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

O_2 mainly due to its triplet ($^3\Sigma_g$) 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 system 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-factors[1].

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 ion[2]. In all the postulated mechanisms for non-heam 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.

[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.

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.*
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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 complex[1].

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 $P2_12_12_1$.

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. $P4_32_12$) whereas the Fd arrangement is orthorhombic (S.G. $P2_12_12_1$).

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