Kynurenine aminotransferase and glutamine transaminase K of *Escherichia coli*: identity with aspartate aminotransferase

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The present study describes the isolation of a protein from *Escherichia coli* possessing kynurenine aminotransferase (KAT) activity and its identification as aspartate aminotransferase (AspAT). KAT catalyses the transamination of kynurenine and 3-hydroxykynurenine to kynurenic acid and xanthurenic acid respectively, and the enzyme activity can be easily detected in *E. coli* cells. Separation of the *E. coli* protein possessing KAT activity through various chromatographic steps led to the isolation of the enzyme. N-terminal sequencing of the purified protein determined its first 10 N-terminal amino acid residues, which were identical with those of the *E. coli* AspAT. Recombinant AspAT (R-AspAT), homologously expressed in an *E. coli*/pET22b expression system, was capable of catalysing the transamination of both L-kynurenine ($K_m = 3$ mM; $V_{max} = 7.9$ μmol·min^{-1}·mg^{-1}) and 3-hydroxy-DL-kynurenine ($K_m = 3.7$ mM; $V_{max} = 1.25$ μmol·min^{-1}·mg^{-1}) in the presence of pyruvate as an amino acceptor, and exhibited its maximum activity at temperatures between 50–60 °C and at a pH of approx. 7.0. Like mammalian KATs, R-AspAT also displayed high glutamine transaminase K activity when L-phenylalanine was used as an amino donor ($K_m = 8$ mM; $V_{max} = 20.6$ μmol·min^{-1}·mg^{-1}). The exact match of the first ten N-terminal amino acid residues of the KAT-active protein with that of AspAT, in conjunction with the high KAT activity of R-AspAT, provides convincing evidence that the identity of the *E. coli* protein is AspAT.

Key words: expression, chromatography, gradient PAGE, *o*-phthaldialdehyde thiol.

INTRODUCTION

Kynurenine aminotransferase (KAT; EC. 2.6.1.7) is an enzyme involved in tryptophan catabolism. The enzyme catalyses the transamination of L-kynurenine and 3-hydroxykynurenine to kynurenic acid (KA) and xanthurenic acid (XA) respectively. In mammals, KAT is a multispecific enzyme that also has glutamine transaminase K (GTK) and aminoadipate aminotransferase (AAT) activities [1–3]. GTK is regarded as a fully reversible glutamine–methionine or phenylalanine–methionine aminotransferase in mammals [4]. KA and XA, produced by KAT-mediated transamination of kynurenine and 3-hydroxykynurenine, have important biological functions in mammals and insects. KA is a nonselective blocker of excitatory receptors and plays an important physiological role in protecting the central nervous system from being overstimulated by excitotoxins in mammals [5,6]. XA is widely distributed in insects [7–9] and has been reported to induce gametogenesis in *Plasmodium* [10,11]. XA is also an ecdysteroidogenesis inhibitor in the crustacean moulting cycle [12,13].

KAT sequences have been determined in human, mouse and rat [3,14–16]. Some sequence data deposited in the National Center for Biotechnology Information (NCBI) protein database (accession numbers T21518, T23861, S56832 and AAK97625) have also been described as KATs, due apparently to their similarities with those of mammalian KATs. In a study dealing with the functional expression of an insect KAT using an *Escherichia coli* expression system (J. Fang, Q. Han and J. Li, unpublished work), we found that *E. coli* cells exhibited high KAT activity. A BLAST search of the *E. coli* databases identified a putative *E. coli* aminotransferase (NCBI protein database accession number P77806), which shared the highest sequence similarity with those of mammalian KATs. The KAT activity in *E. coli* was initially considered a result of the expression of that protein (P77806), but after the putative aminotransferase was homologously expressed, no KAT activity was detected from the recombinant protein. In the present study, to determine the enzyme responsible for catalysing the transamination of kynurenine in *E. coli*, we purified the *E. coli* protein possessing KAT activity and deduced its partial N-terminal amino acid sequence. We describe the isolation of the KAT-active protein and its identification as AspAT. We also discuss the physiological role AspAT might play in *E. coli* tryptophan metabolism.

