Tryptophan Catabolism: Identification and Characterization of a New Degradative Pathway

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A new tryptophan catabolic pathway is characterized from Burkholderia cepacia J2315. In this pathway, tryptophan is converted to 2-amino-3-carboxymuconate semialdehyde, which is enzymatically degraded to pyruvate and acetate via the intermediates 2-aminomuconate and 4-oxalocrotonate. This pathway differs from the proposed mammalian pathway which converts 2-aminomuconate to 2-ketoacidipate and, ultimately, glutaryl-coenzyme A.

Tryptophan has a variety of metabolic functions within the cell. It is incorporated into the polypeptide chains of enzymes and proteins, and it is the biosynthetic precursor of the cofactor NAD (19), the antibiotics anthramycin (15) and actinomycin (16), the siderophore quinolobacin (22), and the neurotransmitters serotonin (2) and melatonin (10, 29). Tryptophan can also be fully catabolized. For example, Bacillus megaterium (1) and Rhodococcus erythropolis (27) can grow with tryptophan as their sole source of carbon and nitrogen, and several pseudomonads are capable of catabolizing tryptophan (26).

Eukaryotes are also capable of breaking down excess tryptophan to CO2, NH3, and H2O. Labeling studies indicate that tryptophan degradation in mammals takes place via the kynurenine pathway, which is also used for NAD biosynthesis in all eukaryotic organisms and in a few bacterial species (9, 21, 24) (Fig. 1). On the kynurenine pathway, the branching point between NAD biosynthesis and complete tryptophan catabolism takes place at the intermediate 2-amino-3-carboxymuconate semialdehyde (ACMS) (Fig. 1). ACMS can cyclize non-enzymatically to yield quinoline (5), the direct precursor to the pyridine ring of NAD, or it can be enzymatically decarboxylated by ACMS decarboxylase (ACMSD) (6, 7, 28). Although the biosynthesis of NAD via the kynurenine pathway is well understood, relatively little is known about the enzymology of tryptophan catabolism after ACMS. The recent discovery of the five enzymes necessary to biosynthesize ACMS from tryptophan in several prokaryotes (17) suggests that a complete tryptophan catabolic pathway, similar to the proposed human pathway, might also exist in bacteria.

To test this hypothesis, we searched for clusters of tryptophan catabolic genes in bacteria by using 3-hydroxyanthranilate-3,4-dioxygenase (HAD) (11, 20, 23) and ACMSD (14, 23, 28) sequences from the NCBI database (http://www.ncbi.nlm.nih.gov) and by using the SEED database (http://theseed.uchicago.edu/FIG/index.cgi) for comparative genome analysis. Several bacteria that contained likely gene candidates for further degradation of ACMS clustered with HAD and ACMSD were identified. In Burkholderia cepacia J2315, HAD and ACMSD orthologs occurred in a cluster with genes of unknown function. Sequence analysis suggested that one of the unknown genes might function as a 2-aminomuconate semialdehyde dehydrogenase (AMHD; EC 1.2.1.32) (12) and another as a 2-aminomuconate deaminase (AMD; EC 3.5.99.5) (13, 14).

A second related genomic cluster was identified immediately upstream of the HAD-AMD cluster (Fig. 2). Within the second cluster were putative homologs of 4-oxalocrotonate decarboxylase (4OCD; EC 4.1.1.77), 2-keto-pentenoate hydratase (KPH; EC 4.2.1.80), 2-keto-4-hydroxypentanoate aldolase (HOA; EC 4.2.1.-), and acetaldehyde dehydrogenase (ADH; EC 1.2.1.3). We later identified all eight genes within a single, uninterrupted cluster in the organism Bacillus cereus 10897, suggesting a shared metabolic function for these genes in tryptophan catabolism. This was further supported by the identification of B. cepacia J2315 homologs of the genes encoding tryptophan-2,3-dioxygenase (TDI; EC 1.13.11.11), kynurenine formamidase (KFA; EC 3.5.1.9), and kynureninase (KYN; EC 3.7.1.3). No gene encoding kynurenine-3-monooxygenase (KMO; EC 1.13.14.9) was found, suggesting the existence of a second nonorthologous form of KMO in Burkholderia.

The identification of these putative enzymatic activities suggests the tryptophan catabolic pathway shown in Fig. 3. This pathway differs from the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway previously proposed for eukaryotes (Fig. 1), where 2-aminomuconate is converted to 2-ketoacidipate (Fig. 1) (25). However, the reported conversion of 2-aminomuconate to 2-ketoacidipate may be regarded as a tentative assignment in the mammalian tryptophan catabolic pathway, since the only evidence is from radiolabeling studies performed with crude preparations of cat liver extract (8, 24). We propose that in B. cepacia J2315, 2-aminomuconate is instead deaminated to 4-oxalocrotonate (Fig. 3).

To determine if B. cepacia J2315 utilized a catabolic pathway for tryptophan under normal growth conditions, the bacterial strain was plated on minimal medium containing 2% trypto-
phan as the sole carbon source. After incubation at 30°C, growth was observed at about half the rate at which colonies appeared on full medium.

