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COMMUNICATIONS

Published on Web 06/01/2007

Remarkable Loop Flexibility in Avian Influenza N1 and Its Implications for Antiviral Drug Design

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The emergence and continuing global spread of the highly virulent avian influenza H5N1 has raised concerns of a possible human pandemic.[1] Several approved anti-influenza drugs effectively target the neuraminidase (NA), a surface glycoprotein that cleaves terminal sialic acid residues and facilitates the release of viral progeny from infected cells.[2] The first crystal structures of group-1 NAs in apo form and in complex with currently available drugs[3] revealed that although the binding pose of oseltamivir (Tamiflu) was similar to that seen in previous crystallographic complexes,[4,5] the 150-loop adopted a distinct conformation, opening a new cavity adjacent to the active site. This open form of the 150-loop was proposed as a new opportunity for drug design.[3] However, under certain crystallization conditions, the 150-loop was found to adopt the same closed conformation as previously seen in group-2 structures, suggesting a slow conformational change may occur upon inhibitor binding.[3] The possible transience of the 150-loop cavity and its proximity to the inhibitor-binding site underscores the importance of dynamic biophysical studies to complement static crystal structures in NA drug discovery efforts. Through explicitly solvated molecular dynamics (MD) simulations of the apo and oseltamivir-bound forms of tetrameric N1, here we show that the 150-loop is able to open into significantly wider conformations than seen in the crystal structures. We find this motion in the 150-loop is coupled to motion in the neighboring 430-loop, which expands the active site cavity even further. In addition, we see that the 150-loop approaches the closed conformation in simulations of the oseltamivir-bound system, suggesting that the loop switching motion may be more rapid than previously proposed.

Two separate MD simulations were carried out using apo and oseltamivir-bound crystal structures.[3] The tetramer structures are exceptionally stable over the course of the 40 ns simulations (Supporting Information Figure S1). A principal components analysis over all the chains for both systems reveals that the monomer subunits within each tetramer sample different regions of configurational space, establishing that their motion can be considered independent of one another (Figure S2). On average, the apo N1 exhibits slightly higher overall per residue C_α root-mean-square fluctuation (rmsf) values than the oseltamivir-bound system (Figure S3). Further analysis reveals that the residues exhibiting the largest rmsf differences between the two systems are located at least 5.0 Å distal to the catalytic pocket, suggesting that the largest conformational changes are due to motions of surface-exposed loops rather than local changes directly within the active site pocket.

Most notably, the MD trajectories suggest that the 150-loop is even more flexible than observed in the crystal structures. For this analysis we adopt the structural descriptions established in ref 3, in which the apo and 150 min, 20 μM oseltamivir-soaked crystal structures (2HTY and 2HU0, respectively) represent the so-called "open" conformation and the 0.5 mM or 3-day oseltamivir-soaked crystal structures (2HU4, 2HT8) represent the "closed" conformation. While both simulations were initiated from the 150-loop in the open conformation, in some of the chains the loop adopted conformations varying from nearly closed to even more open (Figure S4). For example, after 5 ns, the loop configuration of chain D in oseltamivir-bound N1 shifts closer to the closed crystal structure than the open conformation and remains in this nearly closed state for the remaining 35 ns of dynamics (Figure 1).

Although we do not sample the completely closed conformation, salt bridge and hydrogen bond interactions between oseltamivir and residues Asp151 and Arg152 persist throughout the 40 ns of dynamics and help stabilize the 150-loop in the more closed conformation. Across both systems the 150-loop is observed to remain stable (apo chain C and D), shift to an intermediate configuration (apo chain A), or move away from the open conformation without becoming more closed (apo chain B, oseltamivir-bound chain C) (Figure 1).

In the latter case, the loop was seen to open up more widely than observed in the crystal structures. This motion was often coupled to an outward movement in the adjacent strand−loop−strand segment comprising residues Arg430−Thr439, herein called the 430-loop (Figure S4B, S4C). These coupled motions significantly expand the active site cavity, increasing its solvent accessible surface area compared with both the open and closed crystal structures (Figure 2). Together, the motion of the 150-loop and 430-loop cause substantial topological changes to occur directly within the active site pocket (Figure S5). Significant changes in the side-chain conformations of several residues occur, including the following: Glu119, which makes contact with the C4 amide group of oseltamivir; Arg156, which makes a salt bridge with Glu119 and a hydrogen bond with oseltamivir; Tyr406, which participates in a hydrogen bond network with conserved active site

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 10.1021/ja0723535 CCC: $37.00

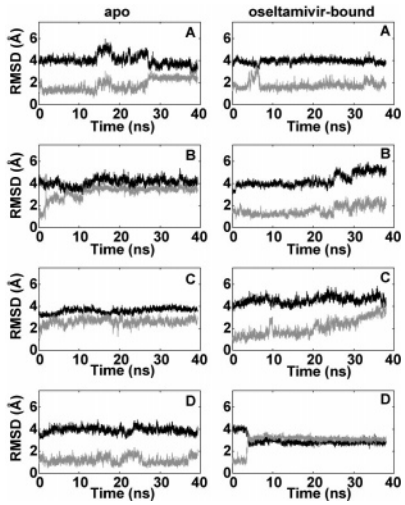

Figure 1. Open−closed loop transition. RMSD of the 150-loop (comprising residues N146−R152) from MD snapshots versus open (gray) and closed (black) crystal structures is shown for each of the chains (labeled A−D in the upper right-hand corner of each plot) in the simulations.