EXPERIMENTAL

KAT activity assays

KAT activity assay was based on previously described methods [9,17]. Briefly, a reaction mixture (50 μl total volume) containing 200 mM potassium phosphate buffer (pH 7.0), 10 mM L-kynurenine or 5 mM 3-hydroxy-DL-kynurenine (a concentration above 6 mM can not be totally dissolved), 12 mM pyruvate, 70 μM pyridoxal 5-phosphate (PLP) and varying amounts of protein sample was incubated for 10 min at 50 °C, and the reaction was stopped by adding an equal volume of 0.8 M formic acid. The mixture was centrifuged at 15000 *g* for 10 min at 4 °C and the

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resulting supernatant was analysed by HPLC with UV detection at 330 nm (HPLC-UV). Protein concentration was determined using a Bio-Rad protein assay kit with BSA as a standard.

Cell growth and protein solubilization

E. coli K12 cells were grown to saturation at 37 °C in 2 litres of Luria–Bertani (LB) broth and were recovered by centrifugation at 3000 *g* for 15 min at 4 °C. The cell pellet was resuspended (4 ml per g of wet cells) in buffer A [10 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM PLP, 10 mM 2-mercapto-ethanol and 0.5 mM PMSF]. Protein was solubilized through repetitive freezing and thawing in solid CO_2 and a 37 °C-water bath respectively. The supernatant was obtained by centrifugation at 20000 *g* for 20 min at 4 °C and used as the starting material for purification of the protein possessing KAT activity.

Protein purification

DEAE-Sepharose, Phenyl-Sepharose and hydroxyapatite chromatography

The soluble protein prepared above in buffer A was applied on to a DEAE-Sepharose column (2.5 cm × 15 cm; Bio-Rad) equilibrated with buffer A. Protein was eluted with 500 ml of a linear gradient of NaCl from 0–300 mM in buffer A. Fractions with KAT activity were pooled, ammonium sulphate was added to a concentration of 0.8 M and the pooled mixture was applied on to a Phenyl-Sepharose column (2.5 cm × 10 cm; Bio-Rad). Proteins were eluted with 500 ml of a decreasing linear gradient of ammonium sulphate from 0.8–0 M in buffer A. Fractions with KAT activity were pooled, concentrated, and then separated by HPLC with a hydroxyapatite column (1 cm × 10 cm; Bio-Rad). Proteins were eluted with 100 ml of a linear gradient of potassium phosphate (pH 7.0) from 10–400 mM in buffer A. Fractions with KAT activity were pooled and concentrated using a Centriprep YM-30 concentrator (Millipore).

Preparative electrophoresis

Concentrated fractions (200 μl) from hydroxyapatite chromatography were mixed with 50 μl of sample buffer containing 25 μl of 200 mM Tris/HCl (pH 8.8) and 25 μl of glycerol, with a minimum amount of Bromphenol Blue for visualization. The mixture was loaded on to a preparative well of a non-denaturing gradient (5–20 %) polyacrylamide gel. Electrophoresis was conducted at a constant voltage of 50 V for 12 h at 4 °C. After electrophoresis, the gel was cut into horizontal strips (2 mm wide). A small piece (2 mm × 2 mm) was cut from individual strips and mixed with 50 μl of substrate buffer [200 mM potassium phosphate buffer (pH 7.0) containing 10 mM L-kynurenine, 12 mM pyruvate and 70 μM PLP] for KAT activity assay. After the gel strip containing KAT activity was identified, the protein in the gel strip was electroeluted using a Centrieluter (Millipore) and concentrated using a Centricon YM-30 concentrator (Millipore).

Uno-Q column chromatography

The protein sample eluted from the gel strip containing KAT activity was separated further by HPLC with a Uno-Q ion-exchange column (7 mm × 35 mm; Bio-Rad) using a linear gradient of KCl from 0–300 mM in 10 mM potassium phosphate buffer (pH 7.0) at a flow rate of 0.5 ml/min over a 50-min period. Individual peaks were collected separately and assayed for KAT activity.

N-terminal sequencing

Following Uno-Q column chromatography, the protein peak containing KAT activity was separated further by SDS/PAGE and the protein was electroblotted on to a PVDF membrane for N-terminal sequencing using a Procise 494 protein sequencer at the University of Illinois Biotechnology Center (Urbana, IL, U.S.A.). The first 10 amino acid residues were identified and BLAST analysis matched the partial sequence with the first ten N-terminal amino acids of *E. coli* aspartate aminotransferase (AspAT).

