Identification of Mammalian Aminotransferases Utilizing Glyoxylate or Pyruvate as Amino Acceptor

PEROXISOMAL AND MITOCHONDRIAL ASPARAGINE AMINOTRANSFERASE*

(Received for publication, April 14, 1987)

Tomoo Noguchi and Satoko Fujiwara

From the Department of Biochemistry, Kyushu Dental College, Kokura, Kitakyushu 803, Japan

The subcellular distribution of asparagine:oxo-acid aminotransferase (EC 2.6.1.14) in rat liver was examined by centrifugation in a sucrose density gradient. About 30% of the homogenate activity after the removal of the nuclear fraction was recovered in the peroxisomes, about 56% in the mitochondria, and the remainder in the soluble fraction from broken peroxisomes. The mitochondrial asparagine aminotransferase had identical immunological properties with the peroxisomal one. Glucagon injection to rats resulted in the increase of its activity in the mitochondria but not in the peroxisomes. Immunological evidence was obtained that the enzyme was identical with alanine:glyoxylate aminotransferase 1 (EC 2.6.1.44) which had been reported to be identical with serine:pyruvate aminotransferase (EC 2.6.1.51) (Noguchi, T. (1987) in Peroxisomes in Biology and Medicine (Fahimi, H. D., and Sies, H., eds) pp. 234-243, Springer-Verlag, Heidelberg). The same results as described above were obtained with mouse liver. All of alanine:glyoxylate aminotransferase 1 in livers of mammals other than rodents, which cross-react with the antibody against rat liver alanine:glyoxylate aminotransferase 1, had no asparagine aminotransferase activity.

Various aminotransferases utilizing pyruvate or glyoxylate as amino acceptor but not 2-oxoglutarate are present in mammalian liver. However, the wide substrate specificity of these several aminotransferases has led to much confusion (1-4). The rat liver aminotransferases, AlaATg, SerATp, phenylalanine:pyruvate aminotransferase (EC 2.6.1.44), glycine:2-oxoglutarate aminotransferase (EC 2.6.1.4), serine:pyruvate aminotransferase (SerATp, EC 2.6.1.51) (2, 4), phenylalanine:pyruvate aminotransferase (EC 2.6.1.58) (1), histidine:pyruvate aminotransferase (EC 2.6.1.58) (1), glutamine:pyruvate aminotransferase (EC 2.6.1.15), and asparagine:oxo-acid aminotransferase (AsnAT, EC 2.6.1.14) (1, 3). They have been examined by various investigators in terms of their substrate specificity and designated accordingly (5, 6). The rat liver aminotransferases, AlaATg, SerATp, phenylalanine:pyruvate aminotransferase, and histidine:pyruvate aminotransferase, are induced by the injection in vivo of glucagon, possibly by a cyclic AMP-dependent mechanism (5). It has been proposed that these four aminotransferases are the same enzyme, designated AlaATg 1 (4). Two forms of AlaATg (AlaATg 1 and AlaATg 2) are present in rat liver (4). AlaATg 1 has two identical subunits of about 40,000 Da and is located both in the peroxisomes and in the mitochondria. AlaATg 2 is composed of four identical subunits of about 50,000 Da and is located only in the mitochondria.

In plant leaves, there are also many aminotransferases utilizing glyoxylate or pyruvate: serine:glyoxylate aminotransferase (EC 2.6.1.45), glycine:2-oxoglutarate aminotransferase (EC 2.6.1.4), serine:pyruvate aminotransferase (SerATp, EC 2.6.1.51), AlaATg (EC 2.6.1.44) (7, 8), tryptophan:glyoxylate aminotransferase (9), and AsnAT (EC 2.6.1.14) (10). Glutamate:glyoxylate aminotransferase is identical to alanine aminotransferase (EC 2.6.1.2) (11, 12). Serine:glyoxylate aminotransferase is identical to SerATp (1, 3), AsnAT (13), and tryptophan:glyoxylate aminotransferase (9). Both glutamate:glyoxylate aminotransferase and serine:glyoxylate aminotransferase have AlaATg activities (9, 11, 12) and are located in the peroxisomes of plants (7, 9, 11, 12).

