Structural insight into Parkinson’s disease treatment gained from drug-inhibited DOPA decarboxylase

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DOPA decarboxylase (DDC) is responsible for the synthesis of the key neurotransmitters dopamine and serotonin via decarboxylation of L-3,4-dihydroxyphenylalanine (L-DOPA) and L-5-hydroxytryptophan, respectively. DDC has been implicated in a number of clinic disorders, including Parkinson’s disease and hypertension and peripheral inhibitors of DDC are currently used to treat these diseases. We present the crystal structures of ligand-free DDC and of its complex with the anti-Parkinson drug carbiDOPA. They reveal the binding mode of carbiDOPA and provide the molecular basis for the development of new inhibitors of DDC with better pharmacological characteristics.

We determined the three-dimensional structure of DDC in its ligand-free form and in complex with the anti-Parkinson drug carbiDOPA [1]. The X-ray diffraction data sets from the native protein and from the Ta6Br12 derivative were collected at 100 K on the X11 beam-line (EMBL, DESY Hamburg) at \( \lambda = 0.91 \) Å close to the absorption edge of bromine (\( \lambda = 0.92 \) Å). The low resolution and the high resolution MAD data sets from the Ta6Br12 derivative of a different crystal form were collected at 100 K on the X31 beamline (EMBL, DESY Hamburg) and the BW6 beamline (MPG-ASMB, DESY Hamburg), respectively, at a wavelength of \( \lambda = 1.25 \) Å close to the absorption edge of Tα. Data were processed, integrated, and scaled using DENZO and SCALEPACK. Initial heavy atom positions were obtained from anomalous difference Patterson maps. Heavy atom parameters were refined using a modified version of the program SHARP including a cluster refinement procedure. Phasing was further improved by multiple crystal averaging, solvent-flattening and histogram matching using the program DMMULTI of the CCP4 suite. The partial initial atomic model was ‘automatically’ built with the program wARP. The model was further improved in a cyclic procedure of i) model building using the graphics program O, ii) refinement using the program REFMAC, and iii) multiple crystal averaging using the model phases of the most recently refined model in one crystal form together with phases obtained from the Ta6Br12 derivatives of two other crystal forms using DMMULTI.

DDC is a tightly associated \( \alpha_2 \)-dimer. Each of the two monomers is composed of three distinct domains (Fig. 1a). Their fold is that of the \( \alpha \)-family of pyridoxal-5’-phosphate-dependent enzymes[2], of which aspartate aminotransferase is the prototype. The structures of two other members of this family with decarboxylase activity have been determined so far, bacterial ornithine decarboxylase and dialkylglycine decarboxylase. The larger domain of DDC contains the PLP binding site and consists of a central, seven-stranded mixed \( \beta \)-sheet surrounded by eight \( \alpha \)-helices in a typical \( \alpha \beta \) fold. The C-terminal small domain comprises a four-stranded antiparallel \( \beta \)-sheet with three helices packed against the face opposite the large domain.

The active site of DDC is located near the monomer-monomer interface but is mainly composed of residues from one monomer (Fig. 1b). In the internal aldimine form of the ligand-free DDC, the cofactor PLP binds to Lys 303 through a Schiff base linkage. The inhibitor carbiDOPA binds to the enzyme by forming a hydrazine linkage with the PLP cofactor through its hydrazine moiety thus mimicking the external aldimine enzyme-substrate intermediate. The catechol ring of carbiDOPA is deeply buried in the active site cleft and penetrates even behind the cofactor ring plane (Fig. 1b).
The crystal structure of DDC would assist in the design of more potent DDC inhibitors compared to carbiDOPA or benserazide. Such new drugs with better pharmacological characteristics would allow to reduce the large dose (up to 1g/day) of L-DOPA needed for efficient treatment of PD and thus also reduce the very undesirable side effects of the most commonly used drugs in its treatment.

Figure 1: a) Stereo view of the polypeptide backbone of DDC as a ribbon diagram. The view is directly down the twofold symmetry axis. One monomer is red whereas the other is green (N-terminal domain), cyan (large domain), and blue (small domain). b) Stereo presentation of the electron density of the inhibitor carbiDOPA. The difference electron density \((|F_o| - |F_c|)\) map with the inhibitor excluded from the phase calculation) in red, contoured at 4\(\sigma\), is superimposed onto the inhibitor model.

References
