Elucidation of the reaction mechanism of hydroxynitrile lyase from *Hevea brasiliensis*

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The crystal structure of the enzyme hydroxynitrile lyase (HNL) from *Hevea brasiliensis* (Hb-HNL) was determined in our laboratory, and refined against data extending to a crystallographic resolution of 1.9 Å [1,2]. Very recently, data extending to 1.1 Å resolution have been collected at beamline BW7B.

The enzyme belongs to the α/β-hydrolase superfamily [3], with an active site that is deeply buried within the protein and connected to the outside by a narrow tunnel. The α/β fold had already been deduced from the primary structure [4], with the catalytic triad consisting of residues Ser-80, His-235, and Asp-207. By analogy with other α/β-hydrolases [3], we proposed a mechanism for the HNL-catalyzed cleavage of cyanohydrins [2], stipulating the formation of a hemiketal or hemiacetal intermediate covalently attached to the nucleophile Ser-80. This mechanism was also consistent with the available mutagenesis data. However, a different mechanism devoid of a covalent intermediate was proposed from another laboratory [5] for the closely related enzyme from *Manihot esculenta*.

Here, we report crystal structures of several of enzyme-substrate and enzyme-inhibitor complexes in order to contribute to the question of the enzyme mechanism. Complexes were prepared by soaking a HNL crystal in a solution containing the inhibitor and subsequent flash-cooling to 100K, at which temperature diffraction data were collected.

**Trichloroacetaldehyde.** The observed density is best interpreted in terms of a covalent hemiacetal formed as a result of a nucleophilic addition of the Ser-80 hydroxyl group to the aldehyde's carbonyl group. However, scrutiny of this structure as a model for an intermediate of the enzyme-catalyzed cyanohydrin reaction reveals a problem with the stereochemistry: if one assumes that the substrate benzaldehyde would form an analogous intermediate in the course of the HNL-catalyzed mandelonitrile synthesis, with the phenyl ring occupying approximately the position of the trichloromethyl group, then a nucleophilic SN2-type substitution of the Ser-80 oxygen by cyanide would lead to the product mandelonitrile with the wrong stereochemistry.

**Acetone.** Unequivocal assignment of the position and orientation of the HNL-bound acetone molecule was difficult since the acetone apparently does not form a covalent bond to the enzyme. Instead, it occupies a position replacing a glycerol molecule observed in the native low-temperature structure. The acetone substrate molecule is observed within hydrogen bonding distance to the Ser-80 oxygen atom.

**Hexafluoroacetone and Hexachloroacetone.** Both perhalogenated acetone derivatives were observed as the corresponding hydrates in similar positions as acetone, also with no covalent attachment to the enzyme.
Crotonaldehyde. Again, no indication of a covalent attachment between the substrate and the enzyme could be detected. However, the orientation of the aldehyde's carbonyl function is quite different from the one observed with acetone.

The combined crystallographic evidence argues against a mechanism involving covalent attachment of the substrate to the enzyme.

![Observed electron density within the active site of the hydroxynitrile lyase from Hevea brasiliensis with a variety of substrate- and inhibitor molecules. Top line from left to right: acetone, native structure at room temperature, trichloroacetaldehyde; Bottom line: hexachloroacetone hydrate, hexafluoroacetone hydrate, crotonaldehyde.](image)

Figure 1

References