Cloning and Functional Expression of a Soluble Form of Kynurenine/α-Aminoadipate Aminotransferase from Rat Kidney*

(Received for publication, August 18, 1995)

Rico Buchli, Daniela Alberati-Gianiti, Pari Malherbe, Christer Köhler, Clemens Broger§, and Andrea M. Cesura¶

From the Pharma Division, Preclinical Research, Nervous System Diseases, and §Computational Chemistry Department, F. Hoffmann-La Roche Ltd., CH-4002 Basel, Switzerland

Several aminotransferases with kynurenine aminotransferase (KAT) activity are able to convert L-kynurenine into kynurenic acid, a putative endogenous modulator of glutamatergic neurotransmission. In the rat, one of the described KAT isoforms has been found to correspond to glutamine transaminase K. In addition, rat kidney α-aminoadipate aminotransferase (AadAT) also shows KAT activity. In this report, we describe the isolation of a cDNA clone encoding the soluble form of this aminotransferase isoenzyme from rat (KAT/AadAT). Degenerate oligonucleotides were designed from the amino acid sequences of rat kidney KAT/AadAT tryptic peptides for use as primers for reverse transcription-polymerase chain reaction of rat kidney RNA. The resulting polymerase chain reaction fragment was used to screen a rat kidney cDNA library and to isolate a cDNA clone encoding KAT/AadAT. Analysis of the combined DNA sequences indicated the presence of a single 1275-base pair open reading frame coding for a soluble protein of 425 amino acid residues. KAT/AadAT appears to be structurally homologous to aspartate aminotransferase (AAT, EC 2.6.1.39), an enzyme involved in the metabolism of lysine (1–7) that catalyzes the reversible transamination reaction between L-2-amino adipate and 2-oxoglutarate to produce 2-oxoadipate and L-glutamate.

Due to the postulated role of kynurenic acid as a putative endogenous modulator of glutamatergic neurotransmission (for review, see Ref. 10), particular attention has recently been devoted to the presence of KAT isoenzymes in cerebral tissues. Kynurenic acid is, in fact, an antagonist at the glycine site of N-methyl-D-aspartate receptors, and increased levels of kynurenic acid may exert a neuroprotective action in some pathological conditions (11). Two different aminotransferases able to produce kynurenic acid appear to be present in human brain (12–14). A cDNA clone from rat brain encoding an aminotransferase with KAT activity has also been described. This protein corresponds to α-aminoadipate aminotransferase (AadAT, EC 2.6.1.39), also referred to as glutamine transaminase K (GTK, EC 2.6.1.64) (5). The human form of this aminotransferase with KAT, GTK, and β-lyase activity has also been cloned and shown to have 82% amino acid similarity to the rat protein (6).

In rat kidney, a 2-oxoglutarate-prefering aminotransferase with KAT activity has also been described. This protein corresponds to α-aminoadipate aminotransferase (AadAT, EC 2.6.1.39), an enzyme involved in the metabolism of lysine (1–7) that catalyzes the reversible transamination reaction between L-2-amino adipate and 2-oxoglutarate to produce 2-oxoadipate and L-glutamate.

Kynurenine aminotransferase (EC 2.6.1.7, KAT) catalyzes the irreversible transamination of the L-triptophan metabolite L-kynurenine to form kynurenic acid. Due to an overlapping substrate specificity, multiple forms of pyridoxal 5'-phosphate (PLP)-dependent aminotransferases are apparently able to catalyze this reaction (1). Differences probably exist in the various tissues and among different animal species regarding which enzyme form is predominantly responsible for the biosynthesis of kynurenic acid. A soluble form of KAT has recently been identified and cloned from rat (2, 3), and it was found that the amino acid sequence of this pyruvate-prefering form of KAT is identical to that reported for rat kidney cysteine S-conjugate β-lyase (4), also referred to as glutamine transaminase K (GTK, EC 2.6.1.64) (5). The human form of this aminotransferase with KAT, GTK, and β-lyase activity has also been cloned and shown to have 82% amino acid similarity to the rat protein (6).

In the present work, we describe the molecular cloning and the functional expression of a soluble aminotransferase from rat kidney displaying both KAT and AadAT activity (KAT/AadAT).

