Human Adenosine Deaminase

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Adenosine deaminase exists in multiple molecular forms in human tissues. One form of the enzyme appears to be "particulate". Three forms of the enzyme are soluble and interconvertible with apparent molecular weights of approximately 36,000, 114,000, and 288,000 (designated small, intermediate, and large, respectively). The small form of adenosine deaminase is converted to the large form only in the presence of a protein, which has an apparent molecular weight of 200,000 and has no adenosine deaminase activity. This conversion of the small form of the enzyme to the large form occurs at 4°C, exhibits a pH optimum of 5.0 to 8.0, and is associated with a loss of conversion activity. The small form of the enzyme predominates in tissue preparations exhibiting the higher enzyme-specific activities and no detectable conversion activity. The large form of adenosine deaminase predominates in tissue extracts exhibiting the lower enzyme specific activities and abundant conversion activity. The small form of adenosine deaminase shows several electrophoretic variants by isoelectric focusing. The electrophoretic heterogeneity observed with the large form of the enzyme is similar to that observed with the small form, with the exception that several additional electrophoretic variants are uniformly identified. No organ specificity is demonstrable for the different electrophoretic forms. The kinetic characteristics of the three soluble molecular species of adenosine deaminase are identical except for pH optimum, which is 5.5 for the intermediate species and 7.0 to 7.4 for the large and small forms.

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Human Adenosine Deaminase

Enzyme Assays—Adenosine deaminase activity was assayed by a radiochemical technique described previously (17). Assays of partially purified enzyme preparations required bovine serum albumin at a concentration of 0.5 mg/ml or above to maintain linearity with respect to time. 5'-Nucleotidase activity was determined by the conversion of [β-14C]inosine monophosphate to [β-14C]inosine, or [β-32P]adenosine monophosphate to [β-32P]adenosine (10). Adenosine phosphoribosyltransferase activity and hypoxanthine-guanine phosphoribosyltransferase were assayed by a radiochemical technique (13). Cytochrome c oxidase activity, assayed by the method of Cooperstein and Lazarow (20). Catalase activity was assayed at 25°C by following the disappearance of hydrogen peroxide at 240 nm with a Zeiss spectrophotometer, as described by Beers and Sizer (21).

Bovine serum albumin, ovalbumin, and α-chymotrypsinogen, when used as standards for calibration of sucrose density gradients or gel filtration columns, were identified by the changes in absorbance at 280 nm. Equine splenic ferritin or human hemoglobin was identified by change in absorbance at 415 and 430 nm, respectively. Protein concentration was determined either by the method of Lowry et al. (22) or by absorbance at 280 nm assuming E280 = 1.0 liter g⁻¹ cm⁻¹.

Sucrose Gradient Centrifugation—Sucrose gradient ultracentrifugation was performed with a Spinco SW41 rotor in a Beckman model L5-50 using isokinetic gradients (10 to 28.2%) of 1.18 ml prepared according to the method of McCarty et al. (23). Bovine serum albumin, human hemoglobin, and catalase were used as standards.

Gel Filtration—Gel filtration was performed at 4°C on an 8% agarose column (1.7 x 80.5 cm) or a Sepharose 4B column (1.7 x 82.5 cm) equilibrated with 10 mM Tris/HC1, pH 7.4 (Buffer A). The Kav of each standard was determined according to the method of Laurent and Killander (24) and the Stokes radii for the molecular forms of adenosine deaminase were calculated by the method of Acker (25). The Stokes radii or s, values for the calibration standards were obtained from the literature (26).

Estimation of Molecular Weight and Frictional Ratio—Molecular weights and frictional ratios of adenosine deaminase were calculated from the s, and Stokes radii by the following formulas.

\[ M = \frac{6 nN_{A} (1 - \beta p)}{f/f_{0} - \alpha /3 \mu M / \mu A \ N_{A} s^{2/3}} \]

where

- \( M \) = molecular weight
- \( s \) = Stokes radius
- \( n \) = sedimentation coefficient
- \( p \) = partial specific volume (a volume of 0.725 cm³/g was assumed as representative of most proteins)
- \( f/f_{0} \) = frictional ratio
- \( \alpha \) = viscosity of the medium
- \( \mu \) = density of the medium
- \( N_{A} \) = Avogadro’s number

Conversion Activity—Definition of factors involved in the interconversion of one molecular species of adenosine deaminase to another was achieved by subjecting preparations under specified conditions either to sucrose gradient ultracentrifugation or by applying an aliquot (50 μl) of the preparation to one of several Sephadex G-100 columns (24 x 0.9 cm) containing 13% agarose and equilibrated with 50 mM Tris/HC1 buffer (pH 7.4) containing 1 mM EDTA. Using column chromatography, the large molecular species, which eluted in the void volume (Vv, 4.4 ml), could be separated from the small molecular form (Vₜ, 8.4 ml) with 90% recovery of enzyme activity.

