Structural and Kinetic Alterations in Adenosine Deaminase Associated with the Differentiation of Rat Intestinal Cells

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SUMMARY

Forms of the enzyme adenosine deaminase (ADase) in rapidly dividing versus differentiated epithelial cells of rat intestinal mucosa were compared. Two variants with isoelectric points at pH ca. 4.85 (ADase I) and 4.80 (ADase II) were resolved by preparative and analytical isoelectric focusing. These forms, which could also be partially resolved by molecular exclusion chromatography, displayed apparent molecular weight values of 37,000 and 33,500, respectively. K_m values for adenosine for the two forms were significantly different (0.07 mM for ADase I and 0.38 mM for ADase II). Small differences were also observed in relative substrate specificity, while the pH-activity profiles for the two forms were essentially identical, with broad maxima between pH values of 6.5 and 9.0. The intensity of ADase I observed in analytical isoelectric focusing increased dramatically with values of 6.5 and 9.0. The intensity of ADase I observed in analytical isoelectric focusing increased dramatically with respect to ADase II as the epithelial cells differentiated. The specific adenosine deaminase activity also increased with cellular differentiation by over 30-fold, expressed per mg DNA, or over 6-fold, expressed per mg protein. Cycloheximide, sharply reduced this increase in specific activity. Cycloheximide was also shown to prevent the increase in the activity of ADase I that is normally associated with differentiation in rat intestines. The data imply the formation of a particular enzyme variant characteristic of the differentiated cell by a mechanism related to active protein synthesis.

INTRODUCTION

ADase (adenosine aminohydrolase, EC 3.5.4.4.) is a ubiquitous aminohydrolase that catalyzes the hydrolysis of adenosine to inosine and ammonia. The control of the concentration and metabolic fate of intracellular adenosine by this enzyme is of critical importance in the regulation of cell growth and differentiation. Relatively low concentrations of adenosine are cytostatic to certain mammalian cells in culture (8, 17). Similarly, the addition of coformycin, a tight-binding inhibitor of ADase (41), strongly inhibits mitogen-induced DNA synthesis in human and mouse lymphocyte cultures (13). In addition, it has been reported that lymphocytic leukemia (40, 47) and certain forms of combined immunodeficiency disease (5, 7, 25, 39) are associated with sharply reduced levels of ADase activity. In the latter case the available data strongly support the existence of a causal relationship between the virtually total absence of this activity and the syndrome, which involves defects in both humoral and cellular immunity.

A number of ADase forms distinct in size and charge have been described. Thus, the human enzyme is genetically polymorphic and is found in 2 electrophoretically distinct forms termed ADA-1 and ADA-2 (6). In addition 3 variants that exhibit apparent molecular weight values in different ranges have been described among vertebrates (21-24, 42). These forms have been designated A (M.W. > 200,000), B (M.W. ~ 100,000), and C (M.W. ~ 35,000). Tissues of higher mammals generally lack the B form and are characterized by varying ratios of A and C. Human erythrocytes (6) contain almost exclusively the type C form, whereas other tissues contain varying amounts of the high-molecular-weight species (24). The 2 forms are apparently interconvertible by a protein conversion factor (11, 29) and also display immunological cross-reactivity (29). One form of cancer has been reported to be associated with an absence of both the conversion factor and the high-molecular-weight ADase that it produces (1).

In light of the potential role of adenosine in the regulation of cell division, we have performed a comparative study of changes in the structure and physicochemical properties of ADase in differentiated versus rapidly dividing cells in rat jejunum. The jejunum was chosen since its epithelial cells can readily be separated at various stages of aging, differentiation, and mitotic activity. The data suggest a model for enzyme regulation in which a particular enzyme variant is preferentially produced in rapidly dividing cells. These results may be of eventual usefulness in the design of drugs for the specific control of adenosine metabolism in cancer, as well as for the prevention of the deamination and consequent inactivation of a variety of adenosine analogs that are potential cancer-chemotherapeutic agents (19, 28, 30, 38, 46).

MATERIALS AND METHODS

Animal Procedures. The animals that provided the intestinal tissues used in all of these experiments were male CFN

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1 This investigation was supported by USPHS Grant CA-14906 from the National Cancer Institute through the National Large Bowel Cancer Project, by Grant CA8748, and by Grant BC-202 from the American Cancer Society.

