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Characterization of molecular interactions between Zika virus protease and peptides derived from the C-terminus of NS2B

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ABSTRACT

Zika virus (ZIKV) protease is a two-component complex in which NS3 contains the catalytic triad and NS2B cofactor region is important for protease folding and activity. A protease construct-eZiPro without the transmembrane domains of NS2B was designed. Structural study on eZiPro reveals that the Thr-Gly-Lys-Arg (TGKR) sequence at the C-terminus of NS2B binds to the active site after cleavage. The bZiPro construct only contains NS2B cofactor region and the N-terminus of NS3 without any artificial linker or protease cleavage site, giving rise to an empty pocket accessible to substrate and inhibitor binding. Herein, we demonstrate that the TGKR sequence of NS2B in eZiPro is dynamic. Peptides from NS2B with various lengths exhibit different binding affinities to bZiPro. TGKR binding to the active site in eZiPro does not affect protease binding to small-molecule compounds. Our results suggest that eZiPro will also be useful for evaluating small-molecule protease inhibitors.

1. Introduction

Zika virus (ZIKV), dengue virus (DENV), West Nile virus (WNV) and some other flaviviruses are important human pathogens. ZIKV was first discovered in 1947 [1] and drew worldwide attention as it caused epidemic since 2007. Similar to DENV and WNV, ZIKV can be transfected by a bite of an infected *Aedes* species mosquito [2]. In addition, studies have shown that ZIKV can be transmitted from human to human. An infected woman may transfer the virus to her baby, which may cause microcephaly and other birth defects [3]. ZIKV infection is also related to Guillain–Barré syndrome in adults [4,5]. It is necessary to develop chemotherapies and vaccines to combat ZIKV infection as there is no specific treatment available.

The single-stranded positive-sense RNA genome of ZIKV encodes a poly-peptide which can be further cleaved into three structural and seven non-structural (NS) proteins. The maturation of viral proteins requires cleavage of the polypeptide by both host and viral proteases. NS proteins including NS2A, NS2B, NS3, NS4A and NS4B are essential for viral replication by forming a replication complex on the endoplasmic reticulum (ER) membrane [6–11]. Preventing the viral polypeptide from maturation is therefore a strategy to inhibit viral infection [12]. Viral protease is a two-component complex formed by a cofactor region from membrane protein NS2B and the N-terminal region of NS3 [13–17]. The cofactor region of NS2B comprises of approximately 40 residues and is important for the folding and protease activity of NS3 [18]. As NS2B is a membrane protein with four transmembrane helices, it is important for the localization of NS3 on the ER membrane [19,20]. The N-terminal region of NS3 contains the catalytic triad formed by residues H51, D75 and S135 [14–16,21,22]. In total, the NS2B-NS3 protease is an important drug target due to its importance in the mutation of viral proteins.

Structural study on the native form of flaviviral protease is challenging [23–25]. In particular, alternative constructs of NS3 and NS2B are used in structural and biochemical studies of the flaviviral protease [17,18,26–28]. The first commonly used construct is the gZiPro in which the cofactor region of NS2B and NS3 are covalently linked via a glycine-rich linker [14,15,22,28]. The second construct is bZiPro in which the cofactor region is co-expressed with NS3 and the resulting complex contains no linker between the two sequences [16]. It is notewothy that bZiPro construct contains an empty pocket and is suitable for studying structures of protease in complexes with different types of inhibitors [16,21,29]. The third is the eZiPro construct which is similar to the native form except that the transmembrane domains of NS2B are not included [17]. Work done in our group has shown that the eZiPro construct adopts a closed conformation [17]. Four residues Thr^{127}-Gly^{128}-Lys^{129}-Arg^{130} (TGKR) of NS2B bind to the active site of protease

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while residues from the prime sites are released after cleavage [17]. The structural information of eZiPro provides a snapshot of ZIKV protease under the native conditions [17]. As the active site of eZiPro is occupied by residues from non-prime sites of the substrate, similar phenomenon may also exist in the native protease. Whether eZiPro is subject to ligand binding needs to be further explored.

In this study, we demonstrate that residues from the C-terminus of NS2B in eZiPro are dynamic in solution. Peptide consisting of residues 127–130 (TGKR) of NS2B binds to bZiPro with a dissociation constant (Kd) of 44 μM determined by Isothermal Titration Calorimetry (ITC). A longer peptide consisting residues 126–130 (KTGKR) of NS2B binds weaker to bZiPro with a Kd of 194 μM. Our binding studies also shows that eZiPro binds to TGKR, AcKR, AcKR-aldehyde and a small-molecule fragment. In addition to bZiPro, this eZiPro construct can also be useful in evaluating inhibitors by NMR spectroscopy as the exchanges in the protease are suppressed.

2. Materials and methods

2.1. Purification of eZiPro and bZiPro

Both eZiPro and bZiPro were purified using the methods described previously [16,17]. Triple (^{13}C, ^{15}N, ^{2}H)-labeled samples were concentrated to 0.4–0.8 mM in a buffer that contained 20 mM HEPES, pH7.3, 150 mM NaCl, and 1 mM DTT for NMR studies. ^{15}N-amino acid-labeled eZiPro samples were prepared using the similar method except that amino acid mixture was added into the medium before protein induction [28].

