s1.m8.p24 **Structural Diversity of Plant Glutathione S-Transferases.** <u>A. G. McEwen</u>[1], D. J. Cole[2], A. J. Lapthorn[1], *[1]Department of Chemistry, University of Glasgow, United Kingdom, [2]Aventis CropScience, United Kingdom. E-mail: alamce@chem.gla.ac.uk*

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Glutathione S-Transferases (GSTs) are a diverse family of catalytic and binding proteins. In plants five main classes of GSTs have been identified. The two main classes are the plant specific Phi and Tau classes. The three smaller classes are Zeta, Theta and Lambda. A sixth class of GST-like proteins with dehydroascorbate reductase (DHAR) activity has also been identified in *Arabidopsis*, soybean and rice [1]. Although GSTs have low sequence identity (40% within class and less than 25% between classes) their structure is well conserved. The well conserved N-terminal domain has a thioredoxin like fold and contains the glutathione (γ-glutamyl-cysteinyl-glycine, GSH) binding site. The C-terminal domain is helical, less well conserved and is responsible for substrate specificity. GSTs are active as homo- or hetero-dimers.

Crystals of a rice Tau class GST *Os*GSTU4 were grown and diffracted to 2.3Å at Daresbury SRS station 14.1. The space group was P$4_1$22 and unit cell dimensions α = β = 56.09Å, γ = 157.15Å. The structure was solved by molecular replacement using the wheat protein *Ta*GST4-4 (PDB id 1GWC) [2] as a search model. The structure has been refined to an R_{work} of 18.2% and an R_{free} of 25.4%. The structure of *Os*GSTU1 has been compared to the Tau class GSTs *Ta*GST4-4 and *Os*GSTU1 (PDB id 1OYJ) as well as to GSTs of other solved plant classes.

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[2]   Thom, R., Cummins, I., Dixon, D. P., Edwards, R., Cole, D. J., Lapthorn, A. J. (2002). *Biochemistry*. **41**. 7008-7020.

s1.m8.p25 **Distorted Sugar Ring in the Active Site of Soybean β-Amylase.** <u>Bunzo Mikami</u>, You-Na Kang, Akira Hirata And Shigeru Utsumi, *Division Of Food Quality Design And Development, Graduate School Of Agriculture, Kyoto University, Japan. E-mail: mikami@kais.kyoto-u.ac.jp*

Keywords: β-amylase; Enzyme mechanism; Sugar distortion

β-Amylase catalyzes the liberation of β-maltose from non-reducing ends of starch. The structures of the enzyme from soybean, sweet potato, barley and *Bacillus cereus* have been determined by X-ray crystallographic analysis [1,2]. In order to elucidate the mechanism of β-amylase, we have determined the structure of β-amylase/maltose complexes at around 1.3 Å resolutions. The data collection of trigonal soybean β-amylase crystals (a, b = 86 Å and c = 144 Å) was carried out in various conditions by soaking the crystals in 0.1~200 mM maltose, 2.1 M ammonium sulfate and 0.1 M acetate buffer for 1h at pH 4~6 and 20oC. The capillary crystal data were collected by a Bruker High-star multi-wire detector, and the data of frozen crystals were collected at SPring-8. The structures of the frozen crystals were refined by SHELXL program package [3] with R factors of 0.11 ~0.12. At a maltose concentration of 200 mM and pH5.4, two maltose molecules bound tandem at subsites -2~-1 and +1~+2 in the active site of the enzyme. The sugar conformations were $^4C_1$ chair forms except for subsite -1 where a distorted boat (^{1,4}B) form with a-anomer was clearly identified judging from the puckering parameters of the sugar ring. In contrast, two maltose molecules bound separately at subsites -2~-1 and +2~+3 at lower pH (pH4.0) or at lower concentration of maltose (1mM). In this case, all sugar conformations were stable $^4C_1$ chair forms, and the glucose residue at subsite -1 turned into mixture of α- and β-anomers. It is remarkable that the O1 of the distorted a-glucose residue at subsite -1 occupies the position of
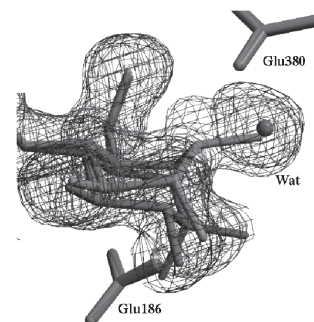

Figure 1 The distorted maltose at the active site of β-amylase.

the assumed catalytic water within 0.35 Å (Fig. 1). It makes a hydrogen bond with OE1 of Glu380 (base catalyst), suggesting that the distorted sugar can not be involved in the catalysis. The formation of this unusual complex found only at optimal pH of the enzyme reaction (pH 5.4) seems to be related with the product inhibition of β-amylase by maltose. Though the Ki value of maltose was reported to be low (~6 mM), it is explained by the energy required for the distortion of the sugar ring. It is concluded that the structure of the distorted sugar is useful for the design of a specific inhibitor of β-amylase.

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