Conversion activity was quantitated by incubating reaction mixtures containing 50 mM Tris/HC1 (pH 7.4), 8 to 10 μg of the small molecular form of adenosine deaminase isolated by gel filtration, and bovine serum albumin (1 mg/ml) in 50 mM Tris/HC1 (pH 7.4) with 50 μl of the sample to be assayed in a final volume of 100 μl. Following incubation of 37°C for 20 min, an aliquot of the reaction mixture was applied to the Sephadex G 100 column as described above. The activity of the large form of adenosine deaminase elaborated under these conditions was determined. The sample to be assayed for conversion activity and the small molecular form of adenosine deaminase were each incubated alone and at the same time with unlabelled adenosine deaminase activity appearing in the void volume in these instances, which was regarded as background, was subtracted from that obtained with the mixture. Units of conversion activity are arbitrarily expressed as the enzyme activity (μmol/h) of the large molecular form of adenosine deaminase elaborated under specified conditions.

Isoelectric Focusing—Isoelectric focusing was performed with an LKB model 7900 Uliphor column electrophoresis system according to the method of Vesterberg and Svensson (27).

Enzyme Preparation—All steps were performed at 4°C unless otherwise specified. Non-naoplastic human tissue obtained post mortem was homogenized in 3 to 4 volumes (w/v) of Buffer A with 15 to 30 strokes of a Dounce homogenizer. The homogenate was subsequently centrifuged at 6,800 x g for 20 min and the supernatant so obtained was stored at −70°C. Storage at this temperature up to 6 months resulted in no loss of activity or alteration in the distribution of the molecular species of adenosine deaminase as determined either by gel filtration or sucrose density ultracentrifugation. After gel filtration, the fractions containing the activity of the appropriate molecular form of adenosine deaminase were pooled and concentrated approximately 5 to 10-fold by ultrafiltration as described above. Preparations were stable when stored at 4°C or −70°C for up to 6 months.

Conversion activity was prepared from human kidney tissue essentially by the method of Nishihara et al. (28). Frozen tissue was thawed and washed in 30 mM phosphate buffer (pH 7.4) (Buffer B). The tissue was then sliced and homogenized at 4°C in 2 volumes (w/v) of Buffer B with a Sorvall blender at full speed for 1 min. The homogenate obtained was centrifuged at 30,000 x g for 30 min and the precipitate discarded. The supernatant was brought to 60% saturation with ammonium sulfate and stirred for 2 h. The precipitate was removed by centrifugation at 30,000 x g for 20 min. Subsequently, ammonium sulfate was added to the supernatant to 80% saturation, stirred for 2 h and centrifuged as above. The precipitate was suspended in approximately 10 ml of Buffer B and subsequently dialyzed against 1000 volumes of Buffer A for 24 h. The dialyzed preparation was clarified by centrifugation at 30,000 x g for 20 min and concentrated 2-fold by ultrafiltration as described above. The preparations were stable at 4°C for up to 3 months.

Tissue Distribution—The appropriate tissues obtained at necropsy were sliced, washed twice with 154 mM sodium chloride, blotted dry with filter paper, homogenized at 4°C in 3 to 4 volumes (w/v) of Buffer A with a Sorvall blender at full speed for 1 min. The homogenate obtained was centrifuged at 30,000 x g for 20 min. The supernatants obtained were dialyzed for 12 h against 1000 volumes of Buffer A and assayed for adenosine deaminase activity as described above.

Subcellular Distribution—The activity and molecular forms of adenosine deaminase in the cytosol and subcellular organelles of human leukocytes was determined by differential centrifugation in isotonic sucrose employing a modification of the method of Morre (29).