In mammals, AsnAT has been purified and characterized from rat liver by Cooper (14) and from mouse liver by Maul et al. (15, 16). This enzyme has been examined in relation to possible anti-tumor effect (17) of asparaginase (EC 3.5.1.1), and it has been postulated that the synthesis of glycine by AsnAT may be crucial in the sensitivity of certain specific tumor cells to treatment by asparaginase (18). However, the substrate specificity of AsnAT from rat and mouse liver is similar to that of AlaATg 1, and rat liver mitochondrial AsnAT was reported to be identical with phenylalanine:pyruvate aminotransferase (20), suggesting the identity of AsnAT with AlaATg 1 (4, 14, 19). In the present study, we attempted to clarify the classification of various aminotransferases utilizing pyruvate or glyoxylate as amino acceptor in mammalian liver.

EXPERIMENTAL PROCEDURES

Enzyme Assays—AsnAT activity was assayed in the presence of glycyglycine to inhibit ω-amidase activity and prevent the degradation of α-ketosuccinamate by the method of Cooper (1). The formation of α-ketosuccinamate was determined by the increase of the absorbance at 290 nm. The assay mixture contained 40 mM L-asparagine, 2 mM glyoxylate, 5 mM EDTA, 200 mM glycylglycine, pH 8.4, and enzyme in a final volume of 0.1 ml. AlaATg, SerATp, phenylalanine:pyruvate aminotransferase and histidine:pyruvate aminotransferase were assayed as described previously (5). The assay mixture contained 40 mM L-amino acid, 2 mM glyoxylate or 20 mM pyruvate, 0.1 M potassium phosphate buffer, pH 8.2, and enzyme preparation in a total volume of 0.4 ml. Glutamine:pyruvate aminotransferase activity was determined as described by Cooper and Meister (21) and glutamine:phenylpyruvate aminotransferase activity as described by Cooper (22). The former method is based on the
determination of glycine and the latter method on the decrease in absorbance at 300 nm of the complex formed between borate and the enol form of the α-keto acid. Catalase (EC 1.11.1.6), glutamate dehydrogenase (EC 1.4.1.3), and acid phosphatase (EC 3.1.3.2) were assayed as described previously (5). A unit of enzyme activity is defined as the amount of enzyme that catalyzes a formation of product or a decrease in substrate of 1 pmol/min at 37°C.

Sucrose Density Gradient Centrifugation of Liver Homogenate with a Vertical Rotor—All procedures were performed at 0-4 °C. Livers were cut into small pieces and homogenized by one excursion in 4 volumes of 0.25 M sucrose in 25 mM glycyglycine, pH 7.5, in a Potter-Elvehjem homogenizer with a Teflon pestle at 500 rpm. The homogenate was filtered through two layers of cheesecloth and centrifuged at 300 × g for 5 min to sediment nuclei and any whole cells. A portion (3 ml) of the resulting postnuclear homogenate was subjected to sucrose density gradient centrifugation with a vertical rotor as described previously (23). Fractions (about 2 ml) were collected from the bottom of the gradient.

Preparation of the Mitochondrial and Peroxisomal Extracts from Rat Liver—All procedures were carried out at 0-4 °C. The homogenates were prepared from rat liver injected intraperitoneally with Triton WR-1339 as described previously (24) and subjected to sucrose density gradient centrifugation as described above. After centrifugation, mitochondrial fractions without peroxisomal catalase and lysosomal acid phosphatase activities were diluted with 4 volumes of 20 mM glycyglycine, pH 7.5, and centrifuged at 20,000 × g for 30 min. The resulting mitochondrial fraction was dissolved in 5 mM potassium phosphate buffer, pH 7.5, and sonicated at 10 kHz for 30 s. After centrifugation at 100,000 × g for 60 min, the resulting supernatant was used as the mitochondrial extract.

Peroxisomes were prepared from the light mitochondrial fractions of rat liver by sucrose density gradient centrifugation according to the method of Osmundsen et al. (25), dissolved in 5 mM potassium phosphate buffer, pH 7.5, and sonicated at 10 kHz for 30 s. After centrifugation at 100,000 × g for 60 min, the resulting supernatant was used as the peroxisomal extract.

Immunotitration of Asparagine Aminotransferase (AsnAT) Activity with the Antibody against Rat Liver Mitochondrial Alanine-Glyoxylate Aminotransferase 1 (AlaATg 1)—The antiserum against rat liver mitochondrial AlaATg 1 was prepared as described previously (6). This antiserum and the serum from control rabbit were added separately to (NH₄)₂SO₄ fractionation and DEAE-cellulose column chromatography to prepare IgG fractions. These IgG fractions were used for the immunotitration. Purified AsnAT was incubated with control IgG fraction or the antibody (IgG) against rat liver mitochondrial AlaATg 1 in 50 mM potassium phosphate buffer, pH 7.5, at 37°C for 30 min. Aliquots of the reaction mixture were used for the determination of AsnAT, SerATp, and AlaATg activities.