2 The abbreviations used are: ADase, adenosine deaminase; APT, 4-amino-8-[(3-p-ribofuranosyl)pyrazolo(1,5-e)-1,3,5-triazine (37); formycin A, 7-amino-3-p-p-ribofuranosylpyrazolo(4,3-d)pyrimidine; ara-A, 9-p-araibino-furanosyladenine.

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The fractions designated tip, upper middle, lower middle, and upper crypt represents cuts of about 125 µm from the top of the villus; a scrape with a glass slide was used to remove the lower crypt fraction. The histological and functional identities of these various fractions have been described previously (15, 16). Each fraction was homogenized in a glass homogenizer as a 10% solution in 0.25 M sucrose: 20 mM imidazole-HCl, pH 7.0.

Cycloheximide and actinomycin D were injected i.p. at a dose of 1.5 and 1.0 mg/kg of body weight, respectively.

Enzyme Assay. ADase activity was determined at 37° in a final volume of 1.0 ml containing 0.2 mM adenosine:100 mM potassium phosphate, pH 7.0 by a modification of the spectrophotometric method of Hopkinson et al. (12). The inosine produced was converted to uric acid catalyzed by the presence of added nucleoside phosphorylase (0.1 unit/ml) and xanthine oxidase (0.2 unit/ml). The uric acid formed was determined spectrophotometrically using a mm extinction coefficient of 12.2 at 293 nm (18). Because of the contamination of ADase present in commercial preparations of nucleoside phosphorylase and xanthine oxidase, it was necessary to run an appropriate background blank before the addition of sample.

For accurate determination of low values of enzymatic activity, a radioactivity assay was used. The enzyme was incubated at 37° for various times with 0.11 mM [8-14C] adenosine in a final volume of 0.2 ml containing 100 mM potassium phosphate, pH 7.0. After the reaction was stopped by boiling, the radioactive products [14C]inosine and [14C]hypoxanthine were isolated by thin-layer chromatography (1-butanol-concentrated ammonium hydroxide, 99:1). Both substrate and product spots were cut out, and the radioactivity was assayed by liquid scintillation.

Other substrates of ADase were also tested for activity by spectrophotometric assays with the following mm extinction coefficients and wavelengths: 2'-deoxyadenosine and 3'-deoxyadenosine, 8.33 (265 nm) (18); 2,6-diaminopurine ribonucleoside, 5.15 (247 nm) (34); 6-chloropurine ribonucleoside, 5.32 (250 nm) (2); APTR, 1.52 (265 nm); formycin A, 6.43 (300 nm); ara-A, 8.28 (265 nm). The extinction coefficients for which no reference is given were determined in this laboratory.

A unit of activity is defined as the amount of enzyme that deaminates 1 µmole of substrate per min at 37° under the specified assay conditions at steady state. Specific activity refers to the number of units of enzyme activity per ml of enzyme solution or per mg protein or DNA. Protein was determined by the method of Lowry et al. (20), using bovine serum albumin as standard. DNA was determined on the particulate material obtained after centrifugation at 15,000 x g according to the method of Burton (3), with calf thymus DNA as standard.

Isoelectric Focusing. Preparative isoelectric focusing was carried out in an LKB Uniphor 7900 in the pH range of approximately 4 to 6. With a Pharmacia peristaltic pump P-3, 150 ml of a 5% to 50% (w/v) linear sucrose gradient containing 1% (w/v) ampholines were produced. The sample was focused in the heavy sucrose solution. The electrode solutions contained 2.9% (v/v) ethanolamine in 64% sucrose and 1% (v/v) sulfuric acid. A typical run was allowed to proceed for about 48 hr with a maximum power output of 3 watts and a typical final voltage of 100 V. Fractions of approximately 1.0 ml were collected.

Analytical isoelectric focusing was carried out in the LKB Multiphor 2117 in a flat bed of 5% polyacrylamide containing 2% ampholines, pH 4 to 6, and 5% sucrose. The electrode solutions were composed of 1 mM barbital and 0.5% ampholines, pH 5 to 7. The sample (about 0.02 ml) was soaked into a 5- x 10-mm cellulose acetate strip, which was applied to the surface of the gel. Focusing was allowed to proceed for about 2 hr with a maximum power output of 40 watts and a final voltage of 900 V. Immediately after termination of the run, the gel was overlaid with a thin layer of 1% agar containing adenosine (1.5 mM), 3-(4,5-dimethylthiazolyl-2)-2,5 diphenyl tetrazolium bromide (0.1 mg/ml), phenazine methosulfate (0.1 mg/ml), xanthine oxidase (0.0075 unit/ml), and nucleoside phosphorylase (0.005 unit/ml) in 25 mM potassium phosphate, pH 7.5, essentially as described by Spencer et al. (35). After a 30- to 60-min incubation at 37°, blue bands corresponding to ADase activity appeared.

