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Role of BC loop residues in structure, function and antigenicity of the West Nile virus envelope protein receptor-binding domain III

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ABSTRACT

Site-directed mutagenesis of residues in the BC loop (residues 329–333) of the envelope (E) protein domain III in a West Nile virus (WNV) infectious clone and in plasmids encoding recombinant WNV and dengue type 2 virus domain III proteins demonstrated a critical role for residues in this loop in the function and antigenicity of the E protein. This included a strict requirement for the tyrosine at residue 329 of WNV for virus viability and E domain III folding. The absence of an equivalent residue in this region of yellow fever group viruses and most tick-borne flavivirus suggests there is an evolutionary divergence in the molecular mechanisms of domain III folding employed by different flaviviruses.

Introduction

The genus *Flavivirus*, family *Flaviviridae*, includes arthropod-borne viruses that are associated with significant human and veterinary diseases, such as West Nile fever/encephalitis, dengue fever, Japanese encephalitis, tick-borne encephalitis and yellow fever. Approximately half of the world's population is at risk for infection with one or more of these viruses, and collectively they are responsible for many hundreds of thousands of human disease cases and tens of thousands of deaths worldwide each year.

Flavivirus virions are relatively smooth, spherical particles approximately 50 nm in diameter (Mukhopadhyay et al., 2003). Their outer surface is comprised of a lipid envelope carrying 180 copies of the viral envelope (E) protein arranged in 90 homodimers, and an equal number of the residual membrane (M) portion of the pre-membrane (prM) protein. The flavivirus E protein is a class II viral fusion protein that mediates the essential functions of attachment to and fusion with host cell membranes and structures solved for several E proteins show strong conservation of the overall fold between tick- and mosquito-borne flaviviruses, presumably mediated via strict conservation of six intramolecular disulfide bridges (Kanai et al., 2006; Modis et al., 2003; Nybakken et al., 2006; Rey et al., 1995).

Structural domain III of the E protein is a beta-barrel/sandwich structure with an Ig-like fold and a single disulphide bridge. Although specific receptors for flavivirus binding to target cells remain elusive, domain III of mosquito-borne West Nile virus (WNV) and dengue virus (DENV) types 1, 2 and 4 have been shown to contribute to binding of those viruses to candidate protein or carbohydrate receptors on target cells (Chin et al., 2007; Chu et al., 2005; Hung et al., 2004; Lee et al., 2006;

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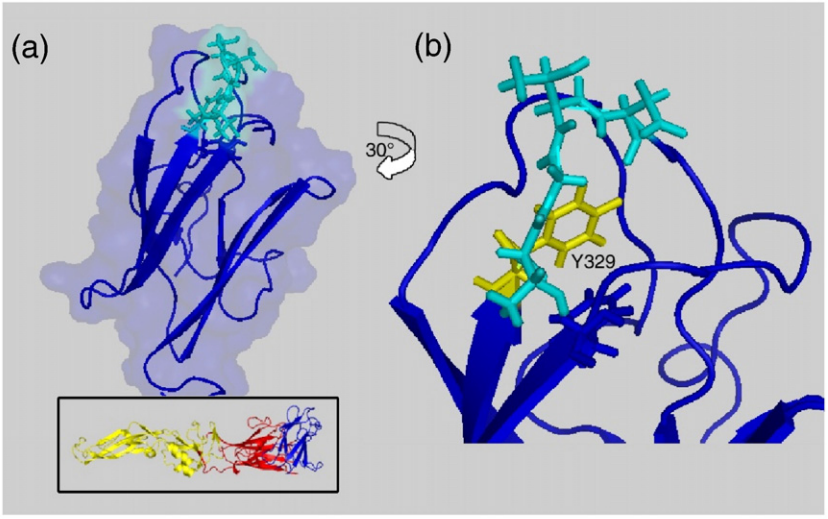
Pattnaik et al., 2007) confirming that domain III is the likely receptor-binding domain and a major determinant of virus tropism. Domain III also encodes critical epitopes recognized by potent, virus type-specific neutralizing antibodies (Roehrig, 2003). As such, the amino acid sequence of domain III for different flaviviruses has presumably been defined by a balance of selective pressures that promote antigenic diversity while retaining the essential receptor-binding functions of the domain.