Other Methods—Ouchterlony double diffusion analysis, isoelectric focusing, and determination of protein were carried out as described previously (26, 27).

RESULTS

Sucrose Density Gradient Centrifugation of Liver Homogenate—Fig. 1, A and B, shows the representative sedimentation profiles of postnuclear homogenates from control and glucagon-injected rat liver in a sucrose density. Three peaks of AsnAT activity were obtained in control rat liver (Fig. 1A). One coincided with catalase activity as the peroxisomal marker and the second with glutamate dehydrogenase as the mitochondrial marker. The third peak was in the soluble top fraction. AsnAT activity showed nearly identical sedimentation profile with SerATp activity; SerATp had been reported to be identical with AlaATg 1 (4). About 30% of the homogenate activity was recovered in the peroxisomal fraction, about 56% in the mitochondrial fraction, and the remaining in the soluble top fraction. The similar ratio for AsnAT and SerATp to catalase in the peroxisomes and in the soluble fraction suggests that AsnAT and SerATp in the soluble fraction are from broken peroxisomes.

The homogenate was prepared from livers of rats pretreated with Triton WR-1339 to ensure separation of lysosomes from peroxisomes and subjected to sucrose density gradient centrifugation because considerable overlapping occurs between the density distribution of the two types of organelles in normal liver; AsnAT and SerATp activities showed different distribution profiles from acid phosphatase activity as the lysosomal marker (not shown).

Compared with control rats, liver from glucagon-injected rats showed virtually identical increases (about 7.5-fold) in AsnAT and SerATp activities of the mitochondria (Fig. 1B). In contrast, the AsnAT and SerATp activities in the peroxisomes and in the soluble fraction were not affected by glucagon injection. This result indicates that only the mitochondrial enzymes are induced by glucagon injection and that most of the two activities in the soluble fraction are from broken peroxisomes but not from broken mitochondria.

Isoelectric Focusing of Peroxisomal and Mitochondrial Extracts—Fig. 2, A-C, shows isoelectric focusing profiles of the peroxisomal and mitochondrial extracts from control rat liver and the mitochondrial extract from glucagon-injected rat liver, respectively; AsnAT, SerATp, AlaATg 1, and glutamine:oxo-acid aminotransferase activities were assayed. With the peroxisomal extract from control rat liver, a single peak of pl 8.0 with these four aminotransferase activities coincident was obtained. These four activities may be from a single enzyme previously designated AlaATg 1 (4). With the mitochondrial extract of control rat liver, three activity peaks were obtained. One is AlaATg 1 of pl 8.0 with the four enzyme activities coincident, and the second is AlaATg 2 of pl 6.2
Identification of Mammalian Asparagine Aminotransferase

was identical with that of glutamine-phenylpyruvate aminotransferase activity and differed from that of glutamine:glyoxylate aminotransferase activity with pH 5.4. On the basis of these data and reports from Cooper and Meister (1, 4) that phenylpyruvate is a relatively poor substrate for glutamine:oxo-acid aminotransferase L, whereas phenylpyruvate is a good substrate, it is concluded that most of histidine:pyruvate aminotransferase 2 and phenylalanine:pyruvate aminotransferase 2 activities are from glutamine:oxo-acid aminotransferase K.

**Copurification of Asparagine Aminotransferase (AsnAT) with Alanine:Glyoxylate Aminotransferase 1 (AlaATg 1) from the Mitochondria and Peroxisomes**—Table I shows the result of the purification of AsnAT from the mitochondrial extract of glucagon-injected rat liver. AsnAT was copurified with AlaATg 1 (SerATp) to homogeneity as described previously (30). The activity ratio of AsnAT to SerATp remained constant during the purification from the mitochondrial extract. The activity ratio of AsnAT to AlaATg remained constant after the removal of AlaATg 2 by DEAE-cellulose column chromatography. Table II shows the result of the purification of AsnAT from the peroxisomal fraction of rat liver. Both the activity ratios (AsnAT/SerATp and AsnAT/AlaATg) remained constant during purification from the peroxisomal fraction because the peroxisomes contain only AlaATg 1 but not AlaATg 2.

