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## Reaction Mechanism and Substrate Recognition of GH-94 Phosphorolytic Enzymes

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*Vibrio proteolyticus* chitobiose (GlcNAc- $\beta$ 1,4-GlcNAc) phosphorylase (ChBP) catalyzes the reversible phosphorolysis of chitobiose into  $\alpha$ -GlcNAc-1-phosphate and GlcNAc with inversion of the anomeric configuration. ChBP and its homologues, cellobiose phosphorylase (CBP) and cellodextrin phosphorylase (CDP), were classified under the glycosyltransferase (GT) class, GT-36, on the finding that they have no hydrolytic activity. As the first known structures of a GT-36 enzyme, we determined the crystal structures of ChBP including the ternary complex with GlcNAc and SO<sub>4</sub>. They are also the first structures of an inverting phosphorolytic enzyme in a complex with a sugar and a sulfate ion, and reveal a pseudo-ternary complex structure of enzyme-sugar-phosphate. ChBP comprises a  $\beta$ -sandwich domain and an ( $\alpha/\alpha$ )<sub>6</sub> barrel domain, constituting a distinctive structure among GT families. Instead, it shows significant structural similarity with glycoside hydrolase (GH) enzymes, glucoamylases (GH-15), and maltose phosphorylase (GH-65). The proposed reaction mechanism of ChBP also shows similarity with those for inverting hydrolytic enzymes with the exception of the molecules attacking the C1 atom. The similarities of overall structures and catalytic mechanisms between ChBP and GH enzymes led to the reclassification of family GT-36 into a novel GH family, namely GH-94. The substrate complex structures of ChBP also provide many structural insights into its oligosaccharide synthesis reaction such as substrate specificity.

**Key words:** crystallography, GH-94, phosphorolytic enzyme, reaction mechanism, structure and function relationship

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