

# Crystal structures of rat neuronal NOS heme domain complexed with substrate or various inhibitors

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## Introduction

Nitric oxide (NO), an important biomediator, is synthesized by nitric oxide synthase (NOS) in mammals. Three isoforms of NOS, neuronal NOS, inducible NOS and endothelial NOS, have been isolated from different tissues and cell types and found involved in important physiological processes such as neurotransmission, immune response, and vascular tone regulation. However, uncontrolled overproduction of NO has been implicated in a wide variety of diseases. Inhibition of NO production would be, therefore, beneficial to the therapeutic treatment of those diseases. Because regulated NO production by three NOS isoforms is essential to many normal physiological functions, it is highly desirable to have NOS inhibitors with isoform selectivity. In this way, inhibition of NO production by one NOS isoform in a pathological state could still leave the physiological NO production of the other NOS isoform untouched. To achieve this goal, a wealth of structural information needs to be accumulated by determining crystal structures of all three NOS isoforms complexed with a wide variety of inhibitors. As part of these efforts, here we report, for the first time, the crystal structures of a neuronal NOS heme domain in the presence of substrate, L-arginine, or of a few other inhibitors including N<sup>G</sup>-nitro-L-arginine, N<sup>G</sup>-allyl-L-arginine, S-ethyl-N-phenylisothiourea, and S-ethyl-N-(4-thifluoromethyl)-phenylisothiourea, respectively.

## Methods

The initial 2.2 Å data of rat neuronal NOS heme domain were collected at ALS BL5.0.2 at liquid nitrogen temperature. The crystals with substrate bound belong to space group of P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> with cell dimensions of a = 52.0 Å, b = 111.4 Å, c = 165.2 Å. The structure of nNOS heme domain was solved by molecular replacement with AMORE using the coordinates of eNOS heme domain as the search model. The nNOS data sets with other inhibitors bound were collected at SSRL BL7-1. The subsequent model building and refinements were carried out with O and CNS, respectively.

## Results and Discussion

Two amino acid based inhibitors, N<sup>G</sup>-nitro-L-arginine (NNA) and N<sup>G</sup>-allyl-L-arginine (ALR), were found at the substrate binding site next to the heme. As expected these two inhibitors share all the H-bonds the substrate has with the protein surroundings. In addition, the nitro- or allyl- group of the two inhibitors provide new hydrogen bonding or hydrophobic interactions with protein, resulting in tighter binding affinity for these ligands compared to the substrate. ALR is actually also a suicide inhibitor. This is because its substituted guanidino nitrogen still bears a proton and locates next to the heme iron which allows the NADPH- and O<sub>2</sub>-dependent turnover to occur, but the heme group being modified concomitantly by the allyl moiety from the inhibitor.

Two isothiourea inhibitors, S-ethyl-N-phenylisothiourea, and S-ethyl-N-(4-trifluoromethyl)-phenylisothiourea, bind to the active site by mimicking the H-bonding interactions of the guanidino group of arginine with their ureido function. The S-ethyl group makes von der Waals contacts with hydrophobic amino acid side chains while the N-phenyl group pointing toward the second heme propionate. The phenyl ring provides an excellent template to attach various functional groups for the structure-activity-relationship (SAR) studies. The trifluoromethyl substituent was found to exhibit good selection for nNOS. The N-substituted isothioureas also provide a mean to further explore the potential of isoform specific inhibitor binding into the open substrate access channel where amino acid variations exist among three NOS isoforms.

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