Methylguanidinase Synthase from Rat Kidney Is Identical to Long-Chain \( L-2 \)-Hydroxy Acid Oxidase

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Dear Sir,

Methylguanidine (MG) is a suspected uremic toxin that accumulates in renal failure [1]. Aoyagi et al. [2] reported that MG was produced from creatinine (Cr) in the presence of active oxygen in an experimental system using isolated hepatocytes. However, Yoko-zawa et al. [3] have demonstrated in vitro that creatol is involved as an intermediate in the conversion of MG from Cr, and purified the enzyme responsible for the conversion of creatol to MG. They isolated MG-synthesizing enzyme from rat liver microsomes and identified as \( L \)-gulono-r-lactone oxidase (EC 1.1.3.8.) [4]. By the way, \( L \)-gulono-r-lactone oxidase is absent in the kidney [5]. Therefore, Yoko-zawa et al. [6] purified MG-synthesizing enzyme from the rat kidney. The enzyme was a flavoprotein with a molecular weight of about 37,000 and oxidized creatol to produce MG, and retrieval from the database (GENETYX) for the protein having this amino acid sequence indicated that the enzyme was a new one which had not been reported previously [6]. We attempted to isolate the cDNA of that enzyme to investigate the metabolism of MG under the genetic level by the PCR cloning method [7]. Rat kidney poly(A)+ RNA was reverse transcribed and used for 30 cycles of PCR with 5 µM each of two mixed oligonucleotides primers corresponding to the 5’ and 3’ ends of the N-terminal segment of amino acid sequence 5’TTCYARGCNAYCNCARAARCA and 5’GCYTCNYTCYTATRARAARTCCCA (N=A+G+T+C; S=G+C; R=A+G; M=A+C; Y=C+T), respectively. Amplification was carried out by 30 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min (concentration of KC1 in the reaction mixture was not 2 but 3 mM). The PCR product was sub-cloned into pGEM-T (TA cloning kit, Clontech Lab. Inc., Palo Alto, Calif., USA). Subclones were characterized by sequencing with an SP6 primer. The cDNA sequences corresponding to the amino acids were confirmed. Using this cloned PCR fragment, 2×105 plaques of rat kidney \( \lambda \text{gt11} \) library (Clontech) were screened. Two clones were purified and subcloned to pGEM-7 (Clontech) and sequenced. Two clones contained about 1,600 bp nucleotides and deduced amino acid sequences to match the N-terminal of MG synthase in rat kidney. Unfortunately, however, from analysis of homology (GenBank), this clone was identical to newly References


References


cloned rat kidney long-chain L-2-hydroxy acid oxidase (EC 1.1.3.15) [8]. Namely, the nucleotide sequence of MG synthase in rat kidney matched perfectly to the oxidase except at the 4th amino acid from the N-terminal as cysteine not serine. This enzyme is a member of the family of FMN-dependent α-hydroxy acid-oxidizing enzymes and located in the peroxisomes in rat kidney [8]. The enzyme oxidizes L-α-hydroxy acid to keto acids with the formation of hydrogen peroxide at the expense of oxygen. The relationship between the enzyme and MG formation is still to be elucidated, and the role of peroxisomes in MG synthesis is also unknown.


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