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UDP-(5F)-GlcNAc acts as a slow-binding inhibitor of MshA, a retaining glycosyltransferase

Patrick A. Frantom^{†,‡,*}, James K. Coward[□], and John S. Blanchard[†]

[†]Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY, 10461

[□]Departments of Chemistry and Medicinal Chemistry, University of Michigan, Ann Arbor, MI 48109

Abstract

Glycosyltransferase enzymes play important roles in numerous cellular pathways. Despite their participation in many therapeutically-relevant pathways, there is a paucity of information on how to effectively inhibit this class of enzymes. Here we report that UDP-(5F)-GlcNAc acts as a slow-binding, competitive inhibitor of the retaining glycosyltransferase MshA from *Corynebacterium glutamicum* ($K_i \sim 1.6 \mu\text{M}$). The kinetic data are consistent with a single-step inhibition mechanism whose equilibration is slow relative to catalysis. We believe this is the first slow-onset inhibitor to be reported for the glycosyltransferase family of enzymes. The potent inhibition of the enzyme by the fluoro-substituted substrate is consistent with the involvement of an oxo-carbenium transition-state structure, which has been previously proposed for this family of enzymes. Additionally, although several members of the GT-B enzyme family, including MshA, have been shown to undergo a conformational change upon UDP-GlcNAc binding, the kinetic data are inconsistent with a two-step inhibition mechanism. This suggests that there may be other conformations of the enzyme that are useful for the design of inhibitors against the large family of GT-B glycosyltransferase enzymes.

pfrantom@bama.ua.edu.

[‡]Current address: Department of Chemistry, The University of Alabama, 250 Hackberry Lane, Tuscaloosa, AL 35487

Supporting Information Available: Experimental procedures and steady-state inhibition plots are described. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Glycosyltransferase (GT) enzymes, which catalyze the transfer of a sugar moiety to an acceptor molecule, are involved in numerous cellular pathways including bacterial cell wall biosynthesis¹, post-translational modification of proteins², and signal transduction³. These enzymes can be classified as either retaining or inverting based on the stereochemical outcome of the anomeric carbon center of the donor sugar.⁴ Despite their participation in many therapeutically-relevant pathways, there is a paucity of information on how to effectively inhibit this class of enzymes. The lack of information can be traced to the observations that only a small percentage of GT enzymes have been characterized, and the affinity of the substrates for characterized enzymes tends to be low. We recently reported the three-dimensional structure and basic kinetic characterization of the retaining glycosyltransferase MshA from *Corynebacterium glutamicum* (CgMshA).⁵ MshA catalyzes the transfer of *N*-acetyl-glucosamine (GlcNAc) from UDP-GlcNAc to 1-L-*myo*-inositol-1-phosphate (L-IIP) in the first committed step in the biosynthesis of the small molecule reductant mycothiol (Scheme 1).

It is generally accepted that the chemical mechanism of glycosyltransferase enzymes involves the development of substantial oxo-carbenium character in the transition state, similar to glycosidase enzymes.⁶ In support of this hypothesis, sugar nucleotides with electron withdrawing substituents are inhibitors of glycosidases. Recently, the synthesis of UDP-(5F)-GlcNAc (Scheme 2) provided the ability to probe the mechanism of glycosyltransferase enzymes as well.^{7,8}

Based on steady-state kinetic data, UDP-(5F)-GlcNAc acts as a competitive inhibitor of CgMshA versus UDP-GlcNAc with a K_{is} value of $1.4 \pm 0.2 \mu\text{M}$, approximately 150-fold lower than the Michaelis constant determined for UDP-GlcNAc ($210 \mu\text{M}$) (Figure S1).⁵ The 5-fluoro compound did not act as a viable donor substrate for the MshA-catalyzed reaction at concentrations up to $200 \mu\text{M}$ under conditions similar to those in the inhibition assays. These results are similar to previous studies demonstrating that the 5-fluoro compound will not act as a donor substrate, but acts as a functional acceptor substrate in glycosyltransferase reactions.⁸ The results shown here provide further support for the formation of substantial charge development on the donor sugar in glycosyltransferase reactions and demonstrate the power of 5-F sugars as mechanistic probes.

