Rice Beta-Glycosidase Structure-function Analysis

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During this period, we investigated the structures of rice Os3BGlu7 (BGlu1), Os3BGlu6, Os3BGlu12, and Os7BGlu26 family GH1 glycoside hydrolases in complexes with various potential ligands, such as substrates and mechanism-based inhibitors by X-ray crystallography. These studies were intended to give insight into the structural basis of the differences in substrate specificity and other properties demonstrated by these enzymes.

For Os3BGlu7, we were primarily studying mutants that were made to engineer the enzyme for synthesis reactions and to view native substrates in the active site. We produced crystal structures of the glycosynthase mutants E386G, E386A and E386S in complexes with the glycosynthase donor alpha-fluoroglucoside, and potential acceptor substrates, such as 4-nitrophenyl-beta-cellobioside, cellotetraose and cellopentaose. However, although the mutant proteins could be crystallized and their structures solved with alpha-fluoroglucoside, none showed significant density for the acceptor substrate 4-nitrophenyl-cellobioside, and only the cellotetraose and cellopentaose seen in the E386G mutant active site were in the positions to act as substrates for hydrolysis, rather than glycosynthase acceptors. Comparison of the different mutants has yet to announce any obvious reasons why the E386G is a much better catalyst for glycosynthase reactions [1]. An acid base mutant of Os3BGlu7 was used to look at the covalent intermediate structure of this enzyme with mannosides rather than glucosides. These crystals yielded structures with clear conformations of the mannoside covalent intermediate, which provides new insights into the mechanism of mannoside hydrolysis.

For Os3BGlu6, the structures of the apo enzyme and the covalent intermediate with 2-fluoro-glucoside were completed. A third structure with n-octyl-beta-glucoside was also solved, which showed the binding of the substrate analogue in the active site, giving clues to the basis of substrate specificity [2]. Interestingly, the inclusion of n-octyl-thioglucoside increased the length of the unit cell in the longest dimension by 10%, although the only significant difference in the protein structure was a change in the position of a loop that does not interact with other molecules (Fig. 1). Subsequently, we collected data for an acid/base mutant of this enzyme in complexes with natural glycoside substrates from rice, but we were unable to obtain the density for these ligands.

Previously, we had collected data to 2.5 Å resolution for Os4BGlu12 and its complex with 2,4-dinitrophenyl 2-deoxy-2-fluoroglucoside. However, the mechanim-based inhibitor did not form a covalent complex, as anticipated. We collected better data for these complexes and proteins soaked for different periods in the inhibitor and were able to obtain density for the covalent 2-fluoro-glucoside intermediate in this period. Mutations of this enzyme were also made in order to produce enzyme-substrate complexes, but most of these gave poor densities for the ligands. The crystallization and preliminary structure determination paper for Os4BGlu12 is currently under revision for publication.

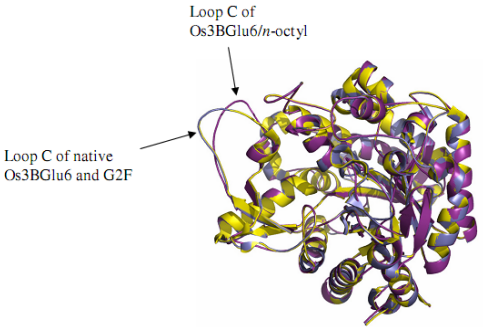

Fig. 1: The structures of rice Os3BGlu6 in complex with 2-deoxy-2-fluoroglucoside (yellow) and with n-octyl thioglucoside (purple) showing the main difference lies in a solvent-exposed loop.

The rice Os7BGlu26 enzyme is primarily a beta-mannosidase, and Os3BGlu7, while primarily a beta-glucosidase, has significant beta-mannosidase activity, however, Os3Bglu6 and Os4BGlu12 do not. In order to delineate the structural basis for beta-mannosidase activity, we have crystallized and solved the structure of Os7BGlu26. The structure was quite similar to that of Os3BGlu7, with which it shares nearly 70% amino acid sequence identity, so we are currently producing complexes of this enzyme with glucosides and mannosides to compare to the Os3BGlu7 complexes. We hope this will allow us to rationalize why Os7BGlu26 has high beta-mannosidase relative to beta-glucosidase activity, while the other enzymes under structural study are primarily beta-glucosidases.

References

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