Cloning of *E. coli* AspAT

A forward primer (5′-GCCTGGCCATGTTTGAGAACATT-ACC-3′) with an *Msc*I restriction site (underlined nucleotides) and a reverse primer (5′-CCGCTCGAGCACTGCCACAAT-CGC-3′) with an *Xho*I restriction site (underlined nucleotides), designed according to the *E. coli* AspAT coding sequence (NCBI protein database accession number AAC74014), were used for PCR amplification of *E. coli* K12 genomic DNA. The PCR product was recovered by ethanol precipitation and digested with *Msc*I and *Xho*I. The digest was gel-purified and ligated between the *Msc*I and *Xho*I restriction sites of the pET22b(+) vector (Novagen). The resulting pET22b(+):AspAT construct was used to transform TOPO 10 *E. coli* cells for proliferation and subsequent sequencing for frame verification.

Protein expression and purification

The BL21(DE3)pLysS strain of *E. coli* (Novagen) was transformed with the pET22b(+):AspAT construct. A single colony was picked from an agar-LB plate containing 50 μg/ml ampicillin, and inoculated into 10 ml of LB broth containing 50 μg/ml ampicillin. After growth for 10 h at 37 °C, the cells were transferred to a large volume of LB broth (1000 ml) containing 50 μg/ml ampicillin and incubated at 37 °C until the D_{600} reached 0.75. The culture then was induced with 1 mM isopropyl-β-D-thiogalactopyranoside ('IPTG') and growth was continued for another 20 h at 25 °C. The cells were harvested, washed twice with 20 mM potassium phosphate buffer (pH 7.0) and resuspended in buffer A. Protein was solubilized through a repetitive freezing and thawing process as described above. The supernatant, obtained by centrifugation at 20000 *g* for 20 min at 4 °C, was mixed with an equal volume of 2 × Ni^{2+}-binding buffer [40 mM Tris/HCl (pH 7.9), 1.0 M NaCl and 10 mM imidazole] and applied on to a Ni^{2+}-charged His-Bind® resin column (1.5 cm × 2.5 cm; Novagen) equilibrated with 1 × Ni^{2+}-binding buffer. The column was extensively washed with a wash buffer [20 mM Tris/HCl (pH 7.9), 0.5 M NaCl and 60 mM imidazole] and the protein was eluted with a linear gradient of imidazole (0.1–1 M) in 20 mM Tris/HCl (pH 7.9) containing 0.5 M NaCl at a flow rate of 0.5 ml/min, and fractions (1.5 ml) were collected. A sample (10 μl) was removed from individual fractions and analysed for KAT activity. Fractions containing KAT activity were pooled and dialysed against 50 mM Tris/HCl (pH 7.0) containing 2 mM EDTA, 10 % glycerol and 70 μM PLP for 12 h at 4 °C.

Substrate specificity

KAT activity of recombinant AspAT (R-AspAT) was determined using either L-kynurenine or 3-hydroxy-DL-kynurenine as amino group donors and pyruvate, α-ketoglutarate, α-ketoadipate, α-keto-γ-methiolbutyrate (αKMB) or oxalacetate as amino group

acceptors. The activity of the R-AspAT against L-kynurenine or 3-hydroxy-DL-kynurenine was based on the detection of KA or XA in the respective reaction mixtures following HPLC-UV as described in the KAT activity assay. GTK activity of the enzyme was determined using phenylalanine and α-KMB as the amino donor and acceptor respectively [18]. Classical AspAT activity, which was originally used to define the enzyme, was measured using glutamate and oxalacetate as the amino donor and acceptor respectively. The reaction mixture (50 μl total volume) consisted of 200 mM potassium phosphate buffer (pH 7.0), 10 mM amino donor (except for 3-hydroxy-DL-kynurenine which was used at 6 mM), 12 mM amino acceptor, 70 μM PLP and 0.5 μg of R-AspAT. GTK and classical AspAT activities of R-AspAT were based on the detection of *o*-phthaldialdehyde thiol (OPT)–methionine and OPT–aspartate conjugates by HPLC-electrochemical detection (HPLC-ED) after their corresponding reaction mixtures were derivatized by the OPT reagent [19]. AAT activity of the enzyme was measured with aminoadipate and α-ketoglutarate as the substrates [20]. The substrates and reagents were purchased from Sigma unless otherwise stated.