MATERIALS AND METHODS

Enzymatic Activity Determination—KAT activity was assayed as described previously (3). For routine analysis, aliquots of the enzyme preparation were incubated (1 h at 37 °C) in the presence of 1 mM...
Cloning of Rat Kynureninase-α-Aminoacidipate Aminotransferase

KAT/AadAT was assayed as measured under “Materials and Methods.” The concentration of l-kynurenine and 2-oxoglutamate was 1 mM.

| Total protein | Total activity | Specific activity |
|---------------|----------------|------------------|
| mg | nmol min⁻¹ | nmol min⁻¹ mg⁻¹ |
| Homogenate supernatant | 2100 | 29590 | 12.4 |
| DEAE-Sephrose | 150 | 5847 | 39.0 |
| Phenyl-Sephrose | 65 | 3170 | 48.8 |
| Mono Q | 5.3 | 1536 | 295 |
| Mono P | 0.11 | 172 | 1563 |

RESULTS AND DISCUSSION

Purification of Rat Kidney KAT/AadAT and Internal Peptide Sequencing—KAT/AadAT was purified from rat kidney following the procedure described in Table 1. The purification of the enzyme was monitored by measuring KAT activity in the presence of 2-oxoglutamate, its preferred aminoaceptor. The purified enzyme also displayed AadAT activity with a specific activity of 9.4 μmol min⁻¹ mg⁻¹ protein⁻¹. After chromatography on DEAE-Sephrose, this aminotransferase form was well separated from KAT/GTK, a distinct enzyme with KAT activity and preference for pyruvate as cosubstrate (Fig. 1). In accordance with previous reports on rat kidney KAT isoforms (9, 26), FPLC chromatofocusing indicated pI values of approximately 3.9 and 4.0 for KAT/AadAT and KAT/GTK, respectively (see also Ref. 3). The native molecular mass of KAT/AadAT was in...
the 90–100-kDa range (as determined by gel filtration on a Pharmacia Superose 12 FPLC column) and was composed of two subunits of equal size. In fact, SDS-polyacrylamide gel electrophoresis analysis of the purified protein under reducing conditions showed a single major band of ~45 kDa (not shown).

To obtain information on the amino acid sequence of KAT/AadAT, the polyvinylidene difluoride-blotted protein was directly submitted to Edman degradation to see if its NH2 terminus was accessible to sequencing. No sequence information could be obtained, indicating that the NH2 terminus of the protein was modified either as the result of a posttranslational event or due to protein handling. The amino acid sequences of internal peptides were then obtained after digestion with trypsin and separation of the resulting peptides by reverse-phase HPLC. The sequence of six tryptic peptides (denominated T2a, T2b, T3, T6, T10, and T12, see Figs. 2 and 3) was determined. No significant matches were found in the Swiss-Prot and Protein Identification Resource Protein data banks.

Isolation of Rat Kidney KAT/AadAT cDNA—Using the amino acid sequences of the analyzed tryptic peptides of purified rat kidney KAT/AadAT, we were able to obtain the corresponding cDNA using RT-PCR. Two amino acid sequences from the least degenerate regions of peptides T3 and T10 were selected to construct synthetic degenerate oligonucleotides. Because the relative order of the two tryptic peptides within the protein was not known, degenerate sense and antisense oligonucleotides corresponding to the tryptic peptide sequences were synthesized (Fig. 2, panels A and B). Poly(A)+ RNA extracted from rat kidney was reverse-transcribed using each degenerate antisense oligonucleotide as a primer, and the resulting cDNA was used as a PCR template with each possible combination of degenerate sense and antisense oligonucleotides from the two tryptic peptides. After 35 rounds of amplification, one distinct PCR product was detected by agarose gel electrophoresis for the primer combination sense-T3/antisense-T10 (Fig. 2C, lane 2). DNA sequence analysis revealed that the 1058-bp fragment contained an open reading frame coding for a polypeptide of 352 amino acids, starting with the codon of the first amino acid phenylalanine (TCT) of peptide T3 and ending with the second nucleotide of the last glutamine codon (CA) of the T10 sequence. Moreover, this cDNA fragment encoded the tryptic peptides T2a, T2b, and T12, confirming that it was indeed a partial cDNA for rat KAT/AadAT.

Molecular Cloning of Rat Kidney KAT/AadAT cDNA—A rat kidney cDNA library (Uni-ZAP™ XR) from Stratagene was screened with the partial cDNA probe of rat kidney KAT/AadAT. Screening of 9 × 10^5 recombinants revealed 10 positive clones with inserts ranging from 950 to 1828 bp. The four longest clones, designated as rkKAT-8, rkKAT-14, rkKAT-3, and rkKAT-9, were chosen for further characterization. DNA sequence analysis showed that all clones were identical, except for two nucleotide differences. Only one of these nucleotide differences was contained within the coding region, and it did not result in a change in amino acid sequence. The nucleotide and deduced amino acid sequences are shown in Fig. 3. Analysis of the combined DNA sequence (nucleotides 1–1828) indicated the presence of a single 1275-bp open reading frame with a predicted initiation codon (ATG) at nucleotide 113 and termination at nucleotide 1388 with the stop codon TAA. This open reading frame codes for a polypeptide of 425 amino acid residues with a predicted molecular mass of 47,789 Da. It is
Cloning of Rat Kynurenine/a-Aminoadipate Aminotransferase

