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Combined molecular dynamics, STD-NMR, and CORCEMA protocol yields structural model for a UDP-galactopyranose mutase–inhibitor complex

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ABSTRACT

UDP-galactopyranose mutase (UGM) is an enzyme involved in the biosynthesis of the *Mycobacterium tuberculosis* cell wall, and is essential for the growth and survival of the organism. A micromolar inhibitor developed by tetrafluorination of the UGM substrate has been previously studied by saturation transfer difference (STD) NMR spectroscopy. To elucidate the bioactive conformation of the inhibitor bound to UGM, we employ molecular dynamics (MD) simulations to construct a structural model. The MD model is subsequently validated by a good fit between experimental and theoretical STD effects, the latter calculated by a complete relaxation and conformational exchange matrix (CORCEMA) analysis. This structural model is used to explain the relative binding affinities of the inhibitor and the parent substrate.

Mycobacterium tuberculosis is the causative pathogen of tuberculosis (TB), which remains a major global health threat that led to 1.5 million deaths in 2013.[1] This threat has also been intensified by the emergence of multidrug resistant and even extensively drug resistant TB,[2] as multidrug resistant TB accounts for 480,000 cases per year with a treatment success rate of only 48%.[1] Thus, there is an urgent need for better TB therapeutics with novel targets.

A newly validated anti-tuberculosis target is UDP-galactopyranose mutase (UGM), an enzyme that catalyzes the interconversion between UDP-galactopyranose (UDP-Gal*p*) and UDP-galactofuranose (UDP-Gal*f*) (Scheme 1).[3] The latter provides galactofuranose (Gal*f*) as the building block of the galactan chain in the *M. tuberculosis* cell wall,[4] whose biosynthesis is essential for the growth and survival of *M. tuberculosis*.[5] In addition, Gal*f* is absent in mammals,[6–8] rendering UGM a promising target for the development of novel agents against tuberculosis.

However, most of the UGM inhibitors developed to date have only moderate binding affinities in the micromolar range,[9–18] unsuitable for clinical application. Hence, understanding their bound geometries could be critical to improving the potency of UGM inhibitors. One such micromolar inhibitor, UDP-(2,3-dideoxy)-2,2,3,3-tetrafluoro-D-galactofuranose (UDP-F4-Gal*f*) (Scheme 1), has been recently developed and shows a K_d of 53 μM against the model UGM enzyme from *Klebsiella pneumoniae*.[18] In addition, its binding epitope in complex with UGM has been mapped by saturation transfer difference (STD) NMR spectroscopy.[18] To reveal the bioactive bound conformation of this tetrafluorinated substrate analogue within the active site, we employ herein molecular dynamics (MD) simulations to construct its binding model with reduced (catalytically active) UGM. This model is subsequently validated by quantitative comparisons between experimental STD effects and theoretical STD values calculated via complete relaxation and conformational exchange matrix (CORCEMA) analysis.[19] This work also sheds some insights into the future design of UGM inhibitors.

MD simulations: Since STD competition experiments suggest that UDP-F4-Gal*f* binds at the active site of UGM,[18] the initial structure for MD simulations was built by replacing the galactopyranose moiety in the crystal structure of *Klebsiella pneumoniae* UGM (PDB

Abbreviations: Gal*f*, galactofuranose; Gal*p*, galactopyranose; UDP, uridine 5′-diphosphate; UDP-Gal*f*, UDP-D-galactofuranose; UDP-Gal*p*, UDP-D-galactopyranose; UGM, UDP-galactopyranose mutase; UDP-F4-Gal*f*, UDP-(2,3-dideoxy)-2,2,3,3-tetrafluoro-α-D-galactofuranose; UDP-[3-F]Gal*f*, UDP-3-deoxy-3-fluoro-D-galactopyranose; FAD, flavin adenine dinucleotide; NMR, nuclear magnetic resonance; STD, saturation transfer difference; TRNOESY, transferred nuclear Overhauser effect spectroscopy; CORCEMA, complete relaxation and conformational exchange matrix; MD, molecular dynamics.

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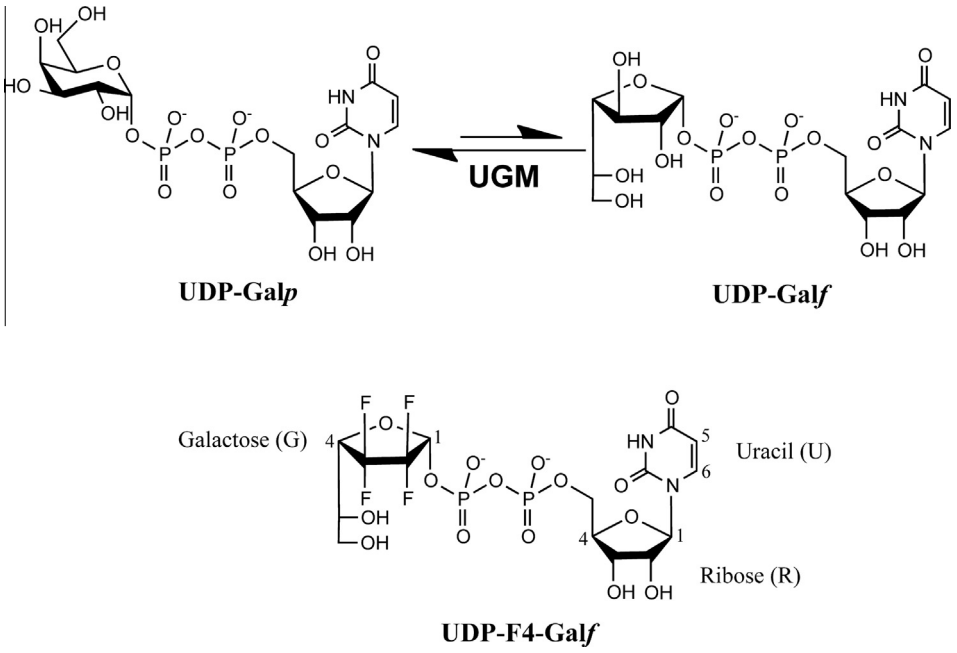

