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Mammalian dopa decarboxylase: Structure, catalytic activity and inhibition ☆

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ABSTRACT

Mammalian Dopa decarboxylase catalyzes the conversion of L-Dopa and L-5-hydroxytryptophan to dopamine and serotonin, respectively. Both of them are biologically active neurotransmitters whose levels should be finely tuned. In fact, an altered concentration of dopamine is the cause of neurodegenerative diseases, such as Parkinson's disease. The chemistry of the enzyme is based on the features of its coenzyme pyridoxal 5′-phosphate (PLP). The cofactor is highly reactive and able to perform multiple reactions, besides decarboxylation, such as oxidative deamination, half-transamination and Pictet–Spengler cyclization. The structure resolution shows that the enzyme has a dimeric arrangement and provides a molecular basis to identify the residues involved in each catalytic activity. This information has been combined with kinetic studies under steady-state and pre-steady-state conditions as a function of pH to shed light on residues important for catalysis. A great effort in DDC research is devoted to design efficient and specific inhibitors in addition to those already used in therapy that are not highly specific and are responsible for the side effects exerted by clinical approach to either Parkinson's disease or aromatic amino acid decarboxylase deficiency.

Background

Dopa decarboxylase (DDC,[1] EC 4.1.1.28) was first identified in 1938 in mammalian kidney tissue, revealing that it catalyzes an essential step in epinephrine biosynthesis. Subsequently, it was reported that the reaction specificity of the enzyme is broader, since, in addition to decarboxylate L-Dopa to dopamine, it is able to transform L-5-hydroxytryptophan to serotonin and, although much less efficiently, also other aromatic amino acids such as p-tyrosine, tryptophan and phenylalanine to the corresponding amines (trace aromatic amines). For this reason, DDC is also described, more correctly, as aromatic amino acid decarboxylase (AADC). Its role is to supply organism with essential neurotransmitters. Despite its low substrate specificity towards different aromatic amino acids, DDC plays a key role in controlling aromatic amines level. Dopamine and serotonin act as neuro-modulators and can influence mood regulation, cognitive and physiological homeostasis as well as motor coordination [1–4]. Not only their synthesis, but also their metabolic and uptake pathways are strictly connected. Serotonin depletion is responsible for some forms of major depression, anxiety and aggression. Dopamine depletion is responsible for lack of motivation and for anhedonia. When there is an imbalance with either one, there is an increased chance of the other becoming unbalanced as a result of their interdependent nature. Balancing serotonin, dopamine, and other biogenic amines such as norepinephrine is the focus of research into treating many mood disorders.

A deficiency in DDC synthesis, due to non-sense or frameshift mutations, or an alteration in its activity, due to single amino acid substitutions generating an aberrant gene product, leads to pathological conditions related to impaired cognitive and physiological homeostasis as well as motor coordination [1–4] and/or neuropsychiatric disorders [5,6]. DDC deficiency is a rare recessive disorder due to mutations in the *DDC* gene and to the related inability to synthesize dopamine and serotonin. This leads to severe developmental delay [7]. A comprehensive review of known variants involved and their possible structure to function relationship is given in [8].

Moreover, DDC is not considered to be rate-limiting in physiological catecholamines or indoleamines synthesis, but it becomes rate-limiting in several pathological states related to aberrant dopamine production, such as Parkinson disease (PD) or the

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[1] *Abbreviations used*: DDC, dopa decarboxylase; AADC, aromatic amino acid decarboxylase; PLP, pyridoxal 5′-phosphate; UPR, unfolded protein response; PD, Parkinson's disease.

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bipolar syndrome [9–11]. In particular, PD is a chronic progressive neurological disorder characterized by tremor, bradykinesia, rigidity and postural instability. These symptons are caused by the low levels of dopamine resulting from the degeneration of dopamine-producing cells in the *substantia nigra* of the brain [12].

DDC is widespread in mammals, insects and plants. In mammals, the enzyme is found in neuronal and non-neuronal tissues. While its presence in neuronal cells is in line with its activity in neuro-modulators synthesis, it is more difficult to explain its abundance in other tissues, for example the kidney, where the enzyme is highly expressed. In insects, in addition to tyramine and octopamine, the major insect hormones, also dopamine and serotonin synthesized by DDC play crucial roles in the behaviour and development of insects [13], and are precursors of compounds contributing to sclerotization of cuticle [14]. Finally, in plants there exist two forms of substrate-specific DDC: a tyrosine decarboxylase and a tryptophan decarboxylase whose peculiar role is related to plant hormone synthesis and plant maturation [15]. Moreover vegetal DDC is also able to produce aromatic alkaloids useful as precursors of pharmaceutically active molecules [15].

Mammalian DDC has been purified from different sources (pig [16], rat [17,18] and human [19–21], although the best studied is the pig kidney enzyme. All three DDCs were cloned and expressed in *Escherichia coli* [19–23] and kinetic, mechanistic and inhibition studies were further performed.

The human versus pig DDC shares 90% sequence identity with 95% positives (this percentage represents the fraction of residues that are either identical or similar), the human versus rat enzyme shares 89% sequence identity with 96% positives. Thus, the three enzymes are closely related and the essential amino acid residues are conserved.