preceded by a 112-nucleotide 5'-untranslated region containing two in-frame stop codons at positions 32 and 71, and a 422-nucleotide untranslated region in the 3' end. The predicted initiation codon (nucleotide 113) is embedded in the sequence GAGACATG, which does not match perfectly with the consen-
sus sequence CCAATG frequently found for eukaryotic translation initiation (27). No further ATG was found upstream of this predicted initiation codon, indicating a complete coding sequence.

Comparison of the complete sequence with the EMBL DNA sequence data base using the Genetics Computer Group sequence analysis software (GCG, University of Wisconsin), indicated that the sequence was unique and had not been isolated previously. Analysis of the hydrophilicity plot of the predicted amino acid sequence for KAT/AadAT showed no evidence for membrane-anchoring or spanning regions, consistent with the soluble nature of the isolated protein. A mitochondrial form of KAT/AadAT has also been described (8, 9). However, no structural features resembling those of leader peptides for mitochondrial import (28) were observed in the predicted amino acid sequence of rat KAT/AadAT. Therefore, whether mitochondrial KAT/AadAT is encoded by a different gene or by a splice variant of the isolated form carrying an additional signal-peptide sequence remains to be established. Four potential N-linked glycosylation sites (Asn, Xaa, Ser/Thr) (29) were found in the predicted amino acid sequence of rat kidney KAT/AadAT at Asn residues 2, 57, 101, and 202. No information, however, is presently available indicating whether the native protein is glycosylated.

KAT/AadAT Sequence Comparison with Other Aminotransferases—Binary alignment of the protein sequences of KAT/AadAT and KAT/GTK (4) using the GAP program contained in GCG showed only 18.4% amino acid identity with 14 gaps located mainly in the N- and C-termini of the two sequences. Considering conservative amino acid substitutions, the two KAT isoforms displayed 46.2% similarity (not shown). Similar degrees of amino acid identity and similarity were observed after comparison with other aminotransferases from different species, including isoenzymes, such as human serine:pyruvate aminotransferase (30), which have been reported to display KAT activity. Sequence homology among aminotransferase isoenzymes is not easily recognizable by standard algorithms for sequence comparison. A multiple sequence alignment of aspartate aminotransferases from various organisms with KAT/AadAT and KAT/GTK showed that several residues in the central region of the sequences are totally conserved, therefore suggesting that the corresponding three-dimensional structures might also be conserved. Despite the low degree of amino acid identity, most aminotransferases appear to constitute a group of structurally homologous proteins that originated from a single universal ancestor protein. The lysine residue forming the aldimine bond with PLP is indicated in bold face type. Note that the Swiss-Prot data base numbering is used for E. coli AAT.

Fig. 3. Nucleotide and predicted amino acid sequence of cloned rat kidney KAT/AadAT. The deduced amino acid sequence of the encoded polypeptide is shown in single-letter code below the nucleotide sequence in bold face type and is numbered beginning with the initiating methionine. Nucleotides are numbered in the 5' to 3' direction. The tryptic peptides (T6, T3, T2a, T2b, T12, T10) isolated from rat kidney KAT/AadAT are boxed. The asterisk denotes the 3'-terminal stop codon. Nucleotide variation between several clones is indicated above the appropriate nucleotide (C→T at nucleotide position 145 (rkKAT-14); C→T at nucleotide position 1784 (rkKAT-8)).

Fig. 4. Sequence alignment of KAT/AadAT and KAT/GTK from rat with E. coli AAT. Asterisks indicate residues conserved among the three sequences. Amino acid identities in KAT/AadAT and KAT/GTK are overlined. The residues involved in cofactor binding (32) are shaded. The lysine residue forming the aldimine bond with PLP is indicated in bold face type. Note that the Swiss-Prot data base numbering is used for E. coli AAT.
Similarities in the NH2- and COOH-terminal regions were too involved in the binding of the cofactor (32) appear to be constrained by the PLP aldehyde group. In addition, several of the AAT residues have been identified as being critical for substrate specificity. The fact that no KAT/AadAT message was detected in the hippocampus might be due to the low mRNA level for this protein in this brain region.

