Comparative characterization of the active site of new intradiol ring cleaving dioxygenases with different substrate specificity involved in biodegradation reactions

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Ring cleaving dioxygenases are ubiquitous in aerobic microorganisms able to use aromatic compounds as energy and carbon sources and generally involved in biodegradation processes.

A variety of intra- and extra-diol dioxygenases with different substrate specificity and oligomeric compositions have been isolated and purified from different microorganisms. We have studied in past years the Fe(II) containing extra-diol cathechol 2,3 dioxygenase and its adducts with substrate and inhibitors.

Recently we have isolated intra-diol ring cleaving dioxygenases, highly specific for various substrates like hydroxyquinol, 3-chlorocatechol, and other substituted catechols.

A highly specific intra-diol dioxygenase has been isolated from *Nocardioides simplex* 3E strain, which cleaves the aromatic ring of hydroxyquinol, or 6-chloro- and 5-chlorohydroxyquinol [1]. This enzyme is an homodimer made of two identical 37-kDa subunits in with only one iron/74 kDa.

We have also purified from *Pseudomonas sp. B13* two catechol 1,2-dioxygenases: one specific for catechol and the other active towards a broad range of halogen substituted catechols. Both are dimers containing about one iron(III) ion per dimer essential for their activity. A chloro-catechol 1,2 dioxygenase has been isolated and purified from *Rhodomonas erhythropolis* 1CP, which specifically cleaves the intradiol bond of 4-chlorocatechol.

A strain of *Acinetobacter radioresistans* has been used for the extraction of two further catechol 1,2-dioxygenases they contain one iron(III) ion in their active site and are the first example of monomeric intra-diol dioxygenases [2].

All the above enzymes have active sites containing mononuclear Fe(III) ions, but have different substrates and different specificity, some of them being highly specific while others show broad substrate specificity.

We have characterized by XAS spectroscopy the hydroxyquinol (1,2,4 trihydroxybenzene) 1,2-dioxygenase (HQ1,2O) from *Nocardiodes simplex* 3E and the chlorocatechol 1,2-dioxygenase (ClC1,2O) from *Rhodococcus erythropolis* 1CP [3]. We have collected high quality data on the two native enzymes and on the adducts with their respective specific substrates. The analysis of the spectra shows that the two native enzymes share a very similar active site structure consisting of five coordinate iron(III) ions bound to oxygen/nitrogen atoms distributed over two distance shells centered at 1.90 and 2.09 Å and 1.92 and 2.12 Å for HQ1,2O and ClC1,2O respectively. The nitrogen atoms are provided by two histidine residues while the short Fe - O distance is consistent with the presence of tyrosinate ligands as indicated by the presence of LTMCT absorption bands in the enzymes visible spectra [1-2]. The reaction with the substrate leaves unaffected the overall coordination of the ferric ion as evidenced by the pre-edge and EXAFS analysis, but causes evident changes in both the XANES and EXAFS regions of the spectra indicative of the substrate binding to

the metal ion. The analysis reveals that the distance spread in the iron first coordination shell has been reduced by at least 0.05 Å originating a more regular distribution of the ligands around the metal. The results clearly indicate that in these enzymes substrate specificity is not influenced by the iron coordinated ligands. Therefore specific interactions with residues in the active site cavity are likely to be involved in the recognition of substrates.
The project has been carried over by performing test experiments on new manganese dependent catechol dioxygenases which seem to contain a mononuclear Mn(II) center in their active site.

References

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