

University of Groningen

Structural basis for the oxygenation of flavonols by flavonol 2,3-dioxygenase.

Steiner, R.A.; Egmond, M.R.; Dijkstra, B.W.

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2000

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Steiner, R. A., Egmond, M. R., & Dijkstra, B. W., (2000). Structural basis for the oxygenation of flavonols by flavonol 2,3-dioxygenase., 1 p.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

s8a.m1.o3 Structural basis for the oxygenation of flavonols by flavonol 2,3-dioxygenase. R.A. Steiner[#], P.I. van Noort[†], M.R. Egmond[†] and B. W. Dijkstra[#] [#] Department of Biophysical Chemistry, University of Groningen Nijenborgh 4, 9747 AG Groningen, the Netherlands[†] Unilever Research Laboratory Olivier van Noortlaan 120, 3133 AT Vlaardingen, the Netherlands
Keywords: enzyme catalysis, protein engineering.

Dioxygenases are enzymes that catalyse the incorporation of both oxygen atoms of molecular oxygen into the substrate.

O₂ mainly due to its triplet (³Σ_g) ground state possesses under physiological conditions a low kinetic reactivity towards organic compounds which generally exhibit a singlet fundamental state. In order to circumvent the spin selection rule biological systems have evolved several pathways. Complexion to a transition metal is a method often employed as activation route. Iron, in both haem and non-haem forms, is the co-factor commonly found in dioxygenases.

Flavonol 2,3 dioxygenase (FDO) from *Aspergillus japonicus* is unique among dioxygenases because it contains only one cupric copper ion per molecule and no other co-factors¹.

FDO catalyses the oxidation of flavonols (3-hydroxy flavones) to yield carbon monoxide and the relative depside (phenolic carboxylic acid ester). Since FDO has been reported for the first time in the degradation pathway of quercetin (3,5,7,3',4'-pentahydroxy flavone) it is also known as Quercetinase.

Relevant to FDO are the two classes of iron dioxygenases (intradiol and extradiol dioxygenases) containing non-haem iron as sole co-factor. It has been proposed that intradiol dioxygenases activate the metal bound substrate whilst the extradiol type activates the dioxygen bound to the ferrous ion². In all the postulated mechanisms for non-haem iron dioxygenases indicate, anyway, a direct co-ordination of molecular oxygen to the metal centre at some stage of the process.

Anaerobic and aerobic structural studies suggest a possible reaction mechanism for this peculiar enzyme.

s8a.m1.o4 Crystallographic studies of the interaction between the Ferredoxin:NADP⁺:Reductase and Ferredoxin R. Morales, M.H. Charon, M. Frey, LCCP, Institut de Biologie Structurale J.P. Ebel, CEA-CNRS, 41 rue J. Horowitz 38027 Grenoble.

Keywords: enzyme catalysis, protein engineering.

Ferredoxin:NADP⁺:Reductase (FNR) catalyses one terminal step of conversion of light energy into chemical energy during photosynthesis. FNR uses two photoproduced high energy electrons conveyed, one by one, from the photosystem I by a ferredoxin (Fd) to catalyse the production of NADPH. Electron transfer between FNR and Fd requires the formation of a ternary NADP⁺/FNR/Fd complex¹.

We have solved the structure of a crystallographic complex between Fd and FNR from the cyanobacterium *Anabaena* PCC7119 at 2.4 Å resolution. This gave the first three-dimensional picture of a Fd/FNR biologically relevant complex.

The crystal cell parameters are a=b= 63.72 Å and c= 158.02 Å; space group P2₁2₁2₁.

The asymmetric unit contains two FNR (FNR1 and FNR2, molecular weight, mw, :2×35 kDa) and one Ferredoxin (mw :11 kDa) molecules. The packing of the FNR molecules displays a nearly tetragonal symmetry (S.G. P4₃2₁2) whereas the Fd arrangement is orthorhombic (S.G. P2₁2₁2₁).

For the computation, the crystal was treated as a merohedral twin with two components related by a [110] dyad axis. This approach proved to be a very powerful tool to locate this elusive ferredoxin and to obtain fully interpretable electron density maps.

[1] Oka, T. and Simpson, F.J. (1971). Quercetinase, a dioxygenase containing copper. *Biochem and Biophys. Research Comm.* **43**, 1-5.

[2] Howard, J.B. and Rees, D.C. (1991). Perspectives on non-heme protein chemistry. *Advan. Protein Chem.* **42**, 199-280.

[1] Batic C. "FERREDOXIN:NADP⁺ OXIDASE Equilibria in Binary and Ternary Complexes with NADP⁺ and Fd.", *J. Biol. Chem.* (1984), 259: 8832 - 8839.