Trapping the covalent intermediate in an α-retaining glycosyl hydrolase

J.C.M. Uitdehaag, R. Mosi[1], S.G. Withers[1] and B.W. Dijkstra

BIOSON Research Institute and GBB Institute, Laboratory of Biophysical Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, the Netherlands.

[1]Department of Chemistry, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z1.

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Glycosyl hydrolases are enzymes involved in the processing of oligosaccharides. Despite the many varieties of carbohydrates put to use by Nature the similarity of their chemical building blocks has led to glycosyl hydrolases employing very similar enzymatic mechanisms. The best known glycosyl hydrolase mechanism is that of hen egg white lysozyme (HEWL), in which two bond cleavage events separated by an intermediate give rise to a product with an anomeric configuration (i.e. β or α) identical to the substrate. This retaining mechanism has been extrapolated to other glycosyl hydrolases that process either β-glycosidic bonds (β-retainers, like HEWL), or α-glycosidic bonds (α-retainers), e.g. the α-amylase family of which the enzyme we study, cyclodextrin glycosyltransferase (CGTase) is a member.

The chemical nature of the intermediate in the lysozyme mechanism is one of the most fascinating controversies in enzymology. Most textbooks claim a charged oxocarbenium ion, stabilized by the carboxylate of a catalytic residue. Alternatively, the intermediate can be covalently bound to that catalytic residue. In the last few years, this latter theory has gained much support by both crystallographic and mass-spectroscopy experiments on different β-retaining enzymes [1,2]. For α-retaining enzymes, a covalent intermediate was trapped by mass-spectroscopy experiments on CGTase [3], but the 3D structure of such an intermediate was never elucidated.

Data Collection	**intermediate**	**Refinement statistics**	
X-ray source	wiggler beamline BW7B, EMBL/DESY Hamburg	No. of amino acids	686 (all)
pH, temperature	6.1, 100 K	active site ligand	4-deoxy maltotriose
Spacegroup	$P2_12_12_1$	No. of solvent sites	666
Cell axes a, b, c (Å)	117.1, 109.3, 65.3	Average B factor (Å^2)	17.7
Resolution range (Å)	58.5 - 1.81	Final R factor (%)	15.5
No. of unique reflections	73,264	Final free R factor (%)	18.9
R_{merge} (%)	5.3	**R.m.s. deviation from ideal geometry**	
Completeness (%)	94.9	bond lengths (Å)	0.006
Completeness (%) in the last resolution shell (Å)	49.9 (1.83 - 1.80)	v.d. Waals contacts (Å)	0.011
		B factor correlations (Å^2)	1.566

Table 1: Data statistics and final model quality

We repeated the experiment that trapped the intermediate in CGTase [3] with a crystal to obtain a 3D structure. For 16 minutes we soaked a crystal of *Bacillus circulans* strain 251 E257Q CGTase in 125 mM 4-deoxymaltotriosyl α-fluoride, 60% MPD and 100 mM MES pH 6.1, and then froze it to 100 K. Data was collected on EMBL beamline BW7B to 1.8 Å, details in Table 1. Shown in Figure 1 is the final OMIT $2F_O$-F_C (1σ contoured) electron density for the intermediate, with an unequivocal covalent bond to the catalytic nucleophile Asp 229, the first such structure for an α-retaining glycosyl hydrolase.

The CGTase-intermediate structure gives much information on how enzymes in the α-amylase family catalyze bond cleavage reactions. Currently we are focusing on how CGTase achieves its unique specificity, the formation of cyclodextrins. In data collected at EMBL Hamburg, we have recently been able to identify the enzyme's product, a cyclodextrin, bound in its active site. To further elucidate product size specificity we are now aiming at binding different sizes of cyclodextrins and linear oligosaccharides.

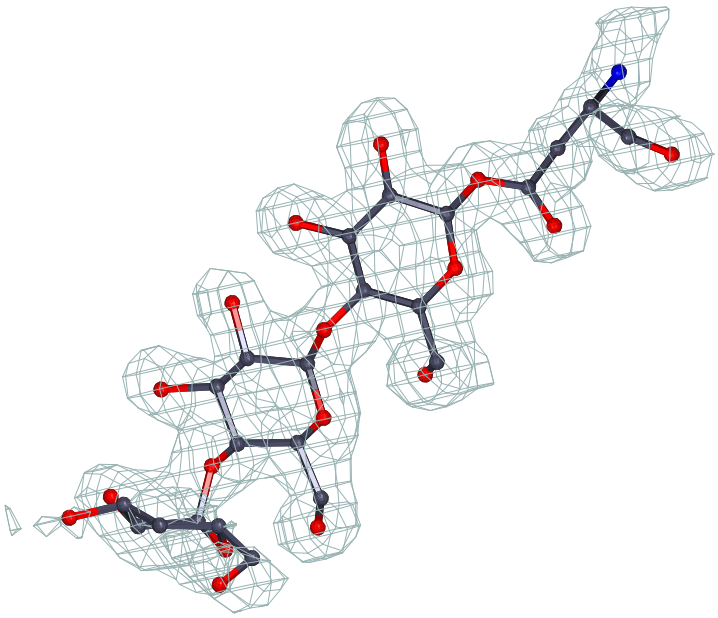

Figure 1: OMIT $2F_O$-F_C electron density contoured around a maltotriose that is covalently bound to the nucleophilic residue of CGTase Asp 229 (upper right in the electron density).

References

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