CRYSTAL STRUCTURE OF HUMAN DOPAMINE BETA-HYDROXYLASE
A crystalline form of dopamine β-hydroxylase is provided. X-ray crystallography reveals the space group and cell dimensions, as well as the atomic coordinates. The information can be used for identifying one or more modulators of dopamine β-hydroxylase, which can then be chemically synthesised and used in treatment. A process for preparing the crystalline form of human dopamine β-hydroxylase is also provided.

The crystal structure of human dopamine β-hydroxylase at 2.9 Å resolution
The norepinephrine pathway is believed to modulate behavioral and physiological processes, such as mood, overall arousal, and attention. Furthermore, abnormalities in the pathway have been linked to numerous diseases, for example hypertension, depression, anxiety, Parkinson’s disease, schizophrenia, Alzheimer’s disease, attention deficit hyperactivity disorder, and cocaine dependence. We report the crystal structure of human dopamine β-hydroxylase, which is the enzyme converting dopamine to norepinephrine. The structure of the DOMON (dopamine β-monooxygenase N-terminal) domain, also found in >1600 other proteins, reveals a possible metal-binding site and a ligand-binding pocket. The catalytic core structure shows two different conformations: an open active site, as also seen in another member of this enzyme family [the peptidylglycine α-hydroxylating (and α-amidating) monooxygenase], and a closed active site structure, in which the two copper-binding sites are only 4 to 5 Å apart, in what might be a coupled binuclear copper site. The dimerization domain adopts a conformation that bears no resemblance to any other known protein structure. The structure provides new molecular insights into the numerous devastating disorders of both physiological and neurological origins associated with the dopamine system.
Expression, purification and characterization of human Dopamine β-monooxygenase

This thesis deals with expression, purification and characterization of the copper containing enzyme dopamine β-monooxygenase (DBM). DBM is an ascorbate dependent protein that requires Cu in the active site in order to be functional. DBM is made of four domains; An N-terminal DOMON domain, the two catalytic domains called ascorbate dependent type IImonooxygenase domains and a C-terminal dimerization domain. DBM is related to peptidylglycine a-hydroxylating monooxygenase (PHM). They are 28 % identical over approximately 300 amino acids (AA) which corresponds to the catalytic domains. This is, among others, one of the reasons why these proteins are considered to follow the same mechanism. DBM converts dopamine (DA) into Norepinephrine (NE). Both substrate and product functions as neurotransmitters and the levels of these are involved in many different disorders such as depression and hypertension and also the reason why DBM in the past decades has been considered an important therapeutic target. The ambition with this project was to develop a solid and efficient expression system for human DBM (hDBM) and subsequent to characterize it with mass spectrometry (MS) and X-ray crystallography.

hDBM has been successfully expressed in the mammalian HEK293S cells in a stable glycosylated form. The protein has been purified to a very high degree of purity. The purification was done with a FLAG-tag purification followed by a size exclusion step. The protein exists in to active homo-oligomer forms, a dimer and a tetramer. The dimer is held together by two intermolecular disulfide bonds (dsb), while the tetramer consists of two dimers held together by noncovalent interaction. The size and extent of glycosylation and other post translational modifications (PTM) has been investigated in both forms using intact MS analysis and it is concluded that they both contain four oligomannose groups primarily in the GlcNAc2MAN5 form. This means that the enzyme despite the extent of glycosylation is very homogenous. The extent of other PTMs is minimal. The equilibrium between the two oligomer forms is likewise looked into with MS, in regards to pH and ionic strength and it is concluded that the tetramer is stable under the investigated conditions. The stability of the fully glycosylated dimer and tetramer and a partly deglycosylated tetramer was investigated with differential scanning fluorimetry (DSF). It was found that the tetramer was significantly more stable than the dimer and that to two tetramer forms were equally stable. The effect of ascorbic acid (Asc) and DA on the stability of the protein was also evaluated with DSF, and the conclusion was that the presence of both substrates had a significant effect on the stability of all forms of hDBM. The results from DSF support the result from MS regarding the equilibrium between the two forms. They are not in equilibrium and the presence of DA and Asc do not affect that. Both oligomers were used for crystallization trials and the dimer formed crystals with a 2.9 Å resolution. The structure could however not be solved from molecular replacement (MR) alone and Selenomethionine (Ssemet) substituted hDBM was hence produced and likewise formed crystals from where the experimental phase could be solved. The first three dimensional (3D) structure of DBM dimer, going from residue 47-596 in each chain, was hereafter manually built. The structure reveals the first structural insights into the DOMON domain and the C-terminal dimerization domain and it shows two different conformations of the catalytic domains. An open conformation, that resembles the structures known from PHM and a closed conformation that brings the two copper sites very close. The closed confirmation has never been observed in PHM and it opens up for a new understanding of how these enzymes functions.

This project has contributed with a lot of new and significant knowledge concerning DBM and in addition provided a stable and efficient expression system which is decisive for future characterization of this fascinating protein.

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A simple two step procedure for purification of the catalytic domain of chicken tryptophan hydroxylase 1 in a form suitable for crystallization

Tryptophan hydroxylase (TPH) [EC 1.14.16.4] catalyzes the conversion of tryptophan to 5-hydroxytryptophan, which is the first and rate-determining step in the biosynthesis of the neurotransmitter serotonin. We have expressed the catalytic domain of chicken (Gallus gallus) TPH isoform 1 in Escherichia coli in high yield. The enzyme was highly purified using only one anion exchange and one gel filtration, with a yield of 11 mg/L culture and a specific activity of 0.60 μmol/min/mg. The Km values were determined to Km,tryptophan = 7.7 ± 0.7 μM, Km,BH4=324±10 μM and Km,O2=39±2 μM. Substrate inhibition by tryptophan was observed at concentrations above 15 μM. Furthermore, the purified enzyme has been crystallized without 7,8-dihydro-l-biopterin and a data set to 3 Å resolution has been collected.
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Udtrykkelse, oprensning og karakterisering af metal-holdige Monooxygenaser involveret i neuropsykiatriske sygdommer

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