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An Objective Assessment of Conformational Variability in Complexes of Hepatitis C Virus Polymerase with Non-Nucleoside Inhibitors

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A major target for antiviral therapy against hepatitis C virus (HCV) is the HCV polymerase nonstructural protein 5B (NS5B). Huge efforts have been devoted to the development of nucleoside and non-nucleoside inhibitors (NNIs) of NS5B. An offshoot of these efforts has been the structural characterization of the interaction of NS5B with NNIs by X-ray crystallography. These works have shown that the conformation of recombinant NS5B is very similar across strains, constructs and complexes, making evaluation of the long-range conformational effects of NNIs nontrivial.

Using procedures appropriate to the evaluation of such minor but potentially important differences, we objectively assessed the conformational diversity in the 78 available genotype 1b NS5B structures in the Protein Data Bank. We find that there are 20 significantly different NS5B conformations available, but all are geometrically close to a closed, RNA synthesis initiation-competent one. Within this fairly restricted range, differences can be mapped to movements of NS5B domains and subregions. Most of this information is actually defined by small but significant changes in complexes with NNIs. We thus establish rigorously the moving parts of the NS5B molecular machine and the previously unrecognized hinge points that come into play upon NNI binding.

We propose that NNIs binding at three of the four distinct sites specifically inhibit the initiation step by the same mechanism: they prevent NS5B's "thumb" from quite reaching the proper initiation-competent position. Furthermore, we suggest that a small number of critical hinges in the NS5B structure may emerge as sites of resistance mutations during future antiviral treatment.

Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma worldwide. There is no available vaccine against HCV, of which six major genotypes exist. Treatment of the chronic infection is costly and poorly effective, especially for patients infected with genotypes 1 and 4. Cure (i.e., a sustained virological response) is now achieved in 50% of cases with the bitherapy of pegylated interferon alpha and ribavirin.[1] There is thus an urgent need to develop

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Abbreviations used: HCV, hepatitis C virus; NS5B, nonstructural protein 5B; NS3, nonstructural protein 3; PDB, Protein Data Bank; esd, estimated standard deviation; RdRp, RNA-dependent RNA polymerase; NNI, non-nucleoside inhibitor.

new potent and nontoxic anti-HCV drugs. The standard of care is nonspecific, and the new therapeutic strategies specifically target the virus (specifically targeted antiviral therapy for hepatitis C).[2] The HCV genome encodes a single large (ca 3000 residues) polyprotein that is processed to yield 10 mature proteins. Among these, two of the main targets of specifically targeted antiviral therapy for hepatitis C have been the viral protease nonstructural protein 3 (NS3) and the RNA-dependent RNA polymerase (RdRp) nonstructural protein 5B (NS5B). HCV is an RNA virus and as such is highly variable. Each patient is infected by a large population of closely related viruses termed a quasispecies, and HCV continuously replicates to very high levels, generating further viral diversity. Accordingly, monotherapy with directly acting antivirals against NS3 and NS5B poses a high risk for selection of resistant variants.[3,4] Nevertheless, huge efforts have been made to develop drugs that target these viral enzymes. Major advances have been recently reported, and a new standard of care will soon be available for patients infected with genotype 1, with tritherapies adding an NS3 inhibitor to the current regimen.[5]

As for NS3, a very large number of drugs raised against NS5B have been tested and characterized *in vitro*, more than 20 have moved into preclinical trials and a few are now in clinical trials.[2,4,6] A strong focus of this drug development effort has been the structural characterization of the interaction of NS5B with non-nucleoside inhibitors (NNIs) by X-ray crystallography. This technique is an onerous but invaluable tool for the drug designer, as it can provide near-atomic details of the interactions between protein targets and lead compounds, greatly facilitating further optimization. The X-ray crystal structure of NS5B was actually the first complete structure of an RdRp to be solved.[7–9] A peculiar feature first revealed by this structure, which turned out to be the hallmark of viral RdRps, is a connection between the so-called "fingers" and "thumb" subdomains through an extension of the fingers ("fingertips", Fig. 1) that closes off the back of RdRps. In the 88 NS5B X-ray structures available in the Protein Data Bank (PDB) as of October 2010, three features stand out: firstly, all of these structures are C-terminal deletions devoid of the 21-residue C-terminal membrane anchor. Crystallized recombinant NS5Bs include both constructs where only this anchor is removed (Δ21 forms) and constructs where the ca 40-residue linker (in gray in Figs. 1 and 2, first column) is also missing (mostly Δ55 forms). Secondly, nearly all structures belong to genotype 1b strains. Finally, most structures are complexes with NNIs. These have pinpointed four inhibitor binding pockets (Fig. 1) in the thumb (sites I and II) and palm (sites III and IV) subdomains.[10]

In this study, we set out to assess objectively the conformational differences between the available genotype 1b NS5B structures. Our objective was twofold: we wanted (i) to establish rigorously the moving parts of the NS5B molecular machine and (ii) to assess any significant conformational changes, however small, associated with the binding of the various classes of NS5B NNIs.

Results

Conformational diversity in available genotype 1b NS5B structures

As of October 2010, the PDB comprised no fewer than 78 genotype 1b NS5B crystal structures.

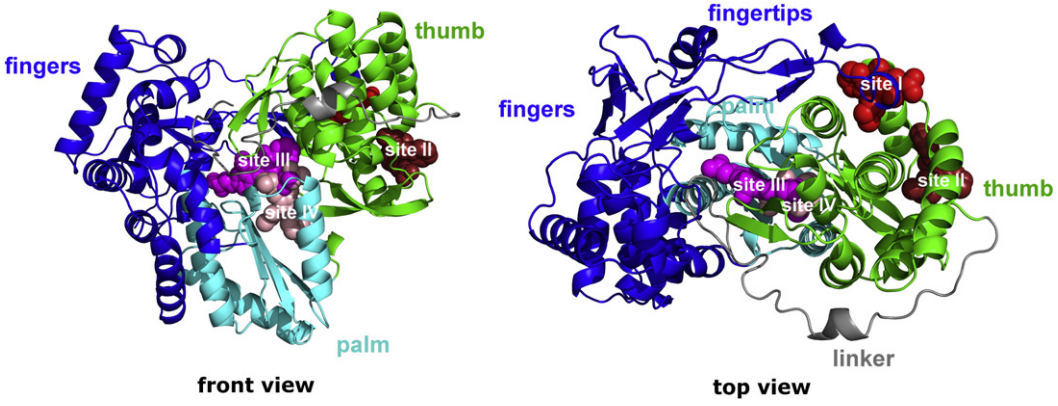

Fig. 1. Cartoon representation of HCV-NS5B colored by subdomains as conventionally assigned. The boundaries of "fingers" (blue), "palm" (cyan) and "thumb" (green) subdomains and those of the "linker" (gray) downstream of the thumb are indicated in Fig. 2, first column. Two views of HCV-BK-NS5B_Δ21 (2GIQ_A) are shown and related by a 90° rotation ("front view," left, and "top view", right). The known binding sites for NNIs are indicated by a representative of each NNI in sphere representation: site I/thumb 1, red (note that this site overlaps with the fingertips, the tip of which it displaces upon binding); site II/thumb 2, brown; site III/palm 1, magenta; site IV/palm 2, light pink.