Chemicals. Xanthine oxidase (crystalline suspension in 2.3 mM ammonium sulfate from buttermilk), nucleoside phosphorylase (crystalline suspension in 3.2 mM ammonium sulfate from calf spleen), bovine serum albumin, calf thymus DNA, 2'-deoxyadenosine, 3'-deoxyadenosine, 6-chloropurine ribonucleoside, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide and phenazine methosulfate were obtained from Sigma Chemical Co. (St. Louis, Mo.). Formycin A was a gift from Meiji Seika Kaisha Ltd. Pharmaceutical Division (Kawasaki, Japan). ara-A was purchased from Parke Davis and Co. (Detroit, Mich.). APTR (37) was a gift from Dr. Jack Fox, and 2,6-diaminopurine ribonucleoside was a gift of Dr. George B. Brown. Ampholines were purchased from LKB Instruments, Inc., Rockville, Md.). Agar (special grade) and sucrose (enzyme grade) were purchased from Schwarz/Mann (Orangeburg, N. Y.). Molecular weight standards (ovalbumin, chymotrypsinogen, RNase) were obtained from Worthington Biochemical Corp. (Freehold, N. J.). [8-14C]-Adenosine (51.2 Ci/mole) was purchased as a 50% ethanol: water solution from New England Nuclear (Boston, Mass.). Thin-layer cellulose plates were obtained from Eastman Kodak (Rochester, N. Y.). Acrylamide and bisacrylamide were purchased from Bio-Rad Laboratories (Richmond, Calif.).

RESULTS

We have studied various ADase electrophoretic variants in a total rat jejunal scrape by preparative isoelectric focusing in a sucrose gradient (Chart 1a). In the pH range of 4 to 6, 2 peaks were partially resolved with approximate isoelectric points of 4.85 (ADase I) and 4.80 (ADase II). Isoelectric focusing in the pH range 3 to 10 indicated the absence of other active species. Complete resolution of these species
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Fig. 1. Analytical isoelectric focusing in a polyacrylamide gel of ADase from different stages of differentiation of a normal rat intestinal mucosa. Upper, mid, and lower, sections cut at 250, 375, and 500 mm from the apex of the jejunal villus, respectively. Details of the procedures are given in “Materials and Methods.”

Chart 1. Isoelectric focusing in a sucrose gradient of ADase from rat intestinal mucosa, a, normal jejunum; b, cycloheximide-treated jejunum. After the tissues were homogenized and centrifuged, the supernatants were subjected to isoelectric focusing as described in “Materials and Methods.” Approximately 2.4 units were applied in a, and 1.6 units were applied in b.

was obtained by analytical isoelectric focusing in a polycrylamide flat bed (Fig. 1). The identity of the 2 bands observed in polyacrylamide gel to the forms resolved in the sucrose gradient was confirmed by electrofocusing each of the sucrose gradient peaks separately in the polycrylamide system. The failure of the preparative system to resolve the forms totally may be explained most simply by the occurrence of diffusion during the collection of fractions. The pl values of the 2 ADase species are too close to allow unequivocal identification by measurement of pH from one run to the next. Simultaneous isoelectric focusing in the polycrylamide flat bed, however, provides a basis for comparison and absolute identification of the 2 forms.

Rat intestinal villi were stratified into various sections from the base to the apex, and the ADase variants were analyzed at the various stages of differentiation (Fig. 1). Although both forms were found simultaneously at all levels, ADase I, which displayed the higher pl value, was consistently observed to increase several fold in activity relative to ADase II as the cells differentiated. Thus in the lower portion of the villus the activity due to ADase I was less than 10% of the total activity, whereas in the upper section of the villus the 2 activities were nearly equal. Interestingly, in some samples the activity of Form I was observed to decline suddenly at the tip of the villus after having steadily increased as the cells differentiated. Although the cause of this phenomenon is not understood, a mechanism based on digestion by proteases derived from the intestinal lumen is a reasonable hypothesis.

