Tryptophan as a Molecular Shovel in the Glycosyl Transfer Activity of *Trypanosoma cruzi* Trans-sialidase

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ABSTRACT Molecular dynamics investigations into active site plasticity of *Trypanosoma cruzi* trans-sialidase, a protein implicated in Chagas disease, suggest that movement of the Trp^{312} loop plays an important role in the enzyme's sialic acid transfer mechanism. The observed Trp^{312} flexibility equates to a molecular shovel action, which leads to the expulsion of the donor aglycone leaving group from the catalytic site. These computational simulations provide detailed structural insights into sialyl transfer by the trans-sialidase and may aid the design of inhibitors effective against this neglected tropical disease.

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The inherently dynamic nature of proteins, encompassing both backbone and side-chain motions, is a well established phenomenon. This protein mobility plays a critical role in a range of biological functions, such as the exchange of ligands or the opening and closing of membrane channels. Diverse demonstrations of the importance of protein flexibility have been documented, for example, in the catalytic mechanism of pathogenic thymidylate synthetase (1), and the resistance of HIV protease (2), making advances in the understanding of protein plasticity of widespread importance. Mobile loop structures, which often host ligand binding sites, confer regions of particular flexibility. Examples recently identified from molecular dynamics (MD) simulations include avian Influenza neuraminidase (3) and *Mycobacterium tuberculosis* 1-deoxy-D-xylulose 5-phosphate reductoisomerase (4). In a similar fashion, conformational fluctuations in the Trp^{312} loop region at the catalytic cleft of the *Trypanosoma cruzi* trans-sialidase (TcTS) enzyme have been observed (5).

In this work, we investigate the implications of active site plasticity on biochemical mechanism by simulating the dynamics of intermediate steps along the sialic acid transfer pathway in TcTS (Scheme 1). From our explicitly solvated MD analysis, we witness varying degrees of movement of the Trp^{312} loop as part of a complex, dynamic, and potentially interlinked series of events involved in the catalytic function of TcTS. Our simulations directly observe the mechanism of expulsion of the aglycone leaving group from the catalytic site, and, by implication, recognition of the new acceptor substrate. This appears to be the result from a previously undocumented molecular shovel action by the flexible Trp^{312} loop.

T. cruzi is the causative agent of Chagas disease, a form of trypanosomiasis presenting a serious health burden to Central and South America (6). The surface-anchored TcTS scavenges sialic acid from host glycoconjugates, and is recognized as crucial for host cell invasion and infection (7). NMR, x-ray crystallographic, and mutagenesis studies on TcTS (8–10) have provided valuable structural and mechanistic insights. Recently, mass spectrometric analysis has confirmed a ping-pong bi-bi kinetic mechanism of sialic acid transfer by TcTS, with formation of a covalent sialyl-enzyme intermediate (11). The early crystallographic work found the TcTS catalytic cleft to comprise a sialic acid binding site and an acceptor binding site. The latter is formed by Tyr^{119} and Trp^{312}, which make CH-π interactions with donor and acceptor sugar residues adjacent to the terminal sialic acid (9,10). The crystal structures also suggest flexibility in the Tyr^{119} side-chain conformation; in the apo enzyme, the phenolic side chain is able to point downward toward the floor of the catalytic pocket. However, with bound substrate, the Tyr^{119} side chain is oriented upwards to form a stack with substrate (Fig. 1). Recent simulation studies (5) additionally suggest flexibility of the Trp^{312} loop (residues 310 to 314) at the mouth of the catalytic cleft, migrating between open and closed conformations (Fig. 1). This confers further plasticity to the apo enzyme and control over solvent exposure of the active site.

MD simulations of I, III and IV (Scheme 1) were performed based on TcTS crystal structures. The structures are stable over the 40 ns simulations (Fig. S1 in the Supporting Material). Tyr^{119} shifts away from its stacked position for each of the three systems studied. Similarly, for each system, Trp^{312} samples conformations with significantly different χ_2 torsion angles. A combination of side-chain rotation with a subtle surface loop motion enables the observed conformational opening and closing of Trp^{312} (Fig. 1). In particular, our simulations suggest that vacation of lactose from the

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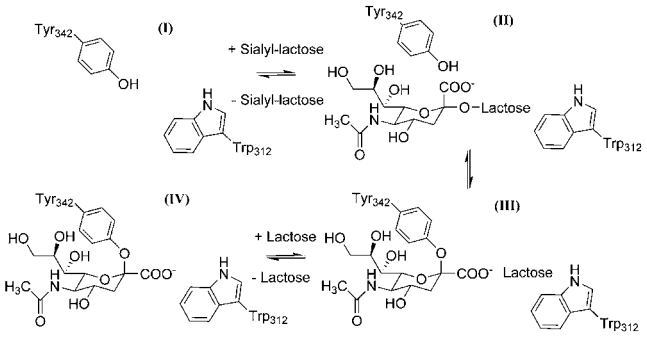

SCHEME 1 First step of sialyl transfer catalyzed by TcTS.

active site of sialyl-enzyme covalent intermediate, III, is linked to opening of the catalytic cleft.

