negative regulator of ribonucleotide reductase. Deoxyadenosine is also known to inhibit the activity of adenosylhomocysteine hydrolase (61). Thus, the presence of ADA may be needed to ensure an environment favorable for the lymph tissue to mediate local immune responses at the mucosal surface (15).

Levels of Adenosine Deaminase at the Fetal-Maternal Interface Are Not Matched by Other Purine Catabolic Enzymes—The production of ADA at the fetal-maternal interface occurs consecutively at two distinct sites, one maternal (the decidua) and the other mostly embryo-derived (the placenta). ADA levels at the fetal-maternal interface peak in the decidua on day 9 of gestation, decline as the decidua regresses, and rise again in the placenta, reaching a plateau by day 13 that is maintained until term. For this reason we analyzed day 9 implantation sites and day 13 placentas for PNP, ADA, and XDH activity. Our results (Fig. 2) indicate that these three enzymes of purine catabolism are not found at high levels in extracts of the day 9 embryo-decidual unit or in extracts from day 13 placenta; their levels are much lower than that found in the proximal small intestine, and are in line with the relatively low levels of these enzymes in other tissues tested. From these data, we conclude that only ADA, and not other purine catabolic enzymes, is high at the fetal-maternal interface. These findings indicate that at the fetal-maternal interface adenosine deaminase is not functioning solely as part of a purine catabolic pathway leading to uric acid production, but rather is functioning to catalyze the deamination of adenosine or deoxyadenosine. Thus, a physiological role may be associated with the removal of substrate or the production of product.

Adenosine receptor agonists have been reported to be effective inhibitors of growth and organogenesis in mouse embryos (62). Thus it might be postulated that ADA at the fetal-maternal interface functions to prevent the inappropriate accumulation of adenosine which could compromise fetal-maternal interaction and interfere with normal embryonic development. The other ADA substrate, deoxyadenosine, has marked lymphotoxic effects as discussed above. A potential function for ADA in decidual cells may be to maintain an environment low in deoxyadenosine to facilitate the immunorecognition necessary to establish and maintain gestation (21). The importance of ADA in fetal-maternal interaction is indicated by experiments showing that administration of deoxycoformycin to pregnant mice on days 7 and 8 of gestation increases markedly the incidence of implantation site resorptions. The elevated level of ADA in the decidua and placenta compared to the low amounts of other purine catabolic enzymes agrees with the proposed protective role of this enzyme in preventing an inappropriate accumulation of endogenous substrates in the implantation site (21). We have confirmed the observation of Witte et al. (63) and extended their findings by using quantitative measurements that allow us to distinguish between enzyme levels of ADA, PNP, GDA, and XDH in several mouse tissues. Our findings indicate that high levels of ADA are present at several tissues that do not display coordinately high levels of other enzymes of purine metabolism. Specifically, in addition to the proximal small intestine, high levels of ADA are found in the forestomach, esophagus, tongue, decidua, and placenta. Recent evidence indicates that genetic elements residing within the first 6.4 kb of 5'-flanking sequences are capable of directing relatively high expression to the placenta and forestomach and moderate expression to the esophagus and tongue (64). Genetic elements required for decidual and small intestinal expression are lacking from this region. One goal of current research is to identify genetic signals associated with the adenosine deaminase gene responsible for directing high expression to the small intestine. In view of the results reported here, such signals are likely to be shared with the genes encoding PNP, GDA, and XDH. Thus it may be possible to identify the genetic signals and mechanisms responsible for coordinate expression of genes encoding purine catabolic enzymes in the small intestine.

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