To further test our proposed pathway, the putative HAD, ACMSD, AMDH, and AMD genes from B. cepacia J2315 were PCR amplified from genomic DNA and cloned into the plasmid pDESTF1, which encodes an N-terminal six-His tag and is under the control of the T7lac promoter. The resulting plasmids were named pBcHAD.XF1, pBcACD.XF1, pBcHM.D.XF1, and pBcAMD.XF1, respectively, and were used to transform Escherichia coli Tuner (DE3). For overexpression, E. coli Tuner (DE3) cells transformed with one of these plasmids were grown at 37°C in Luria-Bertani medium containing 200 mg of ampicillin per liter. When the culture reached an optical density of 0.4 (absorbance at 600 nm), the temperature was lowered to 25°C. When the culture reached an optical density of 0.6, isopropyl-β-D-thiogalactopyranoside was added to a final concentration of 0.1 mM, and the culture was incubated with shaking for 4 to 6 h at 25°C. The cells were then harvested and stored at -20°C until further use. Under these conditions, HAD, ACMSD, AMDH, and AMD all were overexpressed at a high level and were readily purified by nickel-nitrilotriacetic acid affinity chromatography according to the QIAGEN protocol for the purification of poly-His-tagged proteins (Fig. 4).

HAD is a soluble 23-kDa protein. The purified enzyme is oxidized as indicated by a pink color and a UV spectrum consistent with an oxidized rubredoxin Fe-S center (18). The enzyme was reconstituted with FeII in the presence of dithiothreitol; upon removal of reconstituting agents, the protein solution was colorless, consistent with a reduced rubredoxin Fe-S center. HAD catalyzes the oxidation of 3-hydroxyanthranilate to ACMS (λmax = 360 nm); the purified enzyme demonstrated a specific activity of 25 μmol/min/mg (4).

ACMSD was overexpressed as a soluble 41-kDa protein which catalyzed the decarboxylation of ACMS to 2-aminomuconate semialdehyde (AMS; λmax = 380 nm). AMS rapidly cyclizes nonenzymatically to picolinic acid (Fig. 1); therefore, enzymatic activity was monitored either by the decrease in absorbance of the substrate ACMS (λmax = 360 nm) or by the formation of picolinic acid. The identity of picolinic acid as the final reaction product was confirmed by high-pressure liquid chromatography and nuclear magnetic resonance (NMR) analysis (4).

AMDH was overexpressed as a soluble 57-kDa protein which utilized the cofactor NAD to catalyze the oxidation of AMS to 2-aminomuconate. The substrate AMS is unstable, cyclizing rapidly to picolinic acid; therefore, the AMDH reaction was coupled to the ACMSD-catalyzed decarboxylation of ACMS to generate AMS in situ. The formation of 2-aminomuconate was detected as a peak absorbing at 326 nm. At pH 7, 2-aminomuconate hydrolyzes nonenzymatically to the tautomers 2-hydroxymuconate and 4-oxalocrotonate with a half-life of 7 to 8 min. After acidification and extraction, the thermodynamically stable tautomer 2-hydroxymuconate was identified by NMR (4).

FIG. 1. KEGG pathway for tryptophan degradation in eukaryotes. CoA, coenzyme A.

FIG. 2. Region of Burkholderia cepacia J2315 chromosomal DNA containing genomic clusters of tryptophan catabolic genes.
AMD was overexpressed as a soluble 18-kDa protein. The AMD-catalyzed deamination of 2-aminomuconate gave the same products as nonenzymatic hydrolysis, 2-hydroxymuconate and its tautomer, 4-oxalocrotonate. At pH 7.7, AMD accelerates the rate of deamination more than 70-fold over the nonenzymatic hydrolysis. The tautomeric products 4-oxalocrotonate and 2-hydroxymuconate were identified by high-pressure liquid chromatography and NMR (4).

FIG. 3. New tryptophan catabolic pathway in B. cepacia J2315.

FIG. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of tryptophan catabolic enzymes. Lane 1, molecular weight markers. Lane 2, cell extracts of the HAD overexpression strain. Lane 3, purified HAD. Lane 4, cell extracts of the ACMSD overexpression strain. Lane 5, purified ACMSD. Lane 6, cell extracts of the AMDH overexpression strain. Lane 7, purified AMDH. Lane 8, cell extracts of the AMD overexpression strain. Lane 9, purified AMD.
This paper discusses the identification of likely candidates for the genes of a new tryptophan catabolic pathway in *B. cepacia* J2315. As illustrated in Fig. 3, tryptophan is converted to 3-hydroxynanthranilate using the first four enzymatic steps for NAD biosynthesis from tryptophan. 3-Hydroxynanthranilate is then cleaved to ACMS, which does not cyclize to quinolinate but instead is further degraded enzymatically to 2-aminnomuconate and, finally, 4-oxolactonotarate. Enzymatic activities necessary to convert 4-oxolactonotarate to pyruvate and acetate are the same as those involved in the degradation of catechols and antranilate (3). The formation of the intermediate 4-oxolactonotarate differentiates this pathway from the proposed mammalian pathway which converts 2-amominocuanotate to 2-keto-dipate and, ultimately, glutaryl-coenzyme A.

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REFERENCES