Movement of the Trp^{312} residue appears to be fundamental to the process of ligand expulsion seen in the sialylated enzyme/lactose simulation, III (Fig. 2). In the initial pose derived from crystallography, lactose sits in the acceptor site, forming a face-to-face stack with Trp^{312} and a parallel displaced stack with Tyr^{119} (Fig. 2 *a*). This sandwich arrangement appears optimized to promote a reactive orientation for the original α2,3-sialyllactose substrate (II, Scheme 1). However, the absence of a covalent bond between sialic acid and lactose in the sialylated enzyme intermediate (III, Scheme 1) permits a lever-like swing of the lactose/Trp^{312} stack as the Trp^{312} loop opens: rotation of Tyr^{119} away from the stacked position at 22 ns abrogates the lactose/Tyr^{119} stacking interaction (Fig. 2 *b* and Fig. 3 *a*). This is followed by the Trp^{312} motion, which relocates the lactose/Trp^{312} stack to a more solvent-exposed position (Fig. 2 *b* and Fig. 3 *b*). This conformation is maintained for over 10 ns until lactose migration out of the active site and away from the protein surface is observed at 32 ns (Fig. 2 *c* and Fig. 3 *c*). Of three replicate simulations of III with differing initial velocities, two did not observe lactose expulsion (Fig. S2), as expected from the rare nature of this event on the timescale of the simulations.

The predicted persistence of lactose/Trp^{312} interactions in the open solvent exposed pose is interesting. CH-π interactions are common in carbohydrate-protein binding and are estimated to contribute a potential energy of -2 kcal/mol

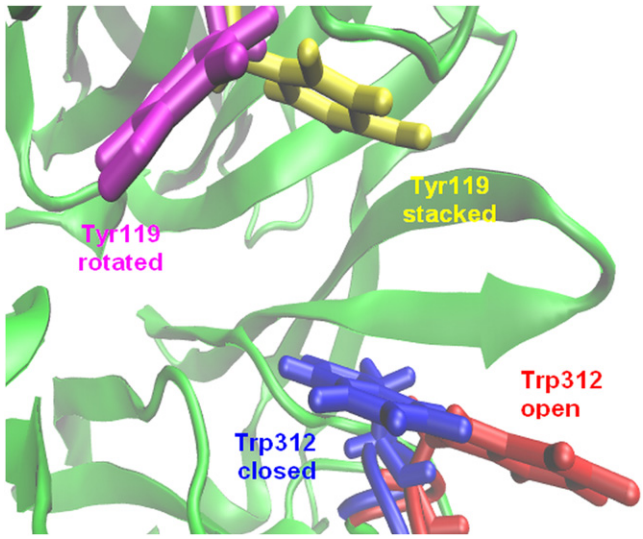

FIGURE 1 Flexibility of the Tyr^{119} and Trp^{312} residues of TcTS during the molecular dynamics simulation of the sialyl enzyme covalent intermediate complexed with lactose: Tyr^{119} switches from the stacked to a rotated position and Trp^{312} moves between stacked and open conformations.

per interaction (12). For further evidence of the feasibility of such water-exposed lactose-aromatic interactions, we recorded solution phase ^{1}H NMR spectra for the disaccharide lactose with phenol added as a simple aromatic partner. We note that previous NMR investigations into carbohydrate-π interactions have partnered various monosaccharides with aromatic entities (13); however, these analyses have not been reported for disaccharides. Complex was detected, with spectra showing marked upfield shifts for galactose H-1, H-3, H-4, H-5 and adjacent α-glucose H-4′ (Table S1), consistent with an aromatic shielding effect on one face of lactose. The NMR experiments also suggest that the complex is weakly bound, consistent with ultimate dissociation of lactose from the enzyme.