On polyacrylamide disc gel electrophoresis at pH 8.9 in 7% gel, each purified enzyme preparation from both the mitochondria and peroxisomes migrated toward the anode as a single protein band, which coincided with AsnAT, SerATp, and with only AlaATg activity previously designated AlaATg 2 (4). The third enzyme peak of pI 5.4 may be glutamine:oxo-acid aminotransferase L, because glutamine:oxo-acid aminotransferase K has much lower activity than glutamine:oxo-acid aminotransferase L for glutamine:glyoxylate aminotransferase; two forms of glutamine aminotransferase (glutamine:oxo-acid aminotransferase L and glutamine:oxo-acid aminotransferase K) have been described in rat liver by Cooper and Meister (22). AlaATg 1 from rat liver after glucagon injection showed virtually identical percentage increases (about 8-fold) in these four enzyme activities, because these four activities are properties of AlaATg 1. However, AlaATg 2 and glutamine:oxo-acid aminotransferase L were not affected by glucagon.

Two activity peaks with histidine:pyruvate aminotransferase and phenylalanine:pyruvate aminotransferase activities coincident were obtained on isoelectric focusing of the mitochondrial extract from control rat liver (not shown). The enzyme with a higher pI (pH 8.0), previously designated histidine:pyruvate aminotransferase 1 or phenylalanine:pyruvate aminotransferase 1, showed an identical focusing profile with AlaATg 1; AlaATg 1 has been reported to be identical with histidine:pyruvate aminotransferase 1 and phenylalanine:pyruvate aminotransferase 1 (4–6, 28, 29). In contrast, the focusing profile of the enzyme with a lower pI (pH 5.15), previously designated histidine:pyruvate aminotransferase 2 or phenylalanine:pyruvate aminotransferase 2,
and AlaATg activities. With each enzyme preparation, Sephadex G-150 gel filtration and sedimentation in a sucrose density gradient for molecular weight determination (28) showed a single peak coincident with protein and enzyme activities.

**Immunological Identification of Asparagine Aminotransferase (AsnAT) with Alanine-Glyoxylate Aminotransferase 1 (AlaATg 1)**—Identification of AsnAT with AlaATg 1 in the peroxisomes and mitochondria of rat liver was also examined by immunotitration with antibody against rat liver mitochondrial AlaATg 1. The results are given in Fig. 3, A and B. Addition of antibody resulted in equivalent losses of AlaATg, AsnAT, and SerATp activities with AsnAT preparation purified from both the peroxisomes (Fig. 3A) and mitochondria (Fig. 3B) of rat liver.

By Ouchterlony double diffusion analysis (Fig. 4), the antibody against rat liver mitochondrial AlaATg 1 yielded a single connecting precipitin among the mitochondrial extract from glucagon-injected rat liver, the peroxisomal extracts from control rat liver, and purified peroxisomal and mitochondrial AsnAT preparations.

**Hepatic Asparagine Aminotransferase (AsnAT) from Different Mammalian Species**—AsnAT was also found to be located both in the peroxisomes and in the mitochondria of mouse liver by the same method as described above. The injection of glucagon in mice resulted in an increase of the mitochondrial AsnAT activity but not of peroxisomal activity. Mouse liver AlaATg 1 has been reported to have similar substrate specificity to rat liver AlaATg 1 (4, 30). AsnAT was also found to be identical with AlaATg 1 in mouse liver by the same experimental procedures as those for rat liver AlaATg 1.

AlaATg 1 which cross-reacts with the antibody against rat liver mitochondrial AlaATg 1 has been reported also to be present in livers of human, monkey, rabbit, pig, cat, and dog (4, 31, 32); AlaATg 1 from these mammalian species is highly specific for L-alanine with glyoxylate as amino acceptor and specific for L-serine with pyruvate. Each of the non-rodent AlaATg 1 enzymes has little or no AsnAT activity.

