Human Adenine Phosphoribosyltransferase

AFFINITY PURIFICATION, SUBUNIT STRUCTURE, AMINO ACID COMPOSITION, AND PEPTIDE MAPPING*

(Received for publication, September 5, 1979, and in revised form, March 13, 1979)

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Adenine phosphoribosyltransferase (EC 2.4.2.7) has been purified 55,000-fold from normal human erythrocytes. The native molecular weight of the enzyme is 38,200 as determined by sedimentation equilibrium centrifugation. The subunit molecular weight is 18,000 as determined by gel filtration in guanidine hydrochloride, suggesting that the enzyme is a dimer in its native state. Cross-linking the enzyme with dimethylsuberimidate confirms the dimeric structure and peptide mapping data suggested that the subunits are quite similar if not identical. The amino acid composition reveals that 33% of the residues are hydrophobic.

Adenine phosphoribosyltransferase (EC 2.4.2.7) catalyzes the condensation of 5-phosphoribosyl-1-pyrophosphate (PP-ribose-P) with adenine to yield adenine phosphoribosyltransferase activity in erythrocytes from patients with the Lesch-Nyhan syndrome (1) and by the inherited deficiency of the enzyme described in a number of families (2-5). In order to better understand the nature of these alterations of adenine phosphoribosyltransferase activity at the molecular level, it is necessary to define the nature of the normal enzyme. The normal enzyme has previously been purified 33,000-fold from human erythrocytes (6). This procedure, however, is lengthy and laborious. In this report we describe a more efficient purification procedure for adenine phosphoribosyltransferase. Some of the characteristics of the highly purified enzyme, not previously reported, are described.

RESULTS*

Purification of Adenine Phosphoribosyltransferase—The purification of adenine phosphoribosyltransferase is shown in Table I. Adenine phosphoribosyltransferase binds to a GMP affinity column. The enzyme appears to be binding to the iminobispropylamine spacer arm rather than the GMP since columns containing only the spacer arm will retain adenine phosphoribosyltransferase but not hypoxanthine-guanine phosphoribosyltransferase. The chromatographic behavior of adenine phosphoribosyltransferase eluted from the GMP column with AMP is shown in Fig. 1. The enzyme obtained from this procedure is about 30% pure as judged by sodium dodecyl sulfate gels. Further chromatography on a Sephadex G-75 column removes contaminating proteins and yields a preparation with a specific activity 55,000 times greater than in hemolsate. The chromatographic behavior of the enzyme on the Sephadex column is shown in Fig. 2. The overall yield is 15% and the enzyme is estimated by sodium dodecyl sulfate gels to be at least 97% pure.

Polyacrylamide gel electrophoresis of the purified enzyme at pH 9.5, pH 8.0, and pH 3.8 yields a single Coomassie blue-staining band (data not shown). Electrophoresis of adenine phosphoribosyltransferase at pH 9.5 on 15%, 12.5%, 10%, and 7.5% polyacrylamide gels also demonstrates only a single Coomassie blue-staining band (Fig. 3). The isoelectric point of the purified enzyme is 4.55.

Subunit Molecular Weight—When the purified enzyme (Step 6) is denatured with sodium dodecyl sulfate and mercaptoethanol and then subjected to sodium dodecyl sulfate gel electrophoresis (14), a single Coomassie blue-staining band is observed (Fig. 4). By comparing the migration of this band to proteins of known subunit molecular weight, the subunit molecular weight of adenine phosphoribosyltransferase appears to be 18,400 ± 1,700 (mean ± S.D. of 19 determinations) as shown in Fig. 5.

We have also purified the enzyme by the method reported previously to yield a subunit molecular weight of 11,000 (6). Sodium dodecyl sulfate gels of the enzyme preparation following the final purification step reveals one major Coomassie blue-staining band which also corresponds to a subunit molecular weight of 18,000. Periodic acid-Schiff staining (15) of a sodium dodecyl sulfate gel containing 50 µg of the 55,000-fold purified adenine phosphoribosyltransferase did not indicate significant staining above the control.

We have also estimated the subunit molecular weight of the enzyme by gel filtration in the presence of 6 M guanidine hydrochloride. Adenine phosphoribosyltransferase elutes in front of hemoglobin at a position corresponding to a subunit molecular weight of 17,300 (Fig. 6).

Amino Acid Analysis—The amino acid composition of purified adenine phosphoribosyltransferase is shown in Table II. The enzyme contains 33% hydrophobic amino acid residues (leucine, valine, isoleucine, phenylalanine, methionine), which is somewhat greater than the average value of 23.9% for most proteins (29). The minimum molecular weight of the enzyme

* This work was supported by National Institutes of Health Grant 5 R01 AM 19045. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviation used is: PP-ribose-P, 5-phosphoribosyl-1-pyrophosphate.