Effect of temperature and pH on the KAT activity of R-AspAT

The effect of temperature on the KAT activity of R-AspAT was determined by incubating the corresponding substrate preparations at 35–70 °C for 5 min prior to the addition of the enzyme and for 10 min after enzyme addition. The effect of pH on the KAT activity of R-AspAT was determined using 10 mM L-kynurenine or 6 mM 3-hydroxy-DL-kynurenine as the amino donor, 12 mM amino acceptor, 70 μM PLP and 0.5 μg of R-AspAT in either 200 mM acetate buffer (pH 5.5), potassium phosphate buffer (pH 6.0–7.5), Tris/HCl (pH 8–9) or 2-amino-2-methyl-1-propanol (pH 9.5–10). The amounts of XA or KA produced following a 10 min incubation were quantitated by HPLC-UV.

Kinetic studies

K_m and V_{max} of R-AspAT for kynurenine, 3-hydroxy-kynurenine and phenylalanine were determined by the addition of various concentrations of kynurenine (1–12 mM), 3-hydroxy-DL-kynurenine (1–6 mM) or phenylalanine (2.5–25 mM) into their corresponding reaction mixtures in the presence of 0.5 μg of enzyme, 70 μM PLP and 12 mM amino acceptor described above. The initial rate was calculated based on the amount of KA, XA or OPT–methionine conjugate formed during a 5 min incubation period at 50 °C. The specific activity was expressed as the amount of product formed $min^{-1} \cdot mg^{-1}$ of protein.

RESULTS

KAT activity of solubilized *E. coli* protein

When solubilized *E. coli* protein was mixed with L-kynurenine in the presence of pyruvate, accumulation of KA was observed in the reaction mixtures (Figure 1A). The specific KAT activity was 5 $nmol \cdot min^{-1} \cdot mg^{-1}$ of crude protein with kynurenine and pyruvate as the amino donor and acceptor respectively. There was a 50% decrease in the specific activity when DL-kynurenine was used as the amino donor, and no KA was produced when D-kynurenine was used in the reaction mixture. Production of XA was also observed when 3-hydroxy-DL-kynurenine was used as the amino donor (results not shown), with a specific activity of 1.5 $nmol \cdot min^{-1} \cdot mg^{-1}$ of crude protein. Because only a mixture of DL-configuration of 3-hydroxykynurenine is available, the specific activity of the *E. coli* protein to 3-hydroxykynurenine
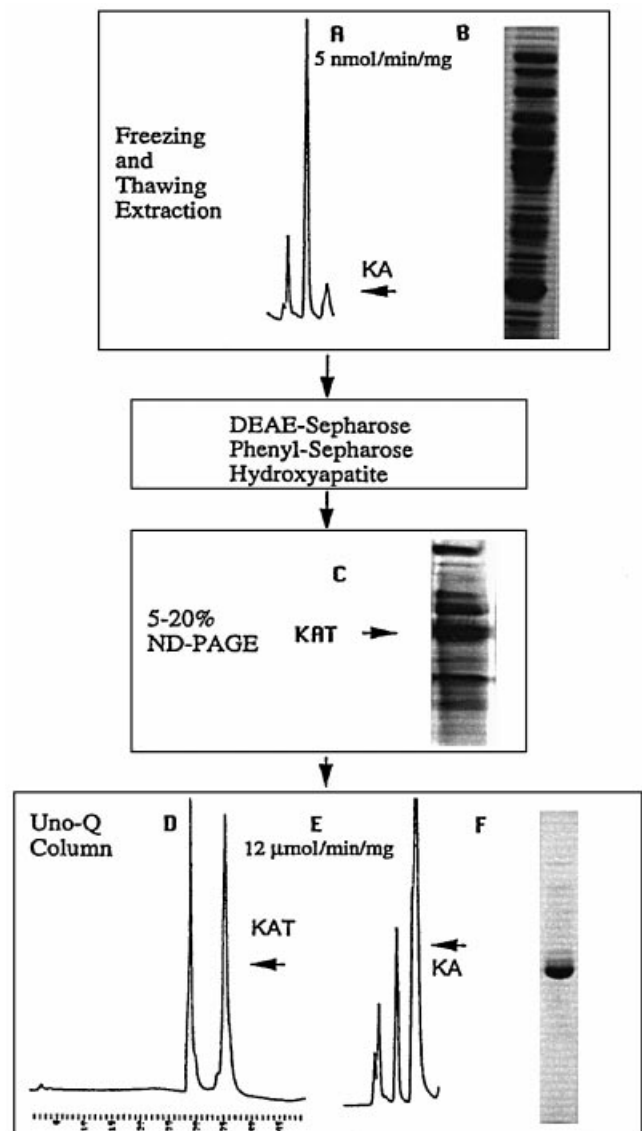