Unlike previous studies however, the time-courses of CgMshA activity obtained in the presence of UDP-(5F)-GlcNAc displayed non-linear kinetics described by a rapid initial rate followed by a slower steady-state rate (Figure 1A). The time-courses were fit using eq 1, where $[P]_t$ is the concentration of product formed, $[E]$ is the total enzyme, v_i is the initial velocity, v_{ss} is the steady-state velocity, t is time, k_{obs} is the exponential rate constant for equilibration, and C is a finite intercept.⁹ The inhibition was demonstrated to be reversible by incubating the enzyme with the inhibitor prior to initiation of the reaction with UDP-GlcNAc; this resulted in a progress curve that consisted of a lag phase followed by reactivation of the enzyme comparable to levels seen in the absence of the inhibitor (Figure 1B).

$$\frac{[P]_t}{[E]} = v_{ss}t + \frac{v_i - v_{ss}}{k_{obs}}(1 - e^{-k_{obs}t}) + C \quad (1)$$

Two possible kinetic mechanisms that can explain non-linear kinetics are shown in Scheme 3.⁹ Mechanism A depicts a two-step mechanism where an initial inhibitory complex is formed followed by a slow equilibration to a more tightly-bound complex (EI*). Mechanism B depicts a single-step mechanism where the formation of the initial complex is the slow step relative to catalysis. A plot of k_{obs} versus inhibitor concentration displays a linear dependence (Figure 2), consistent with the single-step mechanism depicted in mechanism B.

The dependence of k_{obs} on [UDP-(5F)-GlcNAc] can be fit to eq 2, where k_1 and k_2 are the rate constants shown in mechanism B, [I] is the concentration of the inhibitor, [S] is the concentration of UDP-GlcNAc, and K_m is the Michaelis constant for UDP-GlcNAc. From the results of the fit, values of $0.14 \pm 0.01 \mu\text{M}^{-1} \text{min}^{-1}$ and $0.26 \pm 0.06 \text{min}^{-1}$ can be calculated for k_1 and k_2 , respectively. Using these values derived from k_{obs} , the calculated K_i value of $1.8 \pm 0.4 \mu\text{M}$ is in excellent agreement with the K_{is} value determined from the steady-state inhibition experiments ($1.4 \pm 0.2 \mu\text{M}$).

$$k_{\text{obs}} = k_2 + \frac{k_1[I]}{1 + \frac{[S]}{K_s}} \quad (2)$$

Structural studies demonstrate that CgMshA and other GT-B fold glycosyltransferases can adopt both open and closed forms. It has been proposed that a large conformational change takes place after the sugar nucleotide binds to the enzyme, creating the closed form of the enzyme and a binding site for the second substrate.^{5,10–13} One might expect the inhibitor to bind in a similar fashion, consistent with mechanism A. However, the kinetic data indicate that UDP-(5F)-GlcNAc inhibits the enzyme in a single step that occurs slowly relative to catalysis.

Based on steady-state kinetic data, CgMshA follows an ordered bi-bi sequential mechanism with UDP-GlcNAc binding first, followed by L-IIP.⁵ As UDP-(5F)-GlcNAc is a competitive inhibitor versus UDP-GlcNAc, it is expected that both compounds bind to the apo-form of the enzyme. Attempts to cocrystallize CgMshA with the 5-fluoro compound have been unsuccessful. One possible explanation for the slow-onset phenomenon is that UDP-(5F)-GlcNAc binds slowly to the open form of the enzyme. However, given the modest structural change versus UDP-GlcNAc, this seems unlikely. Instead, if UDP-(5F)-GlcNAc binds to a closed form of the apo enzyme, a possible physical mechanism for the slow binding may arise from a small loop (residues 16–22) that becomes ordered in the closed conformation. This loop is proposed to sequester the uridine moiety of UDP-GlcNAc from solvent. It is possible that the movement of this loop limits the rate at which UDP-(5F)-GlcNAc can bind to the apo enzyme resulting in the time-dependent inhibition.