2 Portions of this paper (including Figs. 1 to 10 and Tables I and II) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full-size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 78M-1581, cite author(s), and include a check or money order for $3.15 per set of photocopies.

3 J. Holden, unpublished observations.

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calculated from these data is 20,800. The data also yield a partial specific volume of 0.75 cm$^3$/g (30).

Native Molecular Weight—The $s_{20}$ value of the purified enzyme is 3.32 S as shown in Fig. 7. This represents the average of three determinations (3.30, 3.45, 3.20) and agrees well with the value of 3.35 S previously reported (6). The Stokes radius of the purified enzyme is 26.0 Å. This value represents the average of three determinations (25.2, 24.4, 28.5), and agrees well with the value of 24.9 Å, as previously reported (6). Using the sedimentation coefficient, Stokes radius, and partial specific volume, we have estimated the native molecular weight of the enzyme to be 39,000 and its frictional ratio to be 1.16 (22).

Sedimentation equilibrium centrifugation at 14,000 rpm and at 22,000 rpm yields a linear graph of log $C$ versus $r^2$ (Fig. 8), with better than 95% recovery of sample. The molecular weight calculated from the sedimentation data is 38,200.

A native molecular weight of 38,000 in conjunction with a subunit molecular weight of about 18,000 suggests that the enzyme is a dimer in the native state. In order to test this hypothesis we have cross-linked the purified enzyme with dimethylsuberimidate. Two protein species are observed when the reaction mixture is analyzed by sodium dodecyl sulfate gel electrophoresis (Fig. 9). These two protein species correspond to the monomer (19,300) and the dimer (46,600). The data provide further evidence that the enzyme exists in its native state as a dimer.

Peptide Mapping—The tryptic peptides of the enzyme have been separated by electrophoresis and subsequent chromatography. The result is shown in Fig. 10. The number of peptide spots observed (about 20) agrees well with the predicted number of 23 as determined from the lysine and arginine content of the enzyme if the subunits are identical.

DISCUSSION

In this report we describe a substantially more efficient method for the purification of adenine phosphoribosyltransferase and expand our observations of this enzyme. The enzyme appears to be homogeneous based on the finding that: (a) it chromatographs with a constant specific activity on a Sephadex G-75 column; (b) it yields a single protein band corresponding to enzyme activity during polyacrylamide gel electrophoresis at pH 9.5, pH 8.0, and pH 3.8; (c) it displays a single band on sodium dodecyl sulfate gels; (d) it migrates as a single band during electrophoresis at pH 9.5 on gels of different per cent of acrylamide; and (e) it appears to be homogeneous in the ultracentrifuge. The native molecular weight of the enzyme is 38,200 as determined by sedimentation equilibrium centrifugation which agrees well with the value of 37,000 reported by Raivio and Seegmiller (31) and of 34,000 from this laboratory (6). The former value of 37,000 was based on gel filtration. The latter value of 34,000 was calculated from gel filtration and sedimentation velocity and assumed a $\bar{v}$ of 0.725 cm$^3$/g. Based on the actual $\bar{v}$, 0.75 cm$^3$/g, obtained now that the amino acid composition is known, the data of Thomas et al. (6) give a native molecular weight of 38,000 rather than 34,000 for adenine phosphoribosyltransferase. The subunit molecular weight of the enzyme determined from sodium dodecyl sulfate gel electrophoresis is 18,000; that from gel chromatography in guanidine hydrochloride 17,000; and that estimated from the amino acid composition 21,000. These studies would suggest that the enzyme in its native state exists as a dimer. Further evidence for a native dimeric structure is provided by cross-linking the enzyme with dimethylsuberimidate; this resulted in two protein species corresponding to the monomer and dimer. A dimeric structure for adenine phosphoribosyltransferase is also in accord with conclusions reached from in vitro (32) and in vivo (33) enzyme hybridization studies reported by other investigators. Peptide mapping of adenine phosphoribosyltransferase suggests that the subunits are identical.

A previous report suggested that the enzyme had a subunit molecular weight of 11,000 leading to the hypothesis that the native enzyme existed as a trimer (6). The final specific activity of the enzyme preparation used in that study was 5.08 units/mg with a final recovery of 2.7% as compared to the enzyme obtained from the present purification which has a specific activity of 22 units/mg with a 15% recovery. The differences in the specific activity appear to reflect differences in recovery of enzyme activity. It was not clear why the two different methods of purification should yield protein species of apparently different subunit molecular weight. We therefore purified the enzyme again by the former method. The highly purified enzyme obtained from purification on this occasion yielded a single protein band on sodium dodecyl sulfate gel electrophoresis corresponding to a molecular weight of 18,000.