The data presented in Table 1 demonstrate a dramatic increase in specific ADase activity as the mitotically active crypt cell of rat intestinal jejunum differentiate. A similar trend has also been reported by Imondi et al. (16). The most striking difference between crypt and tip cells is observed if the activity is expressed per mg DNA. These data indicate a greater than a 30-fold increase in the amount of enzyme activity present per cell, compared to about 6-fold increase if the data are expressed per mg protein. Clearly, therefore, ADase activity is increasing at a rate that is several fold higher than the overall rate of protein synthesis. We have also observed that a large increase in specific ADase activity (expressed per mg DNA) of about 8- to 10-fold also occurs in the differentiating cells of the rat duodenum.

Three hr after injection of cycloheximide, an established inhibitor of protein synthesis, the high specific ADase activity in the upper villus regions was sharply diminished, whereas the specific activity in the crypt cells remained essentially unchanged (Table 1). Six hr after treatment all sections of the villus displayed a specific activity similar to that characteristic of the untreated crypt cells. Since the normal migration time for rat intestinal cells from the base to the apex of the villus is about 2 days, it is unlikely that the effects observed are due to changes in cellular migration.

Actinomycin D, a drug that can interfere with the production of new protein by inhibition of RNA biosynthesis, also
specifically diminishes the ADase characteristic of the differentiated state, with little effect on the activity in the crypt cells. Thus, as suggested by Imondi et al. (16). the high ADase activity characteristic of differentiated intestinal mucosa is by some mechanism related to active protein synthesis, most probably from a relatively unstable mRNA template. These data clearly show that only mature intestinal epithelial cells are characterized by a species of ADase that is rapidly turning over.

Preparative isoelectric focusing of the total jejunum from cycloheximide-treated rats demonstrated a dramatic reduction in the activity of ADase I relative to that of ADase II (Chart 1b). This observation was confirmed in analytical isoelectric focusing (Fig. 2). Thus after cycloheximide treatment the various levels of the villus demonstrated only a faint activity band due to ADase I and could be semiquantitatively evaluated as displaying no dramatic changes in the ratio of activity of the 2 forms as the cells differentiated. Therefore, inhibition of protein synthesis causes all the cell types to resemble the normal crypt cells in terms of ADase variants. This result is consistent with the low specific ADase activities in all cell types from cycloheximide-treated rats (Table 1). In addition, although not shown here, simultaneous isoelectric focusing in polyacrylamide gel of native and cycloheximide-treated jejunum showed no change in the electrophoretic nature of the variants after drug treatment, but only in the ratio of 2 activities.

A comparative study of the physicochemical and enzymatic properties of the 2 ADase forms from normal intestinal epithelium was also undertaken. The ADase used for these studies was generally 90 to 95% free of the other form. This preparation was achieved by pooling only side fractions from each of the activity peaks after preparative isoelectric focusing (Chart 1a). Molecular exclusion chromatography indicated a small but significant difference in the apparent molecular weight values of each of the variants (Charts 2a and 3). The apparent molecular weight values for Forms I and II were approximately 37,000 and 33,500, respectively. Certain proportions of the 2 forms could therefore be resolved on Sephadex G-75 (superfine) (Chart 2a, insert). Sephadex chromatography of crude homogenates of cells from the base and apex of the villus, respectively, also indicated an increase in apparent molecular weight associated with cellular differentiation (Chart 2b). This is consistent with isoelectric focusing data, which indicate that the higher-molecular-weight species (ADase I) is in greater proportion in the villus cells near the tip (Fig. 1).

Although isoelectric focusing indicated that both ADase forms are present simultaneously in crude homogenates of intestinal epithelial cells, molecular exclusion chromatography failed to resolve the mixture into distinct peaks (Chart 2b). Total intestinal scrapes generally indicated an apparent molecular weight for ADase between that of the separated species.