Expression of Recombinant Rat KAT/AadAT in HEK-293 Cells and Characterization of the Enzymatic Activity—To confirm that the isolated cDNA indeed encoded for an aminotransferase with both KAT and AadAT activity, the rat KAT/AadAT cDNA was subcloned into the eukaryotic expression vector pBC/CMV (23). HEK-293 cells were chosen for transfection because they did not exhibit either KAT or AadAT activity. A relatively high expression of both KAT and AadAT activities could be achieved after transient transfection of these cells, with most of the enzymatic activity (>90%) being recovered in the soluble fraction of the cells. No activity was observed after transfection of the cells with the antisense cDNA. Determination of KAT kinetic properties of the recombinant enzyme with 2 mM 2-oxoglutarate as amino acceptor showed Km, and Vmax values for L-kynurenine of 0.95 ± 0.33 mM and 135 ± 31 nmol min⁻¹ mg protein⁻¹, respectively. The enzyme also metabolized L-3-hydroxykynurenine to xanthurenic acid with a similar catalytic efficiency (Kmᵣ = 1.36 ± 0.16 mM; Vmaxᵣ = 166 ± 42 nmol min⁻¹ mg protein⁻¹). In the presence of 5 mM L-kynurenine as amino donor, the Kmᵣ of 2-oxoglutarate was 45.5 ± 12.4 μM. When the enzyme was assayed for AadAT activity, the Kmᵣ for L-glutamate was 5.6 ± 2.8 mM, with a Vmaxᵣ of 910 ± 210 nmol min⁻¹ mg protein⁻¹. Notably, under our experimental conditions, the catalytic efficiency of KAT/AadAT appeared to be similar for both KAT and AadAT activity, as it can be inferred from the similar Vmax/Kmᵣ ratios. The observed kinetic parameters are in accordance with those found for the rat native enzyme (1, 8, 19).

The KAT activity of the enzyme measured in the presence of various cosubstrates was highest with 2-oxoglutarate and lowest with pyruvate (Table II). Regarding the specificity of this aminotransferase form toward other L-amino acids, tryptophan, phenylalanine, tyrosine, aspartate, and alanine were found to be substrates for KAT/AadAT. However, the specific activities observed in the presence of these amino acids (up to 10 mM) were relatively low, being 15% (triptophan), 9% (phenylalanine and tyrosine), and 6% (aspartate and alanine) of the activity measured with L-kynurenine. The enzyme did not display significant enzymatic activity toward the other L-amino acids tested (histidine, serine, methionine, glutamine, and leucine). The specificity pattern toward amino acids and cosubstrates of the KAT/AadAT expressed in HEK-293 cells was similar to that observed for the native enzyme purified from rat kidney.

The availability of the cDNA clone for this aminotransferase isoenzyme with KAT activity will contribute to the further elucidation of kynurenine acid disposition in peripheral organs as well as in the central nervous system. For instance, in rat
brain it appears that at least two distinct aminotransferases with KAT activity are expressed: 1) KAT/AadAT, as described in this paper, and 2) the KAT/GTK form, which was directly isolated from cerebral tissues (3, 15). Several factors may determine the role of the various KAT forms in kynurenine acid biosynthesis, such as the different affinity constants of these enzymes for L-kynurenine and the regional and cellular distribution of these enzymes in the different organs. Interestingly, several biochemical characteristics of the two KAT forms identified in rat, including pl value and cosubstrate preference, are similar to those described for the two cerebral KAT forms in humans (12, 13), therefore raising the possibility that these human KAT forms may be similar to the identified KAT forms from rat.

Although our attention has focused mainly on the role of KAT/AadAT in L-tryptophan metabolism, its function in L-lysine metabolism also merits further investigation, for instance, in disorders of the lysine metabolic pathway. Interestingly, L-2-aminoadipate, the substrate for AadAT, is a well known astroglial-specific toxin (18). Thus, knowledge of the cerebral KAT/AadAT in L-tryptophan metabolism, its function in L-lysine metabolism also merits further investigation, for instance, in disorders of the lysine metabolic pathway. Interestingly, L-2-aminoadipate, the substrate for AadAT, is a well known astroglial-specific toxin (18). Thus, knowledge of the cerebral disposition of this compound is instrumental for the elucidation of its mechanism of toxicity and possible relevance in pathology.

Acknowledgments—We are grateful to the late Prof. Mosè Da Prada, Drs. Grayson Richards and Deborah Hartman for critical reading of the manuscript. Dr. Hans-Werner Lahm and Urs Röthlisberger for sequencing of the peptides, and Gabrielle Lang for skillful technical assistance.