RESULTS

Molecular Heterogeneity—Multiple molecular species of adenosine deaminase could be demonstrated using either sucrose gradient ultracentrifugation or gel filtration. Employing the 6,600 x g supernatant from splenic tissue homogenate as starting material, four distinct components of adenosine deaminase activity were demonstrable by gel filtration (Fig. 1). Following gel filtration on either 8% agarose or Sepharose 4B, a portion of the adenosine deaminase activity consistently eluted in the void volume (Vv). The three components of adenosine deaminase activity retarded by gel filtration were designated as the large, intermediate, and small forms of the enzyme, respectively (Fig. 2, Table I). The presence of the intermediate species was variable following gel filtration. Attempts to stabilize this intermediate form by the addition of various compounds to the extraction buffer (Buffer A) such as β-mercaptoethanol (5 mM), dimethyl sulfoxide (5% w/v), dithiothreitol (5 mM), or sucrose (15%, w/v) were unsuccessful. With sucrose gradient ultracentrifugation, the same tissue preparation also yielded four components of adenosine deaminase activity with centrifugation times of 2, 15, or 30 h, one component of activity consistently appeared at the bottom of the tube. The sedimentation coefficients of each of the remaining three components of activity is listed in Table I. The calculated molecular weights and frictional ratios for the soluble molecular forms of adenosine deaminase are also listed.

Tissue Distribution—Adenosine deaminase activity was present in all human tissue extracts examined with preparations from gastrointestinal or splenic tissues exhibiting the higher specific activities (Table II). The molecular form of adenosine deaminase predominating in different tissues as...
Human Adenosine Deaminase

FIG. 1. Molecular heterogeneity of human adenosine deaminase shown by gel filtration. An aliquot (2 ml) of homogenate prepared from human splenic tissue as outlined under "Experimental Procedure" was applied to an 8% agarose column (1.7 x 80.5 cm) equilibrated with 10 mM Tris/HCl (pH 7.4) at 4°C. In all figures, enzyme activity is expressed as nmol of inosine formed/h.

FIG. 2. Stokes radius of the small, intermediate, and large form of adenosine deaminase and conversion activity.

TABLE I

| Physical properties of soluble human adenosine deaminase and conversion activity |
|-----------------------------------------------------------------------------|
| Sedimentation coefficient*  |
| Stokes radius*               |
| Molecular weight            |
| Frictional ratio            |
| Small form                  | $3.8 \pm 0.3 (61)$ | 23.0 $\pm$ 0.9 (10) | 36,000 | 1.06 |
| Intermediate form           | $7.3 \pm 0.2 (11)$ | 38.2 $\pm$ 1.9 (6)  | 114,000 | 1.20 |
| Large form                  | $10.7 \pm 0.4 (42)$ | 67.2 $\pm$ 1.3 (14) | 298,000 | 1.52 |
| Conversion activity         | $9.9 (1)$            | 49 (1)               | 200,000 | 1.27 |

*The value given is the mean $\pm$ 1 S.D. The figure in parenthesis indicates the number of determinations.

determined by sucrose gradient ultracentrifugation of tissue extracts appeared to correlate with the specific activity of adenosine deaminase in the various tissue extracts (Table II). The large form of adenosine deaminase activity predominated in those tissue extracts exhibiting lower enzyme activity, e.g. lung or kidney, while the small molecular form was the major species in those tissue extracts exhibiting higher enzyme activity, e.g. stomach or spleen. Although these patterns of distribution were reproducible for most tissues, substantial variation was observed with extracts prepared from lung. In this tissue from different donors, either the large or small species could predominate despite attempts to maintain identical conditions.

Subcellular Distribution—The subcellular distribution of adenosine deaminase in human leukocyte preparations is shown in Table III. The total recovery of adenosine deaminase activity in the various subcellular fractions was 58% of that present in the initial lysate prepared by freeze-thawing. This loss of activity probably represents inadequate disruption of the leukocytes since the recovery of protein and marker enzymes (cytosol: hypoxanthine-guanine phosphoribosyltransferase; plasma membranes: 5'-nucleotidase; mitochondria: cytochrome c oxidase) was comparable to the recovery of the adenosine deaminase activity. While the bulk of the total adenosine deaminase activity was present in the 100,000 x g supernatant, approximately 2% of the total activity present in the initial homogenate was associated with the 6000 x g and 100,000 x g pellets. This adenosine deaminase activity did not appear to be the result of contamination of these fractions with cytosol activity as no hypoxanthine-guanine phosphoribosyltransferase activity was detected in either fraction. Since the 6000 x g pellet exhibited substantial 5'-nucleotidase activity and attempts to reduce this apparent contamination have proven unsuccessful, the subcellular organelle(s) to which this form of adenosine deaminase is identified cannot be precisely defined. Sucrose gradient ultracentrifugation of the adenosine deaminase associated with the 100,000 x g supernatant revealed the presence of both the large and small forms but, in contrast to tissue extracts, there was no "particulate" adenosine deaminase activity sedimenting to the bottom of the gradient. An extract of the 100,000 x g pellet when subjected to sucrose gradient ultracentrifugation disclosed that the major
Human Adenosine Deaminase 5451