While a number of time-dependent inhibitors of glycosidase enzymes have been reported¹⁴, we believe that this is the first reported glycosyltransferase inhibitor to act in a slow-onset manner. The potent inhibition of CgMshA by the fluoro-substituted compound is consistent with the oxo-carbenium transition-state structure proposed for retaining glycosyltransferase enzymes based on kinetic isotope effects.^{15,16} While structural studies indicate a large conformational change upon sugar nucleotide binding, the kinetic results suggest that more subtle structural changes are involved in the time-dependent inhibition of CgMshA by UDP-(5F)-GlcNAc. Taken together, these conclusions provide data on the electronic structure of the transition state of this retaining glycosyltransferase and the possibility of alternate conformations of the enzyme. This information will aid in the design of future inhibitors for the large family of GT-B glycosyltransferase enzymes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Lovering AL, Gretes M, Strynadka NC. *Curr Opin Struct Biol* 2008;18:534–543. [PubMed: 18721881]
2. Sears P, Wong CH. *Cell Mol Life Sci* 1998;54:223–252. [PubMed: 9575336]
3. Slawson C, Housley MP, Hart GW. *J Cell Biochem* 2006;97:71–83. [PubMed: 16237703]
4. Coutinho PM, Deleury E, Davies GJ, Henrissat B. *J Mol Biol* 2003;328:307–317. [PubMed: 12691742]
5. Vetting MW, Frantom PA, Blanchard JS. *J Biol Chem* 2008;283:15834–15844. [PubMed: 18390549]
6. Lairson LL, Henrissat B, Davies GJ, Withers SG. *Annu Rev Biochem* 2008;77:521–555. [PubMed: 18518825]
7. Hartman MC, Coward JK. *J Am Chem Soc* 2002;124:10036–10053. [PubMed: 12188668]
8. Hartman MC, Jiang S, Rush JS, Waechter CJ, Coward JK. *Biochemistry* 2007;46:11630–11638. [PubMed: 17883281]
9. Morrison JF, Walsh CT. *Adv Enzymol Relat Areas Mol Biol* 1988;61:201–301. [PubMed: 3281418]
10. Buschiazzo A, Ugalde JE, Guerin ME, Shepard W, Ugalde RA, Alzari PM. *EMBO J* 2004;23:3196–3205. [PubMed: 15272305]
11. Boix E, Zhang Y, Swaminathan GJ, Brew K, Acharya KR. *J Biol Chem* 2002;277:28310–28318. [PubMed: 12011052]
12. Mulichak AM, Losey HC, Lu W, Wawrzak Z, Walsh CT, Garavito RM. *Proc Natl Acad Sci U S A* 2003;100:9238–9243. [PubMed: 12874381]
13. Hu Y, Chen L, Ha S, Gross B, Falcone B, Walker D, Mokhtarzadeh M, Walker S. *Proc Natl Acad Sci U S A* 2003;100:845–849. [PubMed: 12538870]
14. Gloster TM, Davies GJ. *Org Biomol Chem* 2010;8:305–320. [PubMed: 20066263]
15. Kim SC, Singh AN, Raushel FM. *J Biol Chem* 1988;263:10151–10154. [PubMed: 3134344]
16. Murray BW, Wittmann V, Burkart MD, Hung SC, Wong CH. *Biochemistry* 1997;36:823–831. [PubMed: 9020780]

Figure 1.

(A) Kinetics of time-dependent inhibition of CgMshA by UDP-(5F)-GlcNAc. Progress curves for MshA in the absence (blue) and presence (black) of UDP-(5F)-GlcNAc. The red lines are a fit to eq 1. Saturating substrate concentrations of 2 mM UDP-GlcNAc and 2 mM L-IIP were used in this experiment. (B) Effect of pre-incubation with UDP-(5F)-GlcNAc on CgMshA activity initiated with UDP-GlcNAc. A solution of 8 nM enzyme, 2 mM L-IIP, and 5 μM UDP-(5F)-GlcNAc was incubated at 25 °C for 10 minutes prior to the addition of 1 mM UDP-GlcNAc (black line). The volume of UDP-GlcNAc added was 1% of the reaction volume. The blue line represents the same experiment in the absence of UDP-(5F)-GlcNAc. See Supporting Information for full experimental details.

Figure 2.

Plot of k_{obs} versus $[\text{UDP-(5F)-GlcNAc}]$. The values for k_{obs} were determined by fits of the progress curves to equation 1. The solid black line is a fit of the data to equation 2.

Scheme 1.

UDP-(5F)-GlcNAc

Scheme 2.

Scheme 3.