Acknowledgments—We thank Dr. Harry Winter for his help with the amino acid analysis and Darrel McCaslin for performing the sedimentation equilibrium centrifugations. We also thank Barbara Claflin for her excellent technical assistance.

REFERENCES

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Human Adenine Phosphoribosyltransferase (AHPT) is a key enzyme in the de novo purine biosynthesis pathway. This pathway is responsible for the synthesis of purine nucleotides from ribose-5-phosphate and ammonia. AHPT catalyzes the transfer of the amino group from ammonia to 5-phosphoribosyl-1-pyrophosphate (PRPP), forming adenylosuccinate (AdoSucc). This reaction is the rate-limiting step in the de novo purine biosynthesis pathway.

The enzyme is encoded by the HAMP gene and is located on chromosome 19. AHPT is a membrane-bound enzyme, predominantly found in the cytosol of mammalian cells. It is synthesized as a preproenzyme and is post-translationally modified by the removal of a signal peptide.

The human AHPT gene is composed of 12 exons and spans approximately 15 kilobases of DNA. The protein product of the gene consists of 431 amino acids and has a molecular weight of approximately 47,500 Da.

The activity of AHPT can be assayed by measuring the formation of adenylosuccinate from PRPP and ammonia. The assay typically involves measuring the increase in absorbance at 340 nm due to the formation of NADPH, which is used in the reaction.

Regulation of AHPT activity is primarily governed by feedback inhibition. The enzyme is strongly inhibited by the end products of purine biosynthesis, including guanosine and inosine. This inhibition is mediated through the activation of a specific guanosine triphosphate (GTP)-dependent regulatory protein, which in turn inhibits the catalytic activity of AHPT.

High concentrations of GTP or guanosine 5'-diphosphate (GDP) reversibly inhibit AHPT, whereas low concentrations of these nucleotides activate the enzyme. This feedback inhibition mechanism allows the cell to regulate the production of purine nucleotides in response to the availability of these end products.

AHPT has been found to be expressed in a variety of tissues, including liver, kidney, and bone marrow. However, the highest levels of expression are found in the liver, where it plays a critical role in the synthesis of purine nucleotides for nucleic acid synthesis.

In summary, AHPT is a critical enzyme in the de novo purine biosynthesis pathway, playing a key role in regulating the synthesis of purine nucleotides and maintaining the balance of purine metabolism in the cell.
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![Figure 1: Affinity chromatography of adenine phosphoribosyltransferase. Column from step 1 was chromatographed on a DEAE-Sephadex A-50 column as described in Experimental Procedures. Fractions were assayed for adenine phosphoribosyltransferase (APRT) and inorganic pyrophosphatase (IPP). Protein was estimated by the method of Lowry et al. (1951).](http://www.jbc.org/)

![Figure 2: Ion-exchange chromatography of adenine phosphoribosyltransferase. Column from step 1 was chromatographed on a Butyl-20 column as described in Experimental Procedures. Fractions were assayed for adenine phosphoribosyltransferase (APRT) and inorganic pyrophosphatase (IPP). Protein was estimated by the method of Lowry et al. (1951).](http://www.jbc.org/)

![Figure 3: Electrophoresis of purified adenine phosphoribosyltransferase (APRT) in gels of different buffer systems. (A) 5% acrylamide gel, (B) 5% polyacrylamide gel, (C) 5% polyacrylamide gel, (D) 5% polyacrylamide gel.](http://www.jbc.org/)

![Figure 4: Selective molecular weight of purified adenine phosphoribosyltransferase. (A) Electrophoresis of purified adenine phosphoribosyltransferase in the presence of sodium dodecyl sulfate and glycerol in 10% gels according to Weber and Osborn (1969).](http://www.jbc.org/)

![Figure 5: Selective molecular weight of purified adenine phosphoribosyltransferase. (B) Electrophoresis of purified adenine phosphoribosyltransferase in the presence of sodium dodecyl sulfate and glycerol in 10% gels according to Weber and Osborn (1969).](http://www.jbc.org/)
Figure 1. Sedimentation equilibrium centrifugation of adenine phosphoribosyltransferase. Sedimentation equilibrium centrifugation of the purified enzyme (Fig. 1) was performed at 34,000 rpm (A) and 25,000 rpm (B).

Figure 2. Polyacrylamide gel of adenine phosphoribosyltransferase. Purified enzyme (Fig. 2) was electrophoresed and stained as described in Experimental Procedure. Unstained gels are shown in A and stained gel is shown in B. The wells lie at the bottom of the gel and migration is from top to bottom.

Figure 3. Chromatography.

Figure 4. Chromatography.

Figure 5. Chromatography.

Figure 6. Chromatography.

Figure 7. Chromatography.
Human adenine phosphoribosyltransferase. Affinity purification, subunit structure, amino acid composition, and peptide mapping.
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J. Biol. Chem. 1979, 254:6951-6955.

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