**TABLE III**

**Adenosine deaminase activities in subcellular fractions of human leukocytes**

| Fraction          | Volume | Protein | Adenosine deaminase | Hypoxanthine-guanine phosphoribosyltransferase | 5'-Nucleotidase | Cytochrome c oxidase |
|-------------------|--------|---------|---------------------|-----------------------------------------------|-----------------|---------------------|
|                   | ml     | mg      |                     |                                               | AMP as substrate | IMP as substrate |
| Homogenate        | 2      | 20      | Adenosine deaminase | 134                                            | 34.8            | 12.3                | 0.026               |
| +2% Triton        | ND     | ND      |                     | ND                                            | ND              | ND                  |
| 6,000 x g pellet  | 1      | 0.86    | Adenosine deaminase | 1.9                                           | <0.001          | 2.9                 | 0.014               |
| +2% Triton        | ND     | ND      |                     | ND                                            | ND              | ND                  |
| 100,000 x g pellet| 1      | 1.10    | Adenosine deaminase | 2.3                                           | <0.001          | 3.0                 | ND                  |
| +2% Triton        | ND     | ND      |                     | ND                                            | ND              | ND                  |
| 100,000 x g supen.| 11     | 9.86    | 5'-Nucleotidase     | 69.0                                          | 23.6            | <0.001              | 3.5                 |
| +2% Triton        | ND     | ND      |                     | ND                                            | ND              | ND                  |
| Total activity    |        |         |                     |                                               |                 |                     |                     |
| Homogenate        | 2      | 20      | Adenosine deaminase | 134                                            | 34.8            | 12.3                | 0.026               |
| +2% Triton        | ND     | ND      |                     | ND                                            | ND              | ND                  |
| 6,000 x g pellet  | 1      | 0.86    | Adenosine deaminase | 1.9                                           | <0.001          | 2.9                 | 0.014               |
| +2% Triton        | ND     | ND      |                     | ND                                            | ND              | ND                  |
| 100,000 x g pellet| 1      | 1.10    | Adenosine deaminase | 2.3                                           | <0.001          | 3.0                 | ND                  |
| +2% Triton        | ND     | ND      |                     | ND                                            | ND              | ND                  |
| 100,000 x g supen.| 11     | 9.86    | 5'-Nucleotidase     | 69.0                                          | 23.6            | <0.001              | 3.5                 |
| +2% Triton        | ND     | ND      |                     | ND                                            | ND              | ND                  |

**Human Adenosine Deaminase Activities**

Adenosine deaminase activities in subcellular fractions of human leukocytes. ND = Not determined.

**Conversion of Large to Small**—The large molecular species of adenosine deaminase was isolated by gel filtration of extracts prepared from lung or kidney tissue, in which this molecular species predominates, and then concentrated by ultrafiltration and subjected to further gel filtration or sucrose gradient ultracentrifugation, a single peak of activity was observed which had the Stokes radius $s_{20,w}$ of the large form. Unsuccessful attempts to dissociate this large molecular species obtained from kidney included incubation at 4°C or 37°C with increasing ionic strength (25 to 200 mM KCl in Buffer A), the substrate or products of the reaction (2.5 to 10 mM adenosine, 5 to 10 mM inosine, 5 mM ammonium chloride, 5 mM $p$-chloromercuribenzoate, or 5 mM $p$-chloromercuribenzoate). However, incubation of the large form of adenosine deaminase in 50 mM sodium succinate (pH 3.4) at 4°C or 37°C resulted in a decrease in the activity of the large form and the appearance of activity with an $s_{20,w}$ of 3.6 (Fig. 3) corresponding in sedimentation coefficient to that of the small form. The large molecular species was not dissociated after incubation under the same conditions in 50 mM sodium succinate (pH 4.4 to 5.0), 50 mM Mes (pH 5.5 to 6.5), or 50 mM Tris/HCl (pH 7.4 to 10.5).