Flaviviruses have an error-prone RNA genome replication strategy, meaning that within any virus population there exists a variable level of sequence diversity. This property has been used in the selection and study of flavivirus variants under a variety of selective pressures, including selection of neutralization resistant antibody escape variants. Structural studies have shown that neutralizing antibodies that bind to epitopes in domain III interact with almost the entire exposed surface of the domain (Nybakken et al., 2005; Wu et al., 2003). However, selection of neutralization resistant variants of WNV using anti-domain III antibodies either *in vitro* (Beasley and Barrett, 2002; Choi et al., 2007) or *in vivo* (Zhang et al., 2009) has been associated with mutations at only a limited number of residues in the amino terminal strand (K307), BC loop (T330 and T332) and DE loop (A367), suggesting that structural and functional constraints may significantly limit the potential range of domain III surface mutations that would otherwise facilitate neutralization escape. Consistent with this, none of these mutations has been associated with measurable differences for growth in cell cultures or mouse virulence phenotypes compared to the parental wild-type viruses (Beasley and Barrett, 2002; Zhang et al., 2009).

In this study, targeted mutagenesis of a WNV infectious clone was used to explore the potential plasticity of domain III BC loop residues (residues 329–333, sequence YTGTD; Fig. 1). Amino acids encoded at residues 330 and 332 are variable and appear to be major determinants of the antigenic differences between WNV strains and between WNV and other flaviviruses (Beasley and Barrett, 2002; Oliphant et al., 2005; Sanchez et al., 2005; Zhang et al., 2009). In contrast, residues Y329 and G331 are highly conserved among mosquito-borne flaviviruses (Fig. 1c), with the exception of most YF group viruses, suggesting that they may be subject to structural and/



(c)

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                    302                                                 337
Mosquito
  WNV       YGVCSKA-FKFLGTPADTGHGTVVLELQYTGTDGPCK
  MVEV      YGMCTEK-FTFSKNPADTGHGTVVLELQYTGSDGPCK
  JEV       YGMCTEK-FSFAKNPADTGHGTVVIELSYSGSDGPCK
  SLEV      YGMCDSA-FTFSKNPTDTGHGTVIVELQYTGSNGPCR
  DENV-1    YVMCTGS-FKLEKEVAETQHGTVLVQVKYEGTDAPCK
  DENV-2    YSMCTGK-FKVVKEIAETQHGTIVIRVQYEGDGSPCK
  DENV-3    YAMCLNT-FVLKKEVSETQHGTILIKVEYKGEDAPCK
  DENV-4    YTMCSGK-FSIDKEMAETQHGTTVVKVKYEGAGAPCK
  YFV       YKMCTDK-MSFVKNPTDTGHGTVVMQVKVPKG-APCR
  WSLV      YSMCKRG-MSFAKQPVETDHGTAVMQIKVTTG-APCR
  EHV       YTMCKGG-FSFVKTPTDTGHGTAVMQVKVSKG-TPCR

Tick
  POWV      YSMCDKAKFKWKRVPVDSGHDTVVMEVSYTGSDKPCR
  KSIV      YSMCEEGKFAWKRPPTDSGHDTVVMEVDYTGTVKPCR
  RFV       YSMCESGKFSWKRPPTDSGHDTVVMEVSYSGATKPCR
  KFDV      YTVCEGSKFAWKRPPTDSGHDTVVMEVDYTGS-KPCR
  LGTV      YTVCDKTKFTWKRAPTDSGHDTVVMEVGFSGT-RPCR
  OHFV      YTMCDKAKFTWKRAPTDSGHDTVVMEVAFSGT-KPCR
  LIV       YTMCDKSKFAWKRTPTDSGHDTVVMEVTFSGS-KPCR
  TBEV(CEE) YTMCDKTKFTWKRAPTDSGHDTVVMEVTFSGT-KPCR
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Fig. 1. Location of BC loop (residues 329–333, in cyan) in the WNV E domain III: (a) lateral view of domain III, oriented as for the complete E protein structure (inset); (b) rotated 30° to the left to show the location of Y329 (in yellow). (c) Amino acid sequence alignment of flavivirus domain III residues equivalent to 302–337 of WNV; flavivirus-conserved cysteines and BC loop residues 329–333 are boxed. Mammalian tick-borne flaviviruses are ordered according to their proximity to the root of that branch in previous phylogenetic analyses of their evolution (Gaunt et al., 2001; Grard et al., 2007) showing replacement of tyrosine with phenylalanine and shortening of the BC loop.