Figure 1   Purification of *E. coli* KAT

(**A**) Chromatogram showing the production of KA in a reaction mixture containing the crude *E. coli* protein sample, L-kynurenine, pyruvate and PLP. (**B**) Protein profile of the freeze–thawed solubilized *E. coli* proteins separated by SDS/PAGE using a 10% gel. (**C**) Protein profile of the fractions containing KAT activity separated by non-denaturing PAGE (ND-PAGE) using a 5–20% gradient gel. The arrow indicates the band with KAT activity. (**D**) Chromatogram of the electroeluted band with KAT activity separated by Uno-Q column chromatography and monitored at A_{280}. The arrow points to the KAT-active protein. (**E**) Chromatogram illustrating the accumulation of KA after 10 min incubation at 50 °C in a 50 μl reaction mixture containing 10 mM kynurenine, 12 mM pyruvate, 70 μM PLP and 0.2 μg of the protein with KAT activity eluted from the Uno-Q column. (**F**) Protein profile of the protein with KAT activity eluted by Uno-Q column chromatography and separated by SDS/PAGE using a 10% gel.

should be much higher than that determined using its mixed DL-forms.

Purification of the protein possessing KAT activity

DEAE-Sepharose, Phenyl-Sepharose and hydroxyapatite chromatography

Under the separation conditions used, the KAT-active protein was eluted from the DEAE-Sepharose column at a gradient of 150–200 mM NaCl, from the Phenyl-Sepharose column by
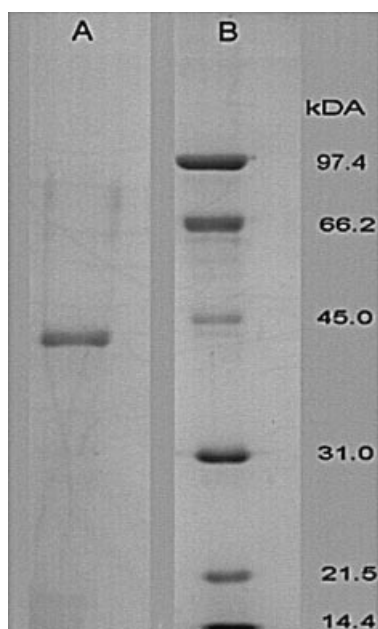
Figure 2 SDS/PAGE-analysis of R-AspAT

Lane A, Ni²⁺-affinity-column-purified R-AspAT sample separated by SDS/PAGE using a 10% gel. Lane B, molecular-mass markers (in kDa). The molecular mass of the recombinant protein is 44 000 Da based on its migration on the gel, which is essentially the same as the sum of the estimated relative molecular mass of AspAT (43 000 Da) and the His₆-tag (1000 Da).

Table 1 Co-substrate specificity of R-AspAT with L-kynurenine and 3-hydroxy-DL-kynurenine as amino donors

Purified R-AspAT was mixed with either L-kynurenine (10 mM) or 3-hydroxy-DL-kynurenine (6 mM) in the presence of different keto acids (12 mM). Data are expressed as a percentage of the enzyme activity with pyruvate as the amino acceptor and are the means of two separate experiments.

Co-substrates	Percentage of KAT activity	
	(L-kynurenine)	(3-hydroxy-DL-kynurenine)
α-Ketoadipate	89	81
α-Ketoglutarate	17	24
αKMB	133	134
Oxalacetate	80	89
Pyruvate	100	100

against the *E. coli* protein databases produced an exact match with the N-terminal sequence of *E. coli* AspAT.

Expression and purification of R-AspAT

The coding sequence of the *E. coli* K12 AspAT was cloned into a pET22b(+) vector and homologously expressed in the *E. coli* BL21(DE3)pLysS strain to produce R-AspAT with a secretion signal peptide at its N-terminus and a His-tag at its C-terminus. After induction, the solubilized protein from pET22b(+): AspAT-transformed *E. coli* showed an approx. 2-fold increase in KAT activity when compared with that of solubilized protein from cells transformed with a pET22b(+) vector without the AspAT insert. Analysis of the pooled fractions with KAT activity from Ni²⁺-affinity chromatography detected a major protein band with a molecular mass of 44 000 Da, which was presumed to be the R-AspAT (Figure 2). This presumption was made because the same protein was not isolated when solubilized proteins from untransformed cells were separated on the affinity column under identical conditions, and because the purified *E. coli* KAT-active protein (lacking the His-tag) did not bind to the Ni²⁺-affinity column.