**Conversion of Intermediate to Small**—The intermediate molecular species was examined by sucrose gradient ultracentrifugation following isolation by gel chromatography. The major peak of activity had an $s_{20,w}$ value of 3.6 while 2 minor components of activity had $s_{20,w}$ values of 7.4 and 10.4. The sedimentation coefficient of these three forms correspond respectively to the small, intermediate, and large forms of adenosine deaminase present in tissue homogenate. The intermediate form of adenosine deaminase ($s_{20,w} = 7.4$) identified after sucrose density ultracentrifugation of tissue homogenate, when pooled, desalted through a Sephacryl G-25 column equilibrated with Buffer A, and subjected to repeat sucrose gradient centrifugation yielded a single major component of adenosine deaminase activity with an $s_{20,w}$ value of 3.6. Due to

Component of adenosine deaminase was "particulate" and sedimented to the bottom of the gradient with a minor component exhibiting the sedimentation velocity of the small form.

Gel filtration of splenic homogenate on 8% agarose revealed 5'-nucleotidase but not adenine phosphoribosyltransferase, hypoxanthine-guanine phosphoribosyltransferase, or cytochrome c oxidase in the void volume with a portion of the adenosine deaminase activity. Isolation of the adenosine deaminase activity eluting in the void volume followed by repeat gel filtration revealed, in addition to activity eluting in the void volume, the appearance of activity corresponding in elution volume to that of the small form (Fig. 3A). On other occasions, in addition to the small form, minor peaks of adenosine deaminase activity were discernable which corresponded in elution volume to the large and intermediate forms. Incubation of material appearing in the void volume with Triton X-100 followed by repeat gel filtration revealed as much as a 3-fold increase in the total adenosine deaminase activity eluting as the small form (Fig. 3, A and B). These observations suggest that the adenosine deaminase activity sedimenting to the bottom of the sucrose gradients or eluting in the void volume with gel filtration is associated with subcellular particulate matter.

Conversion of Large to Small—The large molecular species of adenosine deaminase was isolated by gel filtration from extracts of tissues in which the small molecular form predominates. Following concentration by ultrafiltration, three peaks of adenosine deaminase activity were identified by either gel filtration on 8% agarose or sucrose gradient ultracentrifugation (Fig. 4). The $s_{20,w}$ values and Stokes radii for the large, intermediate and small forms of adenosine deaminase activity observed in the initial homogenate. When the small molecular species of adenosine deaminase newly formed from the large species was isolated by gel filtration and subjected to sucrose gradient centrifugation, a single peak of activity was observed with an $s_{20,w}$ of 3.6. When the large form of adenosine deaminase (Stokes radius = 68, $s_{20,w} = 10.4$) was isolated by gel filtration of extracts prepared from lung or kidney tissue, in which this molecular species predominates, and then concentrated by ultrafiltration and subjected to further gel filtration or sucrose gradient ultracentrifugation, a single peak of activity was observed which had the Stokes radius and $s_{20,w}$ of the large form. Unsuccessful attempts to dissociate this large molecular species obtained from kidney included incubation at 4°C or 37°C with increasing ionic strength (25 to 200 mM KCl in Buffer A), the substrate or products of the reaction (2.5 to 10 mM adenosine, 5 to 10 mM inosine, 5 mM ammonium chloride, 5 mM $p$-chloromercuribenzoate, or 5 mM $p$-chloromercuribenzoate). However, incubation of the large form of adenosine deaminase in 50 mM sodium succinate (pH 3.4) at 4°C or 37°C resulted in a decrease in the activity of the large form and the appearance of activity with an $s_{20,w}$ of 3.6 (Fig. 3) corresponding in sedimentation coefficient to that of the small form. The large molecular species was not dissociated after incubation under the same conditions in 50 mM sodium succinate (pH 4.4 to 5.0), 50 mM Mes (pH 5.5 to 6.5), or 50 mM Tris/HCl (pH 7.4 to 10.5).