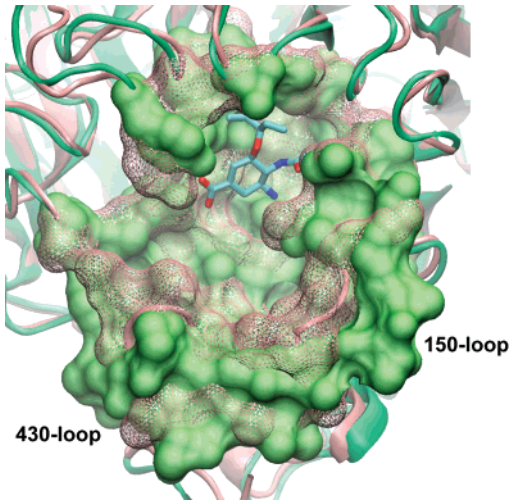

Figure 2. Comparison of the N1 inhibitor-binding pocket. The alignment of the open crystal structure (2HU0, pink cartoon) and a wide-open snapshot extracted from the apo MD simulation after 16 ns (green cartoon), with solvent accessible surface areas depicted in pink wireframe for the open crystal structure and in solid green for the wide-open structure, illustrate the significant cavity expansion during the apo simulation. For reference, oseltamivir is shown in licorice in the binding pocket and the coloring scheme is the same as in Figure S4.

arginines and the oseltamivir carboxylate group; and Gln136, which does not make contact with oseltamivir but is immediately adjacent to Arg156 which does. Nearby Ile427 and Arg428 exhibit a 1.65 Å backbone shift toward the binding site, and the catalytic Asp151 shifts away from the oseltamivir-binding pocket. Significant rotations about the N146C_α−G147C_α and G147C_α−T148C_α bonds (corresponding to dihedrals S145−N146−G147−T148 and N146−G147−T148−V149, respectively) allow the 150-loop to swing outward during the simulations. Within this loop, the position of V149C_α shifts 4.7 Å away from the binding site relative to the open crystal structure (Figures 2 and S5).

Proteins are dynamic and therefore accounting for receptor flexibility plays an important role in structure-based drug design, yet predicting a receptor's novel and unique modes of molecular recognition is still a major challenge.[6] The topological changes and additional expansion of the N1 inhibitor binding pocket that we present here could potentially play an important role in structure-based design of inhibitors for N1. This possibility was demonstrated in the case of HIV integrase, where a previously unknown trench capable of inhibitor binding was identified through MD simulations.[7] The new structural understanding was subsequently exploited in the development of raltegravir (MK-0518), a promising drug in a new class of antiretroviral agents active against the integrase enzyme that is presently in phase III clinical trials.[8] We anticipate that the insight into the 150- and 430-loop dynamics and the new cavity conformations we provide here will be of value in the rational design of inhibitors against the N1 strain. In light of the urgency of antiviral drug discovery efforts against H5N1, we offer a representative wide-open N1 structure (Supporting Information) extracted from the MD simulations to assist in H5N1 drug discovery efforts.

Acknowledgment. R.E.A. is funded in part by National Institutes of Health Grant 1F32 GM077729-01 and MRAC CHE060073N. D.M. is supported by an NIH Molecular Biophysics Training Grant at UCSD. The work is also funded by NIH Grant GM31749, NSF Grants MCB-0506593 and MCA93S013 (to J.A.M.). W.M.L., A.J.O., L.S.C., and W.W.L. are supported by NIH Grant P41 RR08605. L.S.C. is also supported by NSF Grant INT 0407508 to the PRIME program. J.-H.L is supported by the thematic program of Academia Sinica under Grant AS-95-TP-A07, NSC Grants 95-2627-B-002-010 and 95-2112-M-002-007, and by the Research Center for Applied Sciences, Academia Sinica. Additional support from the Howard Hughes Medical Institute, San Diego Supercomputing Center, Accelrys, Inc., the W.M. Keck Foundation, the National Biomedical Computational Resource and the Center for Theoretical Biological Physics is gratefully acknowledged.

Supporting Information Available: Five additional figures, computational methodology details (and corresponding references), and a wide-open snapshot extracted from the MD trajectories. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0723535