Substrate specificities and kinetic parameters

R-AspAT was active against both L-kynurenine and 3-hydroxy-DL-kynurenine, similar to that observed for the purified enzyme. However, the specific activity of R-AspAT was approx. 3-fold lower than that of the purified enzyme, which may be due to the presence of the His-tag at its C-terminus. Several keto acids were tested for their ability to function as amino acceptors, and among them αKMB and pyruvate were found to be the best amino group acceptors for the KAT activities of AspAT (Table 1). R-AspAT also displayed high GTK activity, which is similar to those reported for mammalian KATs [1,3]. However, unlike mammalian KATs, the *E. coli* R-AspAT had no apparent AAT activity. Production of aspartate was clearly observed when R-AspAT was mixed with L-glutamate and oxalacetate, demonstrating the typical AspAT activity of the expressed enzyme (Figure 3). Under identical assay conditions, the specific activity of R-AspAT was 12.4 μmol · min⁻¹ · mg⁻¹ for typical AspAT activity with L-glutamate and oxalacetate as substrates, 12.2 μmol · min⁻¹ · mg⁻¹ for GTK activity with L-phenylalanine and α-KMB, 4.2 μmol min⁻¹ mg⁻¹ for KAT activity with L-kynurenine and pyruvate and 0.9 μmol · min⁻¹ · mg⁻¹ for 3-hydroxykynurenine transaminase ('HKT') activity with

buffer A after the ammonium sulphate gradient, and from the hydroxyapatite column at a gradient of 180–220 mM potassium phosphate (results not shown). Approx. 97% of the total protein was eliminated after the three steps of chromatographic separation with an approx. 40% recovery of the total KAT activity (results not shown).

Preparative non-denaturing PAGE and Uno-Q column chromatography

Separation of the concentrated KAT-containing fractions by non-denaturing PAGE resolved most of the major proteins remaining in the sample and a band with KAT activity was identified on the basis of KAT activity assays of the individual gel strips (Figure 1C). Subsequently, the protein in the identified gel strip was electroeluted, concentrated and separated on a Uno-Q column. Two major absorption peaks at 280 nm were detected (Figure 1D), with the one eluting after 35–37 min (corresponding to a gradient of 210–222 mM NaCl) showing high KAT activity (Figure 1E). The specific activity of the enzyme after Uno-Q column chromatography was increased to 12 μmol · min⁻¹ · mg⁻¹ with a 26% recovery of the total activity. SDS/PAGE analysis of the concentrated protein resulted in the detection of a major protein band (90% of the total protein) with a molecular mass of 43 000 Da (Figure 1F).

N-terminal sequencing

On the basis of its high KAT activity, the 43 000 Da protein band was presumed to be the protein with KAT activity. Therefore, after electrophoresis, the protein was blotted on to a PVDF membrane for sequencing, which resulted in the identification of its first 10 N-terminal residues (Met-Phe-Glu-Asn-Ile-Thr-Ala-Ala-Pro-Ala). BLAST analysis of the partial sequence
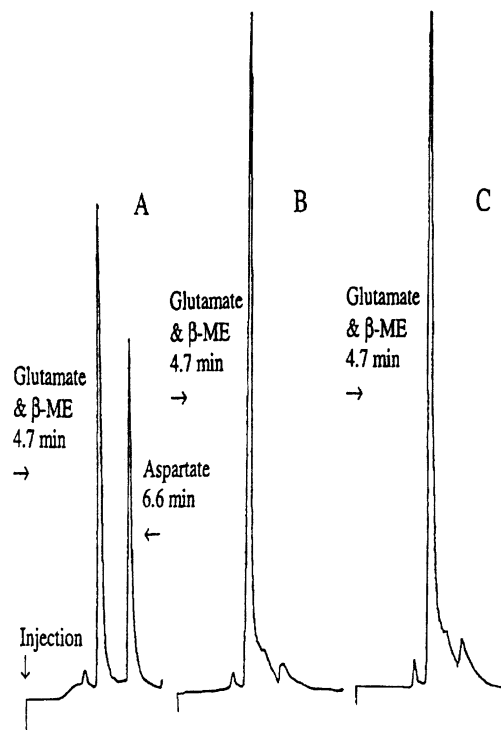
Figure 3   HPLC-ED-analysis of R-AspAT activity