Conversion of Intermediate to Small—The intermediate molecular species was examined by sucrose gradient ultracentrifugation following isolation by gel chromatography. The major peak of activity had an $s_{20,w}$ value of 3.6 while 2 minor components of activity had $s_{20,w}$ values of 7.4 and 10.4. The sedimentation coefficient of these three forms correspond respectively to the small, intermediate, and large forms of adenosine deaminase present in tissue homogenate. The intermediate form of adenosine deaminase ($s_{20,w} = 7.4$) identified after sucrose density ultracentrifugation of tissue homogenate, when pooled, desalted through a Sephacryl G-25 column equilibrated with Buffer A, and subjected to repeat sucrose gradient centrifugation yielded a single major component of adenosine deaminase activity with an $s_{20,w}$ value of 3.6. Due to
FIG. 3. Repeat gel chromatography of "particulate" adenosine deaminase from human spleen with and without treatment with Triton X-100. The enzyme activity that eluted between 62 and 68 ml from the 8% agarose column (see Fig. 1) was pooled and concentrated by ultrafiltration to 2.5 ml. Aliquots (1 ml) of this preparation were incubated at 4°C for 45 min with either Buffer A or 1% Triton and applied to identical 8% agarose columns equilibrated with Buffer A. A, Preparation incubated with Buffer A. B, Preparation incubated with 1% Triton.

FIG. 4. Sucrose gradient ultracentrifugation of the large molecular form of adenosine deaminase from human splenic tissue. The enzyme activity that eluted between 83 and 94 ml from the 8% agarose column (see Fig. 1) was pooled and concentrated by ultrafiltration to 2.5 ml. An aliquot (200 μl) of this sample was applied to a sucrose gradient and centrifuged at 4°C for 30 h as outlined under "Experimental Procedure."

the low activity of the intermediate form of the enzyme, it was not possible to examine its properties by repeat gel filtration.

Conversion of Small to Large—The small molecular form of adenosine deaminase was isolated by gel filtration chromatography from tissues where the small form predominates. Follow-

FIG. 5. Dissociation of the large form of adenosine deaminase from human kidney to the small form at pH 3.4 shown by sucrose gradient ultracentrifugation. The enzyme activity eluted between 83 and 94 ml by gel filtration of human kidney extract on the 8% agarose column equilibrated with Buffer A (see Fig. 1) was pooled and concentrated by ultrafiltration. A portion (0.15 mg) of this enzyme preparation (specific activity 51 nmol/min/mg of protein) was incubated at 4°C for 30 min either in 50 mM Tris/HCl, pH 7.4 (●—●), 50 mM sodium succinate, pH 4.4 (■—■), or 50 mM sodium succinate, pH 3.4 (▲—▲). An aliquot of each reaction mixture was layered on to sucrose gradients made up in Buffer A and centrifuged at 4°C for 15 h as outlined under "Experimental Procedure."
deaminase. This was further supported by the absence of a "particulate" species following incubation of the small or large form with conversion activity.

**Characteristics of the Conversion Reaction**—The distribution of conversion activity in different tissue extracts is shown in Table IV. Conversion activity was present in those tissue extracts in which the large species was the predominant form of adenosine deaminase, and was not detectable in those extracts in which the small form predominated.

The partial purification of conversion activity (see "Experimental Procedure") eliminated more than 95% of the adenosine deaminase activity present in crude kidney extract and the resultant preparation exhibited a 2- to 3-fold increase in conversion specific activity with an overall recovery of 40%.

The time course of the conversion reaction at 4° and 37° and at two different concentrations of the preparation is shown in Fig. 7. The formation of the large form was complete within 5 min at 37° and within 15 min at 4°. Following completion of the reaction, the addition of increasing amounts of the small form led to no further formation of the large form. Under these conditions, the formation of the large molecular species was limited by the amount of protein present with conversion activity. However, by increasing the amount of conversion activity added to a constant activity of the small form, all of the small form could be converted to the large molecular form. Preincubation of conversion activity preparations with the large species of adenosine deaminase did not influence the amount of product formed on subsequent incubation with the small molecular species.

The effect of pH on conversion activity is shown in Fig. 8. The diminished conversion at pH 3.4 was not due to irreversible inactivation of either conversion activity or of the small form since incubation of the preparations at this pH in 50 mM sodium succinate for 20 min at 37° and subsequent dialysis against Buffer A resulted in complete recovery of both conversion activity and the adenosine deaminase activity of the small form. The conversion reaction did not require thiol compounds or bivalent metal ions for activity. Preincubation with 5 mM p-chloromercuribenzoate, 5 mM dithiothreitol, or 5 mM &beta; mercaptoethanol and their subsequent removal by dialysis, did not affect the conversion activity; 5 mM EDTA, 2.5 mM MgCl2, or 2.5 mM CaCl2 similarly were without effect. Various purine nucleosides and nucleotides including adenosine, inosine, AMP, IMP, GMP, cyclic 2′:3′-AMP, ATP, and GTP at a final concentration of 2.5 mM had no effect on the conversion reaction.

