

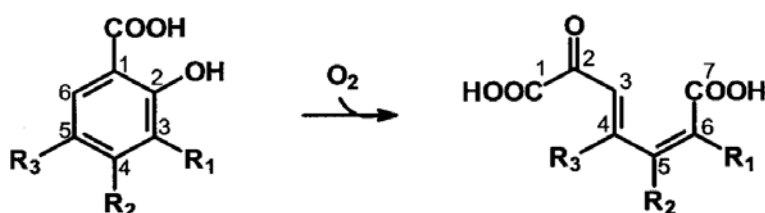
Crystal Structure Determination of a Salicylate 1,2-Dioxygenase from *Pseudaminobacter salicylatoxidans*

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The oxygenolytic cleavage of the aromatic nucleus by bacteria requires in most cases the presence of two hydroxyl groups attached to the aromatic ring. Only a few examples have been previously described in which monohydroxylated aromatic compounds were cleaved by ring fission dioxygenases. Recently a new ring fission dioxygenase from the naphthalenesulfonate-degrading strain *Pseudaminobacter salicylatoxidans*, which oxidized salicylate by a novel ring fission mechanism to 2-oxohepta-3,5-dienedioic acid has been described [1].



The salicylate dioxygenase activity from *P. salicylatoxidans* BN12 is rather unique among the currently known ring fission dioxygenases because the enzyme is able to cleave various substituted salicylates that carry only a single hydroxy group and that are not activated by additional electron-donating substituents for a ring fission reaction. Previous biochemical characterization of the salicylate dioxygenase activity from *P. salicylatoxidans* BN12 demonstrated that the enzyme converted gentisate, 5-aminosalicylate, and 1-hydroxy-2-naphthoate with much higher catalytic activities compared with salicylate and suggested that the ring fission dioxygenase was also structurally similar to gentisate 1,2-dioxygenase or 1-hydroxy-2-naphthoate dioxygenase. This was indicated by the size of the subunits, the structure of the holoenzyme, and the dependence of the enzyme from Fe²⁺ ions. Nevertheless, it became evident that the ring fission dioxygenase from *P. salicylatoxidans* was clearly different from the presently known gentisate 1,2-dioxygenases or 1-hydroxy-2-naphthoate dioxygenases because of its unique ability to oxidatively cleave salicylate and also the ability to cleave gentisate and 1-hydroxy-2-naphthoate with high catalytic efficiencies [1].

The enzyme from *P. salicylatoxidans* BN12 was heterologously expressed in *Escherichia coli* and purified as a His-tagged enzyme variant. The deduced amino acid sequence encoded a protein with a molecular mass of 41,176 Da, which showed 28 and 31% sequence identity, respectively, to a gentisate 1,2-dioxygenase from *Pseudomonas alcaligenes* NCIMB 9867 and a 1-hydroxy-2-naphthoate 1,2-dioxygenase from *Nocardioides* sp. KP7 [1].

In order to allow a more detailed analysis of the relationship between the mechanistic capabilities of this particular ring fission dioxygenase and its structural features, this enzyme was crystallized and x-ray diffraction data were collected.

Crystallization experiments were performed using the sitting drop vapor diffusion method. Diffraction quality crystals were obtained at 277 K from a solution containing 12% w/v EtOH, 4% w/v PEG400, 0.1 M sodium acetate pH 4.6. Drops were prepared using 1 μ l protein solution mixed with 1 μ l reservoir solution and were equilibrated against 100 μ l precipitant solution [2].

A complete data set at 100 K extending to a maximum resolution of 2.9 Å was collected at the EMBL beamline BW7B, Hamburg, Germany. Data were collected adding 30% glycerol to the mother liquor as cryoprotectant, using a MAR345 image plate detector and a wavelength of 0.8423 Å. Crystals belong to the primitive tetragonal space group P4₃2₁2 with unit cell dimensions a=133.3, c=191.51. Assuming one tetramer per asymmetric unit the solvent content is 47% of the unit cell (V_m = 2.3 Å³/Da). Data processing with Mosflm and SCALA gave 38218 unique reflections, an R_{symm} of 12.1 % and an overall completeness of 99.0%. Attempts were made in order to solve the structure of the enzyme by a MAD experiment using the anomalous signal of the catalytic Fe(II). From these experiments resulted that the iron content in the native protein was too

low and prevented us to solve the structure by this technique. Molecular replacement using coordinates of an extradiol dioxygenase structure recently solved, gentisate 1,2-dioxygenase from *Escherichia coli* O157:H7 (PDB accession code 2D40) [3], as a model succeeded in finding a solution for salicylate 1,2 dioxygenase. The two enzymes are both homotetramer and a sequence comparison between the two monomeric subunits shows a relatively low sequence identity (33.3%). Refinement of the model using the program Refmac is still under work. In the active site the Fe(II) ion is coordinated by three histidines (His119, His121, His160) and at least one water molecule. The detailed analysis of the structural data is underway.