Chromatograms show the detection of L-aspartate–OPT conjugate after OPT-reagent derivatization of a reaction mixture (50 μl) containing 10 mM L-glutamate and 12 mM oxoacetate in the presence of 0.5 μg of active R-AspAT (**A**), in the absence of the enzyme (**B**), and in the presence of heat-inactivated R-AspAT (**C**). The reaction mixture was incubated for 10 min at 50 °C prior to addition of the OPT reagent for derivatization and analysed 2 min after it was mixed with the OPT reagent by HPLC-ED. 2-Mercaptoethanol (β-ME; 110 mM) was used to prepare the OPT reagent. Separation of L-glutamate– and L-aspartate–OPT conjugates was achieved by reverse-phase separation using a C$_{18}$ column (4.6 mm × 20 mm). The mobile phase consisted of 50 mM potassium phosphate (pH 4.8) containing 12% acetonitrile with a flow rate of 0.6 ml·min^{-1}. A BAS LC-4C detector (Bioanalytic System) was used for electrochemical detection. The applied potential of the working electrode (versus the Ag/AgCl reference electrode) was maintained at 750 mV with a full sensitivity scale of 1 μA.
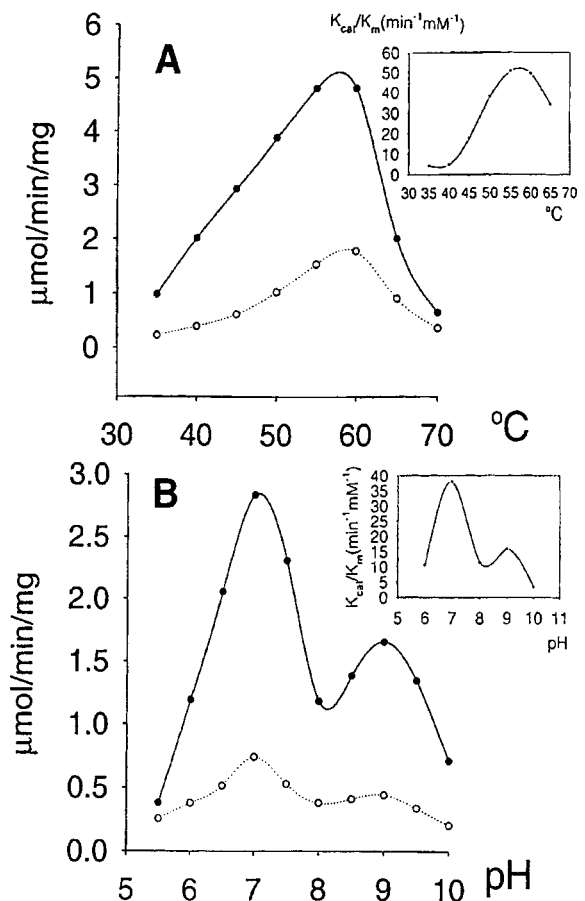

Figure 4   Effect of pH and temperature on KAT activity

The detailed assay methods are described in the Experimental section. (**A**) KAT activity of R-AspAT at temperatures from 35–70 °C with 10 mM kynurenine (●) and 6 mM 3-hydroxy-DL-kynurenine (○) as amino donors respectively. Inset. The calculated K_{cat}/K_m for R-AspAT at temperatures from 35–65 °C with kynurenine as the amino donor. (**B**) KAT activity of R-AspAT at pH from 5.5–10 with 10 mM kynurenine (●) and 6 mM 3-hydroxy-DL-kynurenine (○) as amino donors respectively. Inset. The calculated K_{cat}/K_m at pH from 6–10 with kynurenine as the amino donor.

3-hydroxy-DL-kynurenine and pyruvate. Other kinetic parameters, including K_m, V_{max}, K_{cat} and K_{cat}/K_m are shown in Table 2.

Effect of pH and temperature on KAT activity

KAT activities of R-AspAT were elevated under relatively high temperatures for both L-kynurenine and 3-hydroxy-DL-kynurenine with a maximum activity occurring around 55 °C (Figure 4A). The enzyme showed little activity at pH 5.5, had its maximum activity around pH 7.0, and decreased to less than half of its maximum activity at pH 8.0. However, above pH 8.0, its activity progressively increased again with a second peak activity around pH 9.0 (Figure 4B).