Conversion activity present in kidney extracts was subjected to gel chromatography with 8% agarose on one occasion. The activity partitioned as a single peak which did not correspond to the peak of activity of the large molecular form of adenosine deaminase. The Stokes radius of conversion activity was 49 Å. The conversion activity evident with gel filtration was pooled, concentrated by ultrafiltration, and subjected to sucrose gradient ultracentrifugation. This yielded a single peak of conversion activity with an S20,w of 9.9. The calculated molecular weight and frictional ratio of conversion activity is listed in Table I.

**Isoelectric Focusing**—Isoelectric focusing over a broad pH range (3.5 to 10) of crude tissue extracts exhibiting both the large and small molecular forms of adenosine deaminase disclosed all the enzyme activity between pH 4 and 6. Following isolation by gel chromatography, both the large and small molecular forms of adenosine deaminase also electrophoresed within this pH range. Thus the remaining studies (with the one exception indicated) were carried out using ampholytes in the range from pH 4 to 6.

The small form of adenosine deaminase isolated from either small intestine or spleen exhibited two or three different electrophoretic forms (Table V). Sucrose gradient ultracentrifugation of each electrophoretic variant yielded a single peak of activity with an S20,w value ranging from 3.6 to 3.8, which corresponds to that of the native small form. Isoelectric focusing of the large molecular species of adenosine deaminase obtained by gel filtration from one of several different tissues revealed five to six different electrophoretic forms (Table V). The large form elaborated by incubation of the isolated small form with partially purified conversion activity yielded a pattern similar to that observed with the native large form.
FIG. 7. Effect of temperature and protein on the rate of conversion of the small molecular form of adenosine deaminase to the large molecular form by conversion activity partially purified from kidney tissue. A quantity (10 μg) of the partially purified small form (specific activity 6.3 pmol/min/mg of protein) was incubated with 0.88 units of conversion activity at 37°C (▲—▲) or 2.2 units of conversion activity at 4°C (○—○) and 37°C (■—■) in a final volume of 0.1 ml containing 50 mM Tris/HCl (pH 7.4) and bovine serum albumin (1 mg/ml). The formation of the large form under these conditions was quantitated as outlined under “Experimental Procedure.”

![Graph](image)

FIG. 8. Effect of pH on the conversion of the small molecular form of adenosine deaminase to the large form in the presence of conversion activity. A quantity (1.1 units) of conversion activity partially purified from kidney tissue was incubated with 10 μg of a partially purified preparation of the small form (specific activity 6.3 pmol/min/mg of protein) for 20 min at 37°C in a final volume of 0.1 ml containing 50 mM of the appropriate buffer (see Table VI) and bovine serum albumin (1 mg/ml). The formation of the large form under these conditions was quantitated as outlined under “Experimental Procedure.”

Sucrose gradient ultracentrifugation of each electrophoretic variant from kidney or the large form produced in vitro yielded a single peak of activity with an s~20, w value ranging from 10.6 to 10.8, which corresponds to that of the native large form. In contrast to the kidney gel filtration of the electrophoretic variants with pl value(s) of 4.65 and 4.75 from lung indicated a mixture of the large and small molecular forms (ratio of activities being 0.64:0.36 and 0.37:0.63, respectively) while the electrophoretic variants with pl value(s) of 5.06, 5.14, and 5.24 remained large. The results with large form from liver were essentially the same as observed with the large form from lung.