Lineweaver-Burk plots of the 2 species from normal rat indicated distinct $K_m$'s for adenosine at pH 7.0 of about 0.07 and 0.039 mm for ADase I and II, respectively (Chart 4a). The pH-activity relationships for the 2 forms with adenosine as substrate were very similar in general characteristics (Chart 4b). Each species exhibited a broad optimum in activity between pH values of approximately 6 and 9 with sharp losses in activity noted on either side of these values. A notable difference between the 2 species was observed between pH 5.0 and 5.5, where the activity of ADase I was significantly greater than that of ADase II in comparison to
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Chart 3. Determination of the apparent molecular weight values of ADase variants in rat intestinal mucosa by Sephadex G-75 superfine chromatography. Chromatography conditions were as described in Chart 3. All standards were located by their extinction at 280 nm. ADase forms routinely eluted as sharp peaks with apparent molecular weight values of 37,000 and 33,500.

pH 6.0, e.g., where the activities were observed to be nearly equal.

The substrate specificity of the 2 ADase variants relative to adenosine is summarized in Table 2 for a variety of compounds at a concentration of 0.15 mM. It is clear from these data that, although the overall specificity of the 2 forms is similar, statistically significant differences exist. Thus, 2,6-diaminopurine ribonucleoside, ara-A, and APTR are hydrolyzed at a faster rate by ADase II, while formycin A is the preferred substrate of ADase I. Interestingly, both variants hydrolyze the C-nucleosides, formycin A and APTR, at rates severalfold greater than the other substrates. These data might be significantly different, however, if other substrate concentrations or pH values had been used.
Chart 4. Catalytic properties of ADase variants in rat intestinal mucosa, a, Lineweaver-Burk plots for ADase I and II. Enzyme activity was assayed spectrophotometrically as in "Materials and Methods." Adenosine concentration was determined by absorbance at 259 nm, using a molar extinction coefficient of 15.4 (18). b, pH-activity profiles. Enzyme activity was assayed by the radioactivity method given in "Materials and Methods." The buffers used were: pH 4.0 to 5.4, 0.4 M acetic acid titrated with 0.4 M potassium phosphate (dibasic); pH 6.0 to 7.9, 0.4 M potassium phosphate (monobasic) titrated with 0.4 M potassium phosphate (dibasic); pH 8.5 to 9.0, 0.4 M Tris titrated with 0.4 M potassium phosphate (monobasic); pH 9.5 to 11.0, 0.4 M sodium carbonate titrated with 0.4 M potassium phosphate (monobasic). Enzyme preparations used for each of these catalytic properties were obtained after preparative isoelectric focusing (Chart 1) by pooling column fractions that were >90% free of the other ADase variant.
Data presented here and elsewhere (16) strongly support the concept that the high activity of ADase in the differentiated rat intestinal mucosa is produced by a mechanism related to protein synthesis. The appearance of this high activity shows an apparent correlation with the increase in staining intensity of an ADase variant (ADase I) that is only faintly visible in crypt cell populations. A straightforward interpretation is that there is a greater quantity of ADase I actively synthesized de novo in the differentiated cells. In this case each of the forms is considered a separate gene product. However, the fact that the apparent molecular weight of ADase I (ca. 37,000) is slightly larger than that of Form II (ca. 33,500) suggests an alternative mechanism. Thus, it can be speculated that the addition of a small polypeptide chain to an ADase II enzyme causes a transformation into a catalytically more competent species. This mechanism would represent a resourceful use of a preexisting molecule to produce the level of activity required of the differentiated state in the intestine. This type of regulation could be considered to be the inverse of the postulated involvement of intracellular proteases in regulating the activity of fructose-1,6-bisphosphatase by causing a decrease in molecular weight of about 6,000 daltons (31). However, the different chromatographic behavior of rat intestinal ADase I and II on Sephadex, which is the basis for these speculations, is not unequivocal evidence for a difference in molecular weight. Thus, differences in conformation, degree of hydration, or nonspecific interaction with the column matrix could also account for these data.

Two forms of human ADase have been described that exhibit molecular weight values of approximately 35,000 and greater than 200,000, respectively. An increase in the proportion of the low-molecular-weight form has been associated with 2 examples of rapid cellular growth, namely, a carcinous lung (1) and mitogen-stimulated lymphocytes (13). In contrast, the high-molecular-weight form appears to be more of a "storage" form of the enzyme characteristic of the differentiated state. The changes in the properties of the rat jejunal enzyme reported here to be associated with cellular maturation are an interesting parallel to the data on the human enzyme. Thus far the presence of a conversion factor in animals similar in function to the human protein that produces high-molecular-weight enzyme from the low-molecular-weight form has not been described. It is interesting to speculate that the extra segment of polypeptide chain present in ADase I in rat intestine may be similar in function to the human conversion factor. However, in neither man nor the rat is it yet understood what relationship, if any, exists between alterations in the molecular weight of ADase and catalytic function.