DISCUSSION

AspAT is an interesting paradigm for studies focusing on structure–function–stability relationships. This enzyme is present

Table 2   Kinetic parameters of R-AspAT

The activities were measured as described in the Experimental section. The parameters of R-AspAT against the three substrates were calculated by fitting the experimental data to the Michaelis–Menten equation using the Enzyme Kinetics Module (SPSS Science).

Substrates	K_m (mM)	V_{max} (μmol · min^{-1} · mg^{-1})	K_{cat} (min^{-1})	K_{cat}/K_m(min^{-1} · mM^{-1})
3-hydroxy-DL-kynurenine	3.7 ± 1.5	1.25 ± 0.24	50 ± 9.6	13.5 ± 6.4
L-kynurenine	3 ± 0.9	7.9 ± 0.8	316 ± 32	105 ± 35.6
L-phenylalanine	8 ± 3.6	20.6 ± 3.5	824 ± 140	103 ± 38.9

in all living organisms from bacteria to humans. It catalyses reversible transamination reactions between dicarboxylic amino and keto acids. Its biological functions include, synthesizing aspartate, alanine and aromatic amino acids, and the removal of amino groups from these amino acids during catabolism [21,22]. The *E. coli* AspAT is one of the most extensively studied AspATs. Its three-dimensional structure has been solved [23–26], and its catalytic mechanism has been proposed from X-ray crystallographic and enzymological studies [25–29].

In spite of numerous biochemical and molecular studies of the *E. coli* AspAT, there have been no reports that discuss the KAT and GTK activities of this enzyme. Our present results clearly demonstrate that the *E. coli* AspAT has most of the catalytic functions described for mammalian KATs and GTKs, which expand the catalytic functions of this *E. coli* enzyme. Our present results also provide an additional basis and impetus towards the complete understanding of the structure–function relationships between the *E. coli* AspAT and the same enzyme from other species, as well as between the *E. coli* AspAT and mammalian KATs.

E. coli AspAT catalyses the transamination between glutamate–aspartate and aromatic acids–alanine pairs, which provides a mechanism to maintain a continual equilibrium among the amino acids. GTK is regarded as a fully reversible glutamine-methionine or phenylalanine-methionine aminotransferase in mammals [4]. Accordingly, the GTK activity of *E. coli* AspAT can be considered as a part of its overall physiological role in maintaining a balanced level of different amino acids for protein synthesis. However, the exact physiological role of the KAT activity of *E. coli* AspAT cannot be clearly defined on the basis of results from the present study. Bacteria are likely to have no specific enzymes responsible for oxidizing tryptophan to kynurenine and 3-hydroxykynurenine, but other oxidative enzymes may be able to oxidize tryptophan to kynurenine and then to 3-hydroxykynurenine. In addition, tryptophan can be oxidized to kynurenine under physiological conditions in the presence of O_2. Therefore involvement of the *E. coli* AspAT in tryptophan metabolism cannot be excluded and requires further investigation.

In bacteria, there is only one AspAT. In contrast, an additional mitochondrial AspAT is present in eukaryotic cells. However, the primary sequences of AspAT from bacteria to humans are fairly conserved. The *E. coli* AspAT shares 51–63 % similarity with those of other bacterial AspATs and 37–42 % similarity with those of eukaryotic AspATs. Mammalian AspATs, although sharing approx. 40 % sequence similarity with that of the *E. coli* enzyme, have essentially no KAT or GTK activity. In contrast, the *E. coli* AspAT and mammalian KATs share only 5–8 % sequence similarity, but both are highly active for the transamination of kynurenine and 3-hydroxykynurenine, and both have high GTK activity. Our present results highlight an interesting phenomenon that functionally-related enzymes may share extremely limited sequence similarity, but enzymes with high sequence similarity may not necessarily have the same catalytic functions.

In summary, results from the present study have demonstrated that *E. coli* has possesses an enzyme with high KAT and GTK activities and that the enzyme responsible for these activities is AspAT. These data provide the basis for detailed sequence and structural comparisons of the *E. coli* AspAT, mammalian AspAT and KATs, particularly their three-dimensional structures, which may provide interesting information useful in delineating the structure–function relationships of these enzymes. Such studies may also provide insight towards an understanding of how specific KATs evolved in other species.

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