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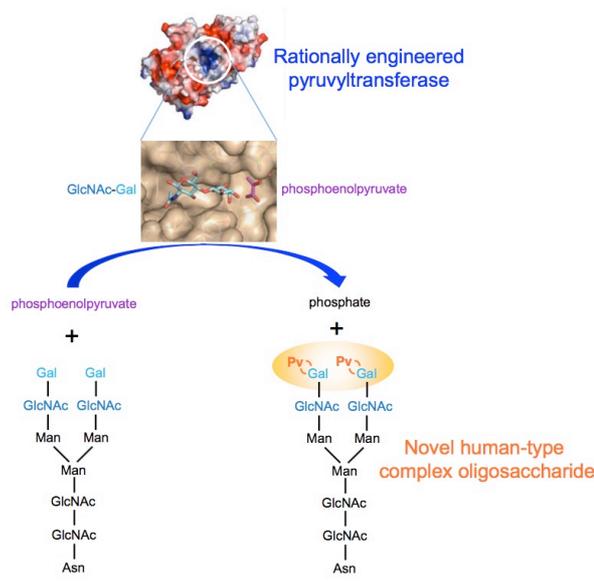
**Grant Title: Enzymatic synthesis of pyruvate-containing glycoproteins using endoglycosidase**

### Abstract

Pyruvylation onto the terminus of oligosaccharide, widely seen from prokaryote to eukaryote, confers negative charges on the cell surface and seems to be functionally similar to sialylation, which is found at the end of human-type complex oligosaccharide. We first determined the crystal structure of fission yeast pyruvyltransferase Pvg1p. By combining molecular modeling with mutational analysis of active site residues, we obtained a Pvg1p mutant (Pvg1p<sup>H168C</sup>) that efficiently transferred pyruvyl moiety onto a human-type complex glycopeptide. The resultant pyruvylated human-type complex glycopeptide recognized similar lectins on lectin arrays as the  $\alpha$ 2,6-sialyl glycopeptides. This newly-generated pyruvylation of human-type complex oligosaccharides would provide a novel method for glyco-bioengineering.



In mammalian cells, sialic acid gets attached to the terminal oligosaccharides. In contrast, model yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* harbor phosphate and pyruvate, respectively, on their cell surface oligosaccharides. Pyruvylation has been observed in both prokaryotic and eukaryotic organisms. Since attachment of sialic acid also provides negative charges on the cell surface, pyruvylation or sialylation on terminal oligosaccharides may confer similar functional effects. The molecular mechanism of pyruvylation has been studied well in the model yeast *S. pombe*. Accordingly, pyruvate is added to the oligosaccharides of glycoproteins by the pyruvyltransferase Pvg1p. In order to elucidate the pyruvyl transfer mechanism of Pvg1p and also to understand the structural basis of its substrate specificity, we first determined the crystal structure of Pvg1p at a resolution of 2.46 Å. The folding pattern of Pvg1p resembled that of the type-B glycosyltransferase, including sialyltransferases. Based on our *in-silico* models of the substrate-enzyme complex and results of mutational analyses, we presented



evidence for the underlying structural basis for the substrate specificity of Pvg1p. In addition, by rational protein engineering of Pvg1p, we were able to create a Pvg1p mutant that could transfer pyruvate moiety onto a human-type complex oligosaccharide efficiently. We observed that the molecular properties of the pyruvylated human-type complex glycopeptide were similar to those of the 2,6-sialyl glycopeptide, suggesting that pyruvylation can mimic sialylation. Based on our results, we showed that this modification offers a strategy for generating novel glycopeptides and glycoproteins.