DDC belongs to the α-family of aminotransferases (Fold-Type I), in particular to the subgroup II of α-decarboxylases to which also glutamate decarboxylase and histidine decarboxylase belong. Their chemistry and reaction mechanism bases on the chemical features of the coenzyme: pyridoxal 5′-phosphate (PLP) [24]. Moreover, they share common features, such as similar active site architectures and conserved essential amino acidic residues. The resolution of the structure of histidine decarboxylase in the presence of the inhibitor histidine methyl ester [25] revealed an active site conformation similar to that of DDC in the complex with carbiDopa [26] except for one residue, i.e. Ser-354, of histidine decarboxylase replaced by glycine in the same position in DDC. Interestingly, the S354G variant of histidine decarboxylase presents a decreased activity towards histidine and an increased activity towards L-Dopa [25]. In addition, both decarboxylases possess a mobile loop considered essential for catalysis containing a tyrosine residue (Tyr-332 in DDC and Tyr-334 in histidine decarboxylase) playing a critical role in catalytic activity [25,27].

Until now, much work has been reported on DDC, although reviews found in the literature are mainly focused on its role in PD or on other pathological states related to enzyme deficiency. Several papers, in fact, deal with the presence or the abundance of DDC in various areas of central nervous system and other neuronal cell types. Another line of research is focused on the search for inhibitors with the aim of slowing down, at least, the progression of PD. Finally, a few reviews are involved in structure to function relationship of DDC, while many research articles on catalysis and mechanism exist and are widespread in literature. The study of the biochemistry of DDC pointing to the molecular approach is worthy since it could aid in filling the gap between enzymatic features and cause-to-effect disease relationship.

In this review, an update on DDC kinetic and mechanistic studies will be provided as well as a possible correlation of the features of mammalian DDCs with their functions.

Structural determinants of mammalian DDC

Up to now, the structures of native holo pig kidney DDC, ligand-bound holo pig kidney DDC and apo human DDC have been solved [26,28]. Overall these structures contribute to provide a picture of active site organization. In all three cases, the enzyme is a structural and functional dimer with the typical arrangement of Fold-Type I PLP-enzymes. Previous structural studies of superimposition of the overall sequence and structure of DDC with aspartate aminotransferase [29], bacterial and human glutamate decarboxylase [30,31] and histidine decarboxylase [25] revealed which residues are required to assure fundamental catalytic activities and which ones are specific for each enzymatic species. The knowledge of this molecular basis is fundamental in aimed pharmacological treatment oriented to a specific enzyme.

The information obtained reveals that: (i) the organization of the PLP binding cleft (Fig. 1) is mediated by some essential conserved residues: Lys-303 is the Schiff base lysine residue which covalently attaches the formyl group of PLP, Asp-271 makes a salt bridge interaction with the protonated pyridine nitrogen of PLP, His-192 is the pyridine stacking residue that sandwiches the aromatic ring of PLP, an extensive network of residues, namely Ser-147, Ser-149, Asn-300, His-302 and Phe-309, contributes to the hydrogen binding interactions with PLP and to hold in place the phosphate group of the cofactor; (ii) the localization of the catechol ring of the ligand (carbiDopa) bound to DDC reveals that residues Ile-101′ and Phe-103′ (from the neighboring subunit), and Trp-71, Tyr-79, Phe-80, Thr-82 are involved in substrate binding and cofactor stabilization, finally Thr-246, which faces the opposite face of the PLP ring, is suggested to play a role in the catalytic mechanism [32]; (iii) a stretch comprising residues 327–354 and 328–339 is missing in both human apo and pig holo structure, respectively, highlighting the presence of a flexible loop containing an important conserved residue, Tyr-332, possibly related to catalytic quinonoid protonation step (Scheme 1, see below) [26] and a protease sensitive consensus [33,34].

The relevance of most of the residues mentioned above arises also from an analysis of mutated variants leading to AADC deficiency (OMIM#608643), an inherited rare neuro-metabolic disease. Point mutations in residues interacting directly or indirectly with PLP or its microenvironment (G102S, S147R, S250F, A275T F309L) lead to modified PLP binding [21,35] as expected by 3D inspection. Moreover, many reports on site-directed mutagenesis studies [32,36] corroborate the proposal advanced on the role of the various PLP-binding residues.

Interestingly, it can be observed that the organization of the active site is different in the apo compared to the holo form, the former being in an "open" conformation with respect to the "closed" arrangement of the latter [28]. When PLP is added, an apo to holo transition takes place, determining the rearrangement of a region containing residues 66–84 [28], important for substrate aromatic side chain location, and leading to a movement of the PLP-binding Lys-303 by 6 Å. The dimeric subunits approach one another by moving 20 Å and closing the active site [28]. This movement involves the whole structure making Trp-304, Phe-80 and Tyr-274 stabilized by aromatic stacking interactions among them. Notably, Tyr-274 in the holo form is hydrogen bonded to His-302 that, in turn, interacts with Tyr-79 (see above). This network is present in the holo form prone to substrate binding, while it is absent in the apo form [28]. The biological consequences of this remarkable open-closed conformational change could be multiple: on the one hand, the selective access of PLP to apoDDC could be driven directly by pyridoxal kinase since it was reported that its steric constraints are complementary to the cleft of the open apoDDC [28], on the other, the open enzyme conformation is easily degraded by proteases, suggesting that apoenzyme concentration levels