2.2. Preparation of peptides for binding studies

The peptides including KTGKR, TGKR, GKR, AcKR and KR were synthesized (GL biochem, Shanghai) with purity more than 95%. AcKR-aldehyde (AcKR-H) was synthesized in house [21]. Stock solutions of these peptides (30 mM) were made by dissolving them into water, respectively. The fragment-EN300 (1H-benzo ([*d*]imidazole-1-yl)methanol) was purchased from Enamine Ltd and dissolved in deuterated dimethyl sulfoxide (DMSO) to make a stock solution (200 mM).

2.3. NMR studies

All the NMR studies were carried out on a Bruker Avance magnet with a proton frequency of 700 MHz or 600 MHz. The data acquisition for eZiPro and bZiPro were performed at 310 K and 298 K, respectively. Relaxation measurement was carried out using the same parameters described previously [16,30]. In the titration experiments, the ^{1}H-^{15}N-HSQC spectra of bZiPro in the presence of different amounts of TGKR peptide were collected and processed. To probe the molecular interactions between eZiPro and peptides/inhibitors, the ^{1}H-^{15}N-HSQC spectra of 0.7 mM eZiPro in the absence and presence of 2.8 mM KTGKR, TGKR, AcKR, AcKR-H and KR were collected, respectively. The ^{1}H-^{15}N-HSQC spectrum of 0.8 mM eZiPro in the presence of 6.4 μl of DMSO and 6.4 mM EN300 were collected and compared. The free NS2B cofactor region was prepared in the sample buffer as eZiPro. The ^{1}H-^{15}N-HSQC spectrum of a ^{15}N-labeled NS2B cofactor region was collected at 310 K. Steady-state heteronuclear NOE (hetNOE) measurements [31] were obtained using two datasets that were collected with (noe) and without (ref) initial proton saturation for a period of 3 s [30]. All the data were acquired using Topspin (version 2.1), processed using NMRPipe [32] and visualized using NMRView [33]. Titration curve and dissociate constant were obtained using NMRView Java version.

2.4. ITC experiments

ITC experiments was performed on an Auto-iTC200 instrument (Microcal Inc.) at 25 °C using the method described previously [34]. Briefly, bZiPro was prepared in a buffer that contained 20 mM HEPES, pH7.3, 150 mM NaCl, and 1 mM DTT. The titration experiment was performed using 18 injections over a period of 40 min. The stirring rate was 1000 rpm. The dissociation constant (K_d) were determined using Origin software provided with the instrument.

3. Results

3.1. The TGKR residues of NS2B are dynamic in eZiPro

The eZiPro construct is similar to the native form of ZIKV protease except that the transmembrane domains of NS2B are not

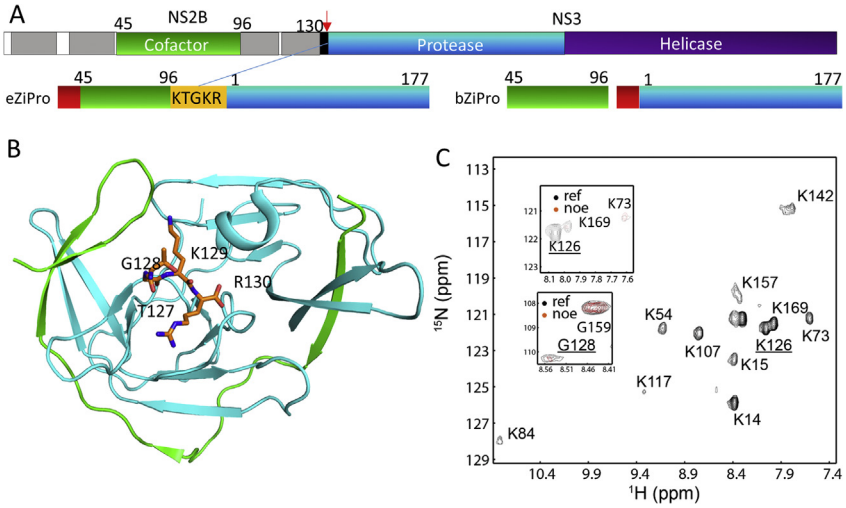

Fig. 1. Structure of eZiPro. A. Diagram of the eZiPro construct. The transmembrane domains of NS2B are shown as open boxes. The cleavage site between NS2B and NS3 is indicated with an arrow. B. Structure of the eZiPro. The X-ray structure (PDB DI 5GJ4) of eZiPro is shown and TGKR peptide in the structure is shown as sticks. C. ^{1}H-^{15}N-HSQC spectra of ^{15}N-Lys labeled eZiPro. Inlet shows the hetNOE analysis of the ^{15}N-Lys labeled bZiPro and G128 of NSB. The spectra before (black) and after (red) saturation are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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