**Kinetic Properties**—Some of the kinetic constants of human adenosine deaminase are summarized in Table VI. The only

| Tissue  | Molecular form | Donor | Iso-electric points | Relative activity |
|---------|----------------|-------|---------------------|-------------------|
| Small Intestine | Small | A | 4-6 | 4.64 | 0.17 |
|          |          |     |                    | 4.75 | 1.00 |
|          |          |     |                    | 4.87 | 0.07 |
| Spleen  | Small     | B   | 4-6 | 4.69 | 0.17 |
|          |          |     |                    | 4.83 | 1.00 |
| Spleen  | Small     | C   | 4-6 | 4.72 | 1.00 |
|          |          |     |                    | 4.84 | 2.86 |
|          |          |     |                    | 4.98 | 3.10 |
| Kidney  | Large     | D   | 4-6 | 4.76 | 1.00 |
|          |          |     |                    | 4.98 | 0.21 |
|          |          |     |                    | 5.02 | 0.27 |
|          |          |     |                    | 5.14 | 0.59 |
|          |          |     |                    | 6.24 | 0.74 |
| Kidney  | Large     | E   | 3-6 | 4.65 | 0.85 |
|          |          |     |                    | 4.72 | 1.00 |
|          |          |     |                    | 4.83 | 0.65 |
|          |          |     |                    | 4.98 | 0.30 |
|          |          |     |                    | 5.04 | 0.47 |
|          |          |     |                    | 5.13 | 0.93 |
| Lung    | Large     | A   | 4-6 | 4.65 | 0.27 |
|          |          |     |                    | 4.72 | 1.00 |
|          |          |     |                    | 5.06 | 0.32 |
|          |          |     |                    | 5.14 | 0.15 |
|          |          |     |                    | 6.24 | 0.07 |
Human Adenosine Deaminase

The present study discloses that adenosine deaminase is widely distributed in human tissues with the highest activity evident in spleen and gastrointestinal tract. While the bulk of the activity is localized to the cytosol, up to 3% of the total cellular activity appears to be associated with a subcellular organelle(s). Mustafa and Tewari (30) previously presented evidence for a mitochondrial form of adenosine deaminase in rat cerebral cortex, a finding questioned by others (31).

Four molecular species of human adenosine deaminase could be defined based on differences in molecular weight. One form appears to be "particulate" in nature based on the findings that (a) it has a molecular weight greater than 20,000,000, (b) it appears to be associated predominantly with a subcellular organelle, (c) treatment with a nonionic detergent is followed by the appearance of activity with a molecular weight of approximately 36,000, and (d) it could not be formed in reconstitution experiments. This form of the adenosine deaminase activity has not been recognized previously.

The remaining molecular species are soluble and have molecular weights estimated to be approximately 36,000, 114,000, and 298,000. The molecular weight of 36,000 for the small form compares favorably with the range of 30,000 to 35,000 reported for partially purified and apparently homogeneous preparations of adenosine deaminase from human erythrocytes (15, 16) or bovine tissue (3, 5, 32, 33), but differs from the previously reported value of 47,000 for an apparently homogeneous preparation from human gastric tissue (14). The apparent molecular weight of 298,000 for the large molecular species in the present study also differs from the previously reported value of 230,000 for an apparently homogenous species from human lung (14). The intermediate species has not been previously reported. On no occasion in this study was the form of adenosine deaminase with a reported molecular weight of 440,000 evident (19). Although multiple molecular forms of adenosine deaminase have been demonstrated in amphibian (9, 10), avian (8), or mammalian tissue preparations (11-14, 32), their interrelationship has remained unclear.

In the present study we have found that the three soluble species of adenosine deaminase are interconvertible. The large and intermediate forms of the enzyme dissociate spontaneously to the small form. Conversion of the small form of the enzyme to the large form appears to require another protein with a molecular weight of approximately 200,000. The interaction of conversion activity with the small form of adenosine deaminase does not appear to be a catalytic process since the reaction is only minimally temperature dependent and the conversion activity appears to be consumed in the process. Some of the characteristics of the conversion reaction reported here are similar to those described for the protein termed "conversion factor" purified from human lung (28). However, the data presented here suggest a molecular weight of 200,000 for conversion activity present in human kidney tissue, a value substantially different from the value of 139,000 for "conversion factor" described by Nishihara et al. (28).

In addition to species of different molecular weights, adenosine deaminase also exhibits substantial electrophoretic heterogeneity. Several forms of adenosine deaminase have been distinguished previously in erythrocytes by their electrophoretic mobility on starch gel electrophoresis (34). Tissues other than erythrocytes have been reported to exhibit additional forms termed "tissue-specific" isoenzymes which vary in their electrophoretic mobility in a manner specific for that particular tissue (13, 35). While considerable electrophoretic heterogeneity was demonstrated with each molecular form of adenosine deaminase using preparative isoelectric focusing, we were unable to confirm the presence of tissue-specific isoenzymes. The molecular basis for the electrophoretic heterogeneity of human adenosine deaminase also remains undefined.

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Human Adenosine Deaminase

5456

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