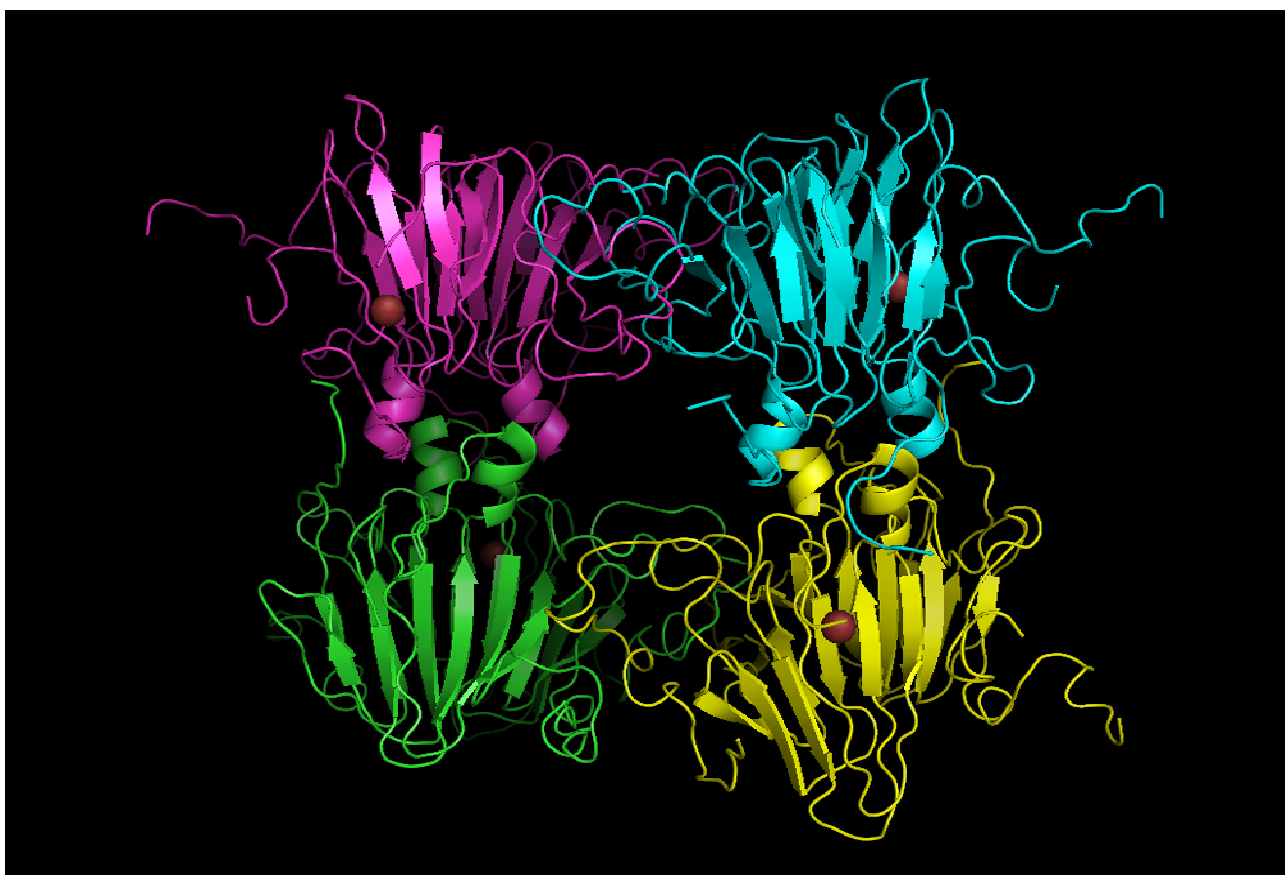


Figure 1: Crystallographic model of salicylate 1,2-dioxygenase from *Pseudaminobacter salicylatoxidans*

References

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