Scheme 1. The interconversion catalyzed by UGM and a substrate-mimicking inhibitor, UDP-F4-Gal*f*.

3INT)[20] with the tetrafluorinated galactofuranose. All MD simulations were conducted using GROMACS 4.6,[21] using similar procedures and parameters as previously reported.[22] The Verlet cutoff scheme[23] was used to evaluate short-range, non-bonded interactions, with both van der Waals and electrostatic interactions truncated at 1.0 nm. To model the catalytically active UGM, the cofactor FAD was modeled in its reduced state $FADH^-$. The UGM:inhibitor complex was first subjected to MD simulations of 100 ns in triplicate at 298 K. After clustering conformations (at 0.5 ns intervals) from the last 60 ns of all three 100 ns-long trajectories, the central conformation of the largest cluster was used to initiate subsequent MD simulations at 285 K so as to match the experimental temperature. These MD simulations were performed in triplicate again for 100 ns, and analyses of these trajectories were mostly done for the final 80 ns. A representative snapshot was generated via clustering of the final 80 ns of triplicate MD trajectories for visualization.

CORCEMA calculations: Residues within 1.5 nm distance of the inhibitor were included for the calculation of theoretical STD effects. A total of 240 snapshots were taken from the last 80 ns of three replicate trajectories (at 285 K) at 1 ns intervals, and STD intensities were calculated for each of the 240 structures before averaging to obtain the theoretical STD effects for the entire MD trajectories. Concentrations of UGM and the inhibitor, their equilibrium constant K_A, spectrometer frequency, and finite delays between scans were all set according to experimental values. The association rate constant k_{on} and the free ligand rotational correlation time were estimated to be 10^6 s^{-1} M^{-1} and 0.5 ns, respectively. The bound ligand rotational correlation time was optimized to be 74 ns. Other parameters were set as previously reported.[22]

MD simulations at 285 K showed good convergence as root-mean-square deviations of complex heavy atoms stabilized within 20 ns for all triplicate trajectories. The fluctuations of the bound inhibitor were extremely small, especially those of the uridine and pyrophosphate portions. Only the F4-Gal*f* moiety exhibited noticeable fluctuations with a root-mean-squared fluctuation value of 0.11 nm (Supplementary data). As expected, the overall bound structure of UDP-F4-Gal*f* from MD simulations was very similar to that of the native substrate, and the major difference was the conformation of the galactose moiety (Fig. 1).

Before further analyzing the interactions between UGM and the inhibitor, the MD trajectories were subjected to validation by CORCEMA calculations. The calculated STD enhancements demonstrated a reasonable match with experimental values (Fig. 2). The NOE *R*-factor, the magnitude of which correlates with the discrepancy between experimental and calculated STD effects,[24] was 0.28, indicative of a good match. In addition, TRNOESY experiments have suggested a H1–H4 distance of less than 3 Å for the F4-Gal*f* moiety when the inhibitor is bound.[18] Although the initial structure of F4-Gal*f* minimized in the active site showed a H1–H4 distance of 3.6 Å before MD simulations, the average H1–H4 distance in our MD trajectories was 2.8 Å, corroborating the TRNOESY results and indicating a ^4E conformation for the furanose ring (Fig. 3). Therefore, our MD model of bound UDP-F4-Gal*f* is validated by agreement with both STD and TRNOESY experiments.

While the uridine and pyrophosphate portions of the inhibitor were shown to bind UGM in a similar fashion to that of UDP-Gal*p* in the crystal structure (Fig. 1), the F4-Gal*f* moiety interacted with

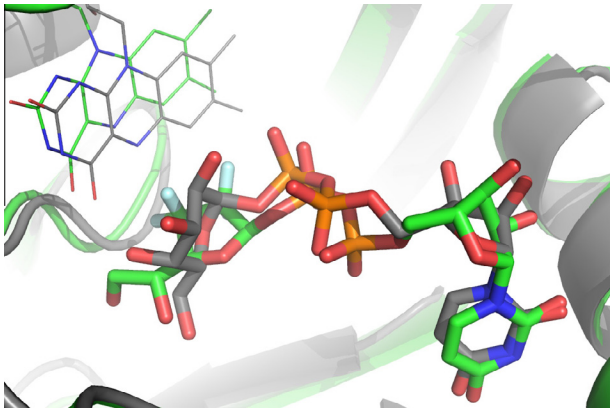

Figure 1. Overlay of the representative structure from MD simulations of UGM:UDP-F4-Gal*f* (green carbon atoms) and the crystal structure of UGM:UDP-Gal*p* (grey carbon atoms). Bound ligands are shown in sticks, while the cofactor FAD is indicated by thin lines. Other atoms are colored: oxygen, red; nitrogen, blue; fluorine, pale cyan.