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Structure, Mechanism and Engineering of a Nucleotidyltransferase as a First Step Toward Glycorandomization

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ABSTRACT: Metabolite glycosylation is effected by three classes of enzymes: nucleotidyltransferases, which activate sugars as nucleotide diphospho-derivatives; intermediate sugar-modifying enzymes; and glycosyltransferases, which transfer the final derivatized activated sugars to aglycon substrates. We have determined the first crystal structures of an enzyme responsible for the first step in this cascade, alpha-D-glucopyranosyl phosphate thymidyltransferase, or E_p , from *Salmonella* in complex with product (UDP-Glc) and substrate (dTTP), at 2.0 Å, and 2.1 Å resolution, respectively. These structures, in conjunction with the kinetic characterization of E_p , clarify the catalytic mechanism of this important enzyme class. We have also utilized structure-based engineering of E_p to produce modified enzymes capable of utilizing “unnatural” sugar phosphates not accepted by wild-type E_p . This demonstrated ability to alter nucleotidyltransferase specificity by design is an integral component of *in vitro* glycosylation systems developed for the production of diverse glycorandomized libraries.