Structural Enzymology on *E. coli* Beta-Galactosidase

D.H. Juers and B.W. Matthews
Institute of Molecular Biology, Department of Physics, and Howard Hughes Medical Institute, University of Oregon, Eugene, OR 97403

Beta-galactosidase from *E. coli* is a large tetrameric enzyme which carries out two reactions on its disaccharide substrate lactose. The hydrolysis reaction produces the monosaccharides galactose and glucose which then function in various metabolic pathways. The transglycosylation reaction produces the disaccharide allolactose, which acts to induce the production of more beta-galactosidase. In this manner *E. coli* can both respond to and utilize lactose as a food source.

Previous crystallographic studies to a resolution of 2.5 Å in a $P2_1$ crystal form using data collected at the Photon Factory have shown the beta-galactosidase monomer to be constructed from 5 distinct domains organized around a central alpha/beta barrel[1] (Figure 1). The active site is shaped from residues of 4 of the domains into a pocket which complements the relatively small size of lactose. Structures of inhibitors bound to the active have suggested a general mode of action consistent with previous biochemical studies. However, the resolution of the complexes (2.6-3.0 Å) has precluded detailed arguments concerning the mechanism.

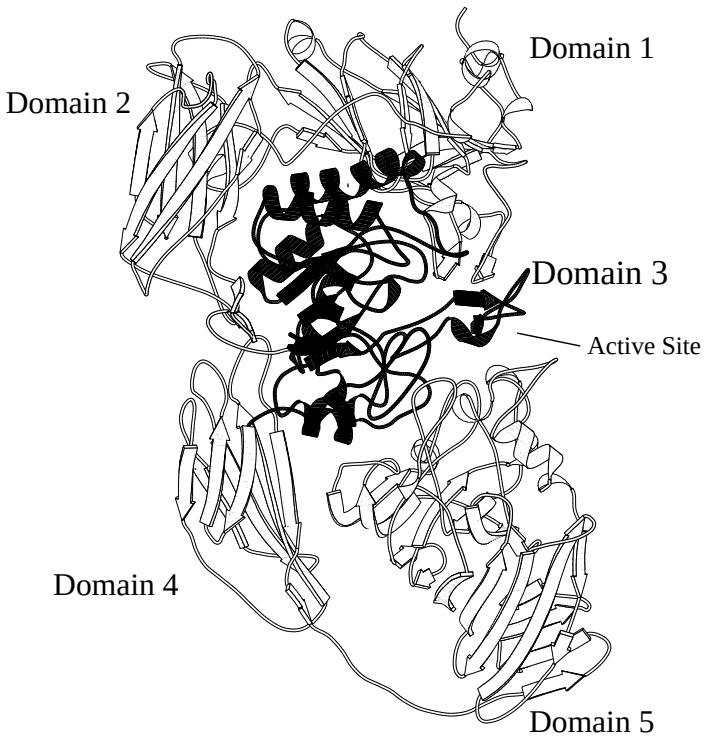

Figure1. Ribbon diagram (MOLSCRIPT) of one beta-galactosidase subunit from the tetramer. There are 5 distinct domains formed by a single 1023 residue polypeptide chain. The domain folds are jelly roll (1), immunoglobulin (2&4), $(alpha/beta)_8$(3-in black), and supersandwich (5). Domains 1-4 are common folds occurring in a large variety of proteins. The active site is located at one end of domain 3 and includes residues from domains 1&5 and domain 2 from a neighboring subunit.

Use of ALS beamline 5.0.2 has now permitted major progress on the native enzyme and a series of complexes in a new $P2_12_12_1$ crystal form of beta-galactosidase. This form diffracts to higher resolution, allowing the structural studies to be extended to 1.5 Å. The x-ray source and fast CCD detector at beam line 5.0.2 have permitted short collection times of < 2 hours/data set to 1.5 Å, despite the relatively large unit cell dimensions of 201 x 168 x 150 Å.

Refinement of the native structure to 1.7 Å in the $P2_12_12_1$ crystal form has been completed. The model includes 4044 amino acid residues and more than 4200 solvent molecules and has a crystallographic R-factor of 16.2 %. Based on liganding patterns, several candidate Mg++ and Na+ binding sites have been identified, including a Na+ in the active site which was previously modeled as a water molecule.

Data sets have been collected on various complexes in an effort discern the details of the reaction coordinate. These include both native and mutant enzymes with inhibitors designed to explore ground state, transition state, intermediate, and product state binding interactions. The complexes

address questions concerning the decision to follow one reaction path over another, the roles of Mg++ and Na+, and the detailed interactions that provide transition state stabilization.

Refinement of these complexes is underway. Figure 2 shows the electron density at 1.7 Å for a 2-deoxy-galactosyl enzyme complex, a reaction coordinate intermediate which has been trapped by flash freezing crystals soaked in galactal. This structure clearly shows a covalent connection to the nucleophile Glu 537 and the normal chair conformation of the sugar. Additionally, noncovalent interactions between the sugar and the enzyme and the sugar and various solvent molecules are clear. It is expected that the higher resolution of these complexes will allow a more detailed description of the reaction coordinate and to answer outstanding questions concerning the mechanism.

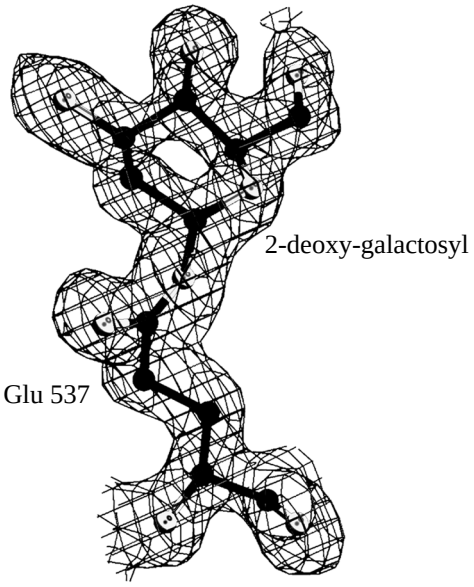

Figure 2. Electron density (2Fo-Fo, 1.0 sigma) showing the binding of a trapped reaction coordinate intermediate. There is clearly a covalent connection between the sugar and the nucleophile, Glu 537. The ring pucker is evident suggesting the sugar binds covalently in its relaxed chair conformation. Additionally, the positions of the ring substituents can be clearly seen and their binding interactions to protein and solvent molecules (not shown) accurately determined.

REFERENCES

1. R.H.Jacobson, X.J.Zhang, R.F.DuBose, and B.W.Matthews, Nature **369**, 761 (1994).

ACKNOWLEDGMENTS

We would like to thank D. Wigley (Oxford University) for providing the $P2_12_12_1$ crystallization conditions and R.Huber (University of Calgary) for help in reproducing these crystals. Also, we would like acknowledge the following for kindly providing beta-galactosidase inhibitors for these studies: T. Heightman and A. Vasella (ETH-Zentrum, Zurich). J. McCarter, L. McKenzie, and S. Withers (University of British Columbia), and G. Jäger (University of Stuttgart).

This work was supported in part by NIH grant GM20066 to B.W.M.

Principal investigator: Brian W. Matthews, Institute of Molecular Biology, University of Oregon. Email: brian@uoxray.uoregon.edu