We, therefore, surmise a dual role for Trp^{312}. Firstly, it assists Tyr^{119} in orienting substrate for sialylation of TcTS. Secondly, due to its lever-like motion, Trp^{312} behaves like a molecular shovel: it displaces donor product from the active site following delivery of sialic acid, and, implied by the

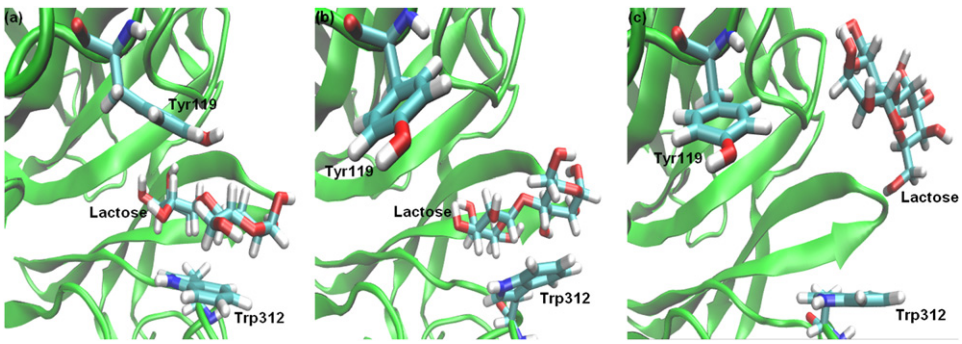

FIGURE 2 Selected steps from along the MD trajectory of the sialylated-enzyme/lactose complex, III. (*a*) Initially Tyr^{119} is in the stacking position and the Trp^{312} loop is stacked (*b*) At 22 ns, the Tyr^{119} rotates away from the stack, followed by a Trp^{312} motion to shift the Trp^{312}/lactose portion of the stack (*c*) Full opening of the Trp^{312} loop enables lactose to vacate the active site by 32 ns.

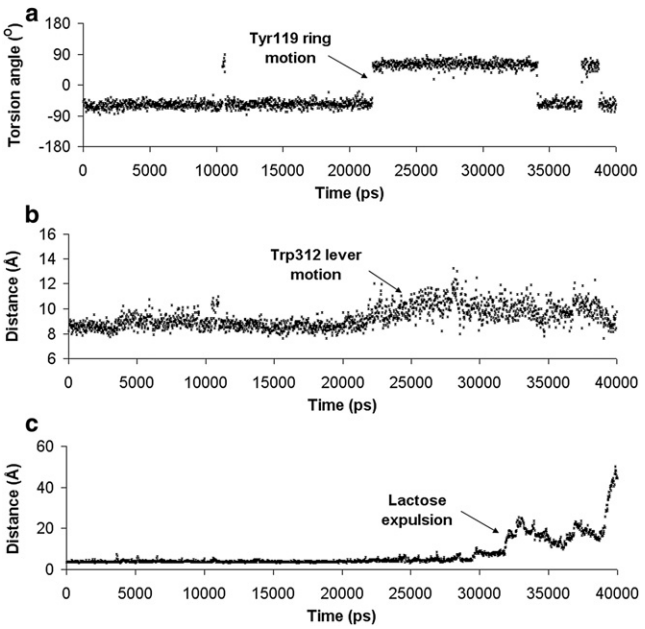

FIGURE 3 Dynamics of sialylated TcTS/lactose (III): time series of (*a*) side-chain dihedral angle, χ_1, of Tyr^{119}; (*b*) intercentroid distance between Tyr^{119} and Trp^{312} rings; and (*c*) distance between centers of mass of sialic acid and lactose.

microscopic reversibility of MD, loads acceptor substrate into position for subsequent molecule binding. This critical role in orientation of acceptor may aid explanation of the observation by Paris et al. (14) of complete abolition of trans-sialidase activity in Trp312Ala TcTS mutant.

Due to the dynamical nature of proteins, enzyme activity is likely to present a more complex picture than static crystal structures may imply. Our simulations suggest that arrival, occupancy, and departure of donor and acceptor substrates at the catalytic cleft of TcTS is a process supported by structural fluctuations in key active site residues. Trp^{312} loop flexibility appears to have a mechanistic role during sialyl transfer, with Trp^{312} position and orientation acting as a lever in discharging donor and recognizing acceptor aglycone. The detailed computational insights into sialyl transfer obtained here also provide much needed new avenues for structure-based design against TcTS, an enzyme which to date has proved stubbornly resistant to rational design attempts (15). By building on early work developing the dynamic pharmacophore (16) and relaxed complex (17) methods, incorporation of receptor flexibility into molecular design of strong binders is proving increasingly fruitful and our hope is that this deepened structural understanding of TcTS will lend new impetus to the design of anti-Chagas inhibitors effective against this neglected yet devastating tropical disease.

SUPPORTING MATERIAL

Two figures and one table are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(09)00101-3.

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