

Class: SARS-CoV NTPase / Helicase

Attributes: (1)

Accession #: NP_828870

Synonyms: nsp13

Molecular weight: unknown

Number of amino acids: 600

Structure:

1) Homology:

- primary sequence places it in superfamily-1 of the five major groups of helicases
- form a distinct class within SF-1
- has similar properties to arterivirus, porcine reproductive and respiratory syndrome virus

2) Domains:

- within the N-term region is a cysteine-rich putative metal binding domain

3) Substrate Specificity:

- all eight common NTPs and dNTPs were hydrolyzed by the SARS helicase in a magnesium-dependent reaction
- consistent with the triphosphate moiety forming the basis of the recognition factor for binding and hydrolysis as has been suggested for hepatitis C ns3 helicase
- ATP may bind to a weaker set of ATP-binding sites in a putative homomultimeric enzyme complex, thereby inhibiting the net ATPase activity of the enzyme
- stimulated by the presence of either single-stranded DNA or RNA
- preference for ATP, dATP and dCTP over other NTP/dNTP substrates
- homopolynucleotides significantly stimulated the ATPase activity (15-25 fold)
- RNA synthesis augmenting proposed base-pairing factors
- exception was poly(G) and poly(dG)
- dT₂₄ bound 10 times more strongly than dA₂₄

4) Catalytic Activity:

- in the absence of ssDNA or RNA there was a basal rate of ATPase activity of $\sim 0.4s^{-1}$
- ATPase activity was absolutely dependent on Mg²⁺
- Mn²⁺ could substitute for Mg²⁺ but with reduced efficiency ($\sim 40\%$ at 2.5 mM)
- Apparent half-maximal stimulation values (K_m^{app}) for the binding of poly(dA)₂₄, poly(dC)₂₄, poly(dT)₂₄ and poly(U)₂₄ were 900, 165, 37 and 150 nM, respectively

Processing:

- released by 3CLpro cleavages of ₅₃₀₁Gln|Ala₅₃₀₂ and ₅₉₀₂Gln|Ala₅₉₀₃

Location: unknown

Functions:

- implicated in subgenomic mRNA synthesis, genome replication and virion biogenesis in equine arteritis virus
- 5' to 3' polarity of unwinding
- stimulation of ATPase activity by single stranded polynucleotides
- unwinds double stranded DNA
- dependent on the presence of a 5' single-stranded overhang indicating a 5' to 3' polarity
- suggests helicase may aid the replicative process by disrupting secondary structure or displacing bound proteins and annealed RNA fragments
- specificity for homopolynucleotides may play a role in the localization of the complex to selected genomic locations during subgenomic

Abundance: unknown

Responsibilities:	Collaborators:
Hydrolize bonds to disrupt secondary structure or displace bound proteins and annealed RNA fragments	NTPs
Hydrolize bonds to disrupt secondary structure or displace bound proteins and annealed RNA fragments	dNTPs
Unwind DNA to allow access of polymerase	dsDNA
Functional consequence unknown	caveolin-1 (2)

- 1) Thiel