**DISCUSSION**

We have classified the various aminotransferases utilizing pyruvate or glyoxylate as amino acceptor other than 2-oxoglutarate. In mammalian liver, the broad substrate specificity of several pyruvate (glyoxylate) aminotransferases had led to much confusion (1–4). It has been reported that rat liver contains several pyruvate (glyoxylate) aminotransferases such as AsnAT, SerATp, AlaATg 1, AlaATg 2, histidine:pyruvate aminotransferase 1, histidine:pyruvate aminotransferase 2, phenylalanine:pyruvate aminotransferase 1, phenylalanine:pyruvate aminotransferase 2, glutamine:oxo-acid aminotransferase K, glutamine:oxo-acid aminotransferase L, aromatic amino acid:glyoxylate aminotransferase (EC 2.6.1.60), and glutamate:glyoxylate aminotransferase (1–4). They can be put into five groups. 1) AlaATg 1 is identical with SerATp, histidine:pyruvate aminotransferase 1, and phenylalanine:pyruvate aminotransferase 1 and is located both in the mitochondria and in the peroxisomes. AlaATg 1 in both organelles has identical physical, enzymatic, and immunological properties and shows very broad substrate specificity. Effective L-amino acids are alanine, serine, glutamine, methionine, asparagine, leucine, phenylalanine, tyrosine, and histidine. Effective amino acceptors are glyoxylate, pyruvate, and phenylpyruvate, and 2-oxoglutarate is inactive (4, 6, 30). 2) AlaATg 2 is specific for L-alanine and glyoxylate and is located only in the mitochondria. AlaATg 1 and 2 have been purified and characterized in detail from rat and mouse liver (4). 3) Both glutamine:oxo-acid aminotransferase K and glutamine:oxo-acid aminotransferase L show broad substrate specificity and are located in the mitochondria and in the cytosol. The two enzymes from both organelles have been purified and characterized in detail from rat liver (1, 3, 14, 21, 22). 4) Aromatic amino acid:glyoxylate aminotransferase is identical with AlaATg 1 (4, 28, 33). 5) Glutamate:glyoxylate aminotransferase is identical with cytosolic alanine aminotransferase (EC 2.6.1.2) and the specific glutamate:glyoxylate aminotransferase is not present in rat and mouse liver (34).

In the present study, AsnAT was found to be identical with AlaATg 1 in rat liver by the following evidence. 1) AsnAT had the same subcellular localization as AlaATg 1; it was located both in the mitochondria and in the peroxisomes. 2) Only the mitochondrial AsnAT activity was increased by the injection of glucagon to rats, but the peroxisomal one was not. The mitochondrial AsnAT and AlaATg 1 activities showed nearly identical percentage increases after glucagon injection. 3) The activity ratio, AsnAT/AlaATg 1 (SerATp), remained constant throughout the purification of AsnAT from both the mitochondrial and peroxisomal fractions. 4) Antibody against rat liver mitochondrial AlaATg 1 yielded equivalent losses of AsnAT and AlaATg 1 activities with AsnAT preparations from both the mitochondria and the peroxisomes. 5) The activity ratio was unchanged by a variety of treatments of...
both the two purified enzyme preparations. The same results were obtained with mouse liver.

On the other hand, mammalian liver contains two forms of AlaATg, designated AlaATg 1 and AlaATg 2, respectively. All of AlaATg 1 from mammalian liver consists of two identical subunits with a molecular weight of about 40,000 and is immunologically cross-reactive. However, intracellular organelles containing hepatic AlaATg 1 and its substrate specificity vary from species to species: it is located in the peroxisomes in human, monkey, rabbit, and guinea pig, in the mitochondria in dog and cat, and in both organelles in rodents (rat, mouse, and hamster). Recently, Maul et al. (15) reported that AsnAT is located both in the mitochondria and in the cytosol but not in the peroxisomes in mouse liver. However, the localization of AlaATg 1 in the peroxisomes and in the mitochondria in rodent liver has also been confirmed by the immunohistochemical method with protein A-gold (35). On the other hand, AlaATg 1 from rodents (rat, mouse, and hamster) catalyzes transamination between various L-amino acids and pyruvate or glyoxylate (4). In contrast, AlaATg 1 from other mammalian species is highly specific for L-alanine with glyoxylate as amino acceptor and for serine with pyruvate and has no AsnAT activity (4). However, the low activity of AsnAT was detected in beef, guinea pig, and pigeon liver by Maul et al. (15). This report suggests that specific AsnAT or other aminotransferases with AsnAT activity are present in mammals and birds other than rodents.

It is unknown whether AlaATg 1 functions as AsnAT in vivo in rodent tissues. Recently, Cooper et al. (36) found that α-keto-succinamate, a transamination product of asparagine, occurs in rat liver and, to a lesser extent, in rat kidney, suggesting that the transamination of asparagine by AlaATg 1 occurs in rat tissue in vivo.

REFERENCES