A number of interesting parallels exist between the ADase forms in rat intestine and the forms characteristic of alkaline phosphatase in the intestinal villi of the mouse (26, 27). The latter protein is also characterized by 2 species with decidedly different substrate specificities. These variants also change in their relative proportions as the cells migrate along the villus, but they exhibit no difference in their molecular weights. Inhibition of protein synthesis, however, does not inhibit alkaline phosphatase activity but, in fact, enhances it. Thus, although both systems display progres-

### DISCUSSION

Various hypotheses have been considered to explain on a molecular level the mechanism by which cells acquire the enzyme activity and specificity characteristic of the differentiated state (9, 10, 14, 32, 33). The intestinal epithelium represents an excellent model for studying enzyme changes associated with differentiation. Thus, there is always present in this system a gradient of cells in various states of differentiation from the crypt to the apex of the villus. In the process of migration along the villus the mitotically active, undifferentiated crypt cells transform into functionally mature villus cells, which are eventually desquamated into the intestinal lumen. A number of procedures (e.g., Refs. 15 and 44) are available for the ready isolation of these variously differentiated cell populations. In this system changes in protein structure and function can be readily correlated with cellular maturation.

The level of a number of enzyme activities has been shown to vary as differentiation occurs (4, 26, 27, 36, 43–45). The most general mechanism can be considered to involve an alteration in the amount of enzyme protein present. This change in the quantity of protein could be accomplished by an effect on either (a) the rate of synthesis or degradation of the mRNA template, (b) the rate of protein synthesis, or (c) the rate of proteolytic degradation of existing enzyme. Alternatively, the changes in activity could be attributed to a modification of preexisting protein, e.g., by the binding of a newly synthesized allosteric activator or inhibitor, or by the activation of thezymogen. In the latter case, the total amount of enzyme protein would be expected to be constant despite the fact that large activity changes were observed.

### Table 2

<table>
<thead>
<tr>
<th>Substrate</th>
<th>ADase I</th>
<th>ADase II</th>
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<tbody>
<tr>
<td>Adenosine</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>2'-Deoxyadenosine</td>
<td>1.07 ± 0.13</td>
<td>1.25 ± 0.07</td>
</tr>
<tr>
<td>3'-Deoxyadenosine</td>
<td>0.64 ± 0.06</td>
<td>0.68 ± 0.08</td>
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<td>6-Chloropurine ribonucleoside</td>
<td>0.36 ± 0.04</td>
<td>0.39 ± 0.02</td>
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<tr>
<td>2',6-Diaminopurine ribonucleoside</td>
<td>0.40 ± 0.05</td>
<td>0.56 ± 0.05</td>
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<tr>
<td>Formycin A</td>
<td>3.29 ± 0.25</td>
<td>2.34 ± 0.25</td>
</tr>
<tr>
<td>ara-A</td>
<td>0.08 ± 0.01</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>APTP</td>
<td>2.61 ± 0.20</td>
<td>3.43 ± 0.33</td>
</tr>
</tbody>
</table>

* "ADase I" and "ADase II" refer to forms with isoelectric points of ca. 4.85 and 4.90 (see Chart 1).

- Mean ± S.E.
- Statistically significant difference in comparison with ADase II at p < 0.025.
- Statistically significant difference in comparison with ADase II at p < 0.005.
- Statistically significant difference in comparison with ADase II at p < 0.05.
sive shifts in the ratio of enzyme variants associated with cellular maturation, the underlying mechanisms are apparently dissimilar.

Regardless of the molecular details of the mechanism of their production, an important conclusion of this study is that distinct ADase variants characterize nondiving versus mitotically active intestinal cells. These forms are distinguishable by physicochemical and kinetic properties. These data suggest the feasibility of finding inhibitors to reduce ADase activity specifically in certain kinds of cell populations. In particular, since a number of potent tumor growth inhibitors are detoxified by action of this enzyme [e.g., ara-A (19)], the choice of the proper inhibitor might also provide for an increased specificity of the antitumor drug for the malignant cell.

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