REFERENCES
1. Deshmukh, D. R., and Mungre, S. M. (1989) Biochim. Biophys. Acta 1012, 221–227
2. Mosca, M., Cozzi, L., Breton, J., Speciale, C., Okuno, E., Schwarz, R., and Benatti, L. (1994) FEBS Lett. 335, 21–24
3. Alberati-Giani, D., Malherbe, P., Köhler, C., Lang, G., Kiefer, V., and Cesura, A. M. (1995) J. Neurochem. 64, 1448–1455
4. Perry, S. J., Schofield, M. A., MacFarlane, M., Lock, P. A., King, L. J., Gibson, G. G., and Goldfarb, P. (1995) FEBS Lett. 360, 277–280
5. Takeuchi, F., Otsuka, H., and Shibata, Y. (1983) Biochim. Biophys. Acta 864, 323–330
6. Hartline, R. A. (1985) Methods Enzymol. 133, 664–672
7. Stone, T. W. (1993) Pharmacol. Rev. 45, 309–379
8. Carpenedo, R., Chiarugi, A., Russi, P., Lombardi, G., Carlà, V., Pelliccioni, R., Mottoli, L., and Moretti, F. (1994) Neuroscience 61, 237–244
9. Okuno, E., Nakamura, M., and Schwarz, R. (1991) Brain Res. 542, 307–312
10. Okuno, E., Guidetti, P., Okuno, E., and Schwarz, R. (1993) Neuroscience 55, 177–184
11. Baran, H., Okuno, E., Kido, R., and Schwarz, R. (1994) J. Neurochem. 62, 730–738
12. Malherbe, P., Alberati-Giani, D., Köhler, C., and Cesura, A. M. (1995) FEBS Lett. 367, 141–144
13. Okuno, E., Du, F., Ishikawa, T., Tsujimoto, M., Nakamura, M., Schwarz, R., and Kido, R. (1990) Brain Res. 534, 37–44
14. Malherbe, P., Köhler, C., Da Prada, M., Lang, G., Kiefer, V., and Cesura, A. M. (1994) J. Biol. Chem. 269, 13792–13797
15. Baw, G., Van Damme, J., Puyoo, M., Vandekerckhove, J., Geser, B., Ratz, G. P., Lauridsen, J. B., and Celis, J. E. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 7701–7705
16. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299
17. Okuno, E., Schmid, W., Parks, D. A., Nakamura, M., and Schwarz, R. (1991b) J. Neurochem. 57, 533–540
18. Huck, S., Grass, F., and Hörtmagn, H. (1984) J. Neurosci. 4, 2650–2657
19. Nakatani, Y., Fujioka, M., and Higashino, K. (1970) Biochim. Biophys. Acta 198, 219–228
20. Malherbe, P., Köhler, C., Da Prada, M., Lang, G., Kiefer, V., Schwarz, R., Lahm, H.-W., and Cesura, A. M. (1994) J. Biol. Chem. 269, 13792–13797
21. Bauw, G., Van Damme, J., Puyoo, M., Vandekerckhove, J., Geser, B., Ratz, G. P., Lauridsen, J. B., and Celis, J. E. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 7701–7705
22. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299
23. Bertocci, B., Migliore, V., Da Prada, M., Dembic, Z., Lahm, H.-W., and Malherbe, P. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1416–1420
24. Huynh, T. V., Young, R. A., and Davies, R. W. (1984) in DNA Cloning: A Practical Approach (Glover, D. M., ed) pp. 49–78, IRL Press, Oxford
25. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
26. Mawal, M. R., Mukhopadhyay, A., and Deshmukh, D. R. (1991) Biochem. J. 279, 595–599
27. Koza, S. (1984) Nucleic Acids Res. 12, 857–872
28. von Heijne, G. (1989) Eur. J. Biochem. 180, 535–545
29. Marshall, R. D. (1972) Annu. Rev. Biochem. 41, 673–702
30. Okuno E., Ninomiya, T., Nakamura, N., Nakashima, J., Makino, M., and Kido, R. (1980) Biochem. J. 180, 581–590
31. Mehta, P. K., Hale, T. I., and Christen, P. (1993) Eur. J. Biochem. 214, 549–561
32. Jäger, A. U., Mose, M., Sauer, U., and ansonius, J. N. (1994) J. Med. Biol. 239, 285–305
33. Gerber, P., and Müller, K. (1995) J. Comput. Aided Mol. Des. 9, 251–268
34. Lüthy, R., Bowie, J. U., and Eisenberg, D. (1992) Nature 356, 83–85
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J. Biol. Chem. 1995, 270:29330-29335.
doi: 10.1074/jbc.270.49.29330

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