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Project 4. Enzymes of the flavonoid biosynthic pathway in *Vitis vinifera* :
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PROPOSAL

Proteins under investigation concern enzymes involved in the flavonoid biosynthesis (1) in grape (*Vitis vinifera*), a plant model of particular interest, according to its high content in two different flavonoid subclasses : anthocyanins and condensed tannins. The former are mainly responsible for the wine colour attributes whereas the latter provide sensorial properties such as astringency or bitterness. Therefore, their relative content is a key parameter which affects both quality and wine behaviour during aging. Besides potential important economic applications in the winemaker's world, grape is considered as a perfect candidate to understand the biochemical mechanisms driving the flavonoid expression and levels during fruit development. The biosynthetic pathway of anthocyanins and condensed tannins includes :

- **(i) three NADPH dependent enzymes (DFR : dihydroflavonol reductase, LAR : leucoanthocyanidin reductase, ANR : anthocyanidin reductase) which belong to the short-chain dehydrogenase/reductase (SDR) family (2),**
- **(ii) the anthocyanidin synthase (ANS), a 2-oxoglutarate iron-dependent oxygenase which initiates the biosynthesis of anthocyanins via a multicomponent enzymatic reaction.**

DFR, LAR, ANR and ANS enzymes (Mw 40 kDa) are expressed in E. coli and purified. Wild type with substrate and cofactor and Se-Met derived DFR datasets were obtained (2.5 Å resolution P212121; 87 x 89 x 93 Å). First crystallites for ANS and LAR have been obtained. MAD data collections are required.

REPORT

Dihydroflavonol 4-reductase (DFR; EC 1.1.1.219) is a key enzyme of the flavonoid biosynthetic pathway leading to both anthocyanins and condensed tannins in plants. This enzyme (39.4 kDa, 354 a.a.), known to belong to the Short-chain Dehydrogenase Reductase (SDR) family, yields flavan-3,4-diols by the reduction of corresponding dihydroflavonols in presence of NADPH. Despite the fact that a number of DFR cDNAs have been isolated from various plants and that the corresponding proteins were expressed in *E. coli* and purified, no structural data were available up to now.
In the course of our structural investigation of enzymes involved in the flavonoid pathway in *Vitis vinifera*, we succeeded in growing two different crystal forms of the native enzyme obtained by co-crystallisation with $NADP^+$ and dihydroquercetin.

Several crystals of the first form (diamond shaped) were tested. All exhibited anisotropic diffraction, diffracted to a poor resolution limit and appeared to be twinned.

Crystals of the second form (needles) diffracted up to 1.8 Å on BM30A and lead to the orthorhombic $P2_12_12_1$ space group (a = 87.4 Å, b = 89.62 Å, c = 92.81 Å) with 2 molecules in the asymmetric unit. To solve the structure, five MAD data sets were recorded at respectively E= 12642, 12640 and 12740 eV for crystals of the corresponding Se-met substituted protein. One of these allowed us to find the positions of the 26 Se atoms of the dimer, using the SHELX program suite and to build a model starting from the obtained electron density map.

Crystallographic data of the phasing data set:

	Peak	Edge	Remote
Resolution	**2.33**	**2.33**	**2.33**
Rmerge	**0.051 (0.126)**	**0.048 (0.158)**	**0.049 (0.196)**
MeanI/σ	**17.6 (4.1)**	**17.5 (3.8)**	**15.4 (3.2)**
Completness	**92.2 (49.8)**	**92.1 (48.4)**	**91.3 (48.7)**
Multiplicity	**2.0 (1.2)**	**2.0 (1.1)**	**2.0 (1.2)**

According to these data, the structure of the native enzyme has been refined ($R_{working\ set}$: 0.192; R_{free} : 0.244) and the atomic coordinates have been deposited at the PDB (PDB code: 2C29).

(1) Winkel-Shirley, B. (2001). Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol 126(2) 485-93.
(2) Jornvall, H., B. Persson, et al. (1995). Short-chain dehydrogenases/reductases (SDR). Biochemistry 34(18) 6003-13.

2C29 : First structure determination of a dihydroflavonol reductase. A typical example from *vitis vinifera* at 1.8 Å.