

## Substrate-product trafficking in the active site of a plant enzyme: Conventional and Laue crystallographic approaches

**M. Hrmova<sup>1</sup>, J. N. Varghese<sup>2</sup>, V. A. Streltsov<sup>2</sup>, H. Driguez<sup>3</sup>, B. J. Smith<sup>4</sup>, G. B. Fincher<sup>1</sup>**

<sup>1</sup> *Plant Genomics Centre, University of Adelaide, Glen Osmond, SA, Australia*

<sup>2</sup> *MHT, CSIRO, Melbourne, VIC, Australia*

<sup>3</sup> *CERMAV, CNRS, Grenoble, France*

<sup>4</sup> *WEHI, Melbourne, VIC, Australia*

A glucose molecule, which represents the final hydrolytic product of a plant  $\beta$ -D-glucan glucohydrolase enzyme, is bound in the active site of the native enzyme until a new, incoming substrate molecule approaches [1, 2]. If we could synchronize dissociation of glucose from the active site throughout a crystal, time-resolved X-ray Laue crystallography could be used to monitor simultaneously the glucose product diffusing away and the new substrate entering the enzyme's active site. We have prepared a photo-caged, non-hydrolyzable substrate analogue, methyl-*O*-thio-gentiobioside (G6sG-OMe) for synchronizing of the release of the bound glucose, following laser-mediated removal of a blocking caged group. Preliminary photolytic and Laue crystallographic studies indicated that with collimated light directed through a Xenon-lamp at 350 nm and delivered through a fibre during a 2 min illumination period, we could achieve photolytic cleavage of the caged non-hydrolyzable substrate analogue G6sG-OMe. Further, with Laue polychromatic light (1.05-1.40 Å) we have collected several data sets of the native crystals of  $\beta$ -D-glucan glucohydrolase at 10 °C with 1.5° oscillations per frame through 90°, in the presence and absence of the caged and free G6sG-OMe ligands. The structure of the native enzyme collected from Laue diffraction images was refined to 2.7 Å. During structure solution of populations of intermediates that are formed during substrate/product trafficking we might benefit from structure solutions of several stable intermediates along the hydrolytic pathway of the  $\beta$ -D-glucan glucohydrolase [2-5].

(1) Varghese JN, Hrmova M, Fincher GB (1999) *Structure* 7, 179.

(2) Hrmova M, Varghese JN, DeGori R, Smith BJ, Driguez H, Fincher GB (2001) *Structure* 9, 1005.

(3) Hrmova M, De Gori R, Smith BJ, Vasella A, Varghese JN, Fincher GB (2004) *J Biol Chem* 279, 4970.

(4) Hrmova M, Streltsov V, Smith BJ, Vasella A, Varghese JN, Fincher GB (2005) *Biochemistry* 44, 16529.

(5) Hrmova M, De Gori R, Smith BJ, Driguez H, Varghese JN, Fincher GB (2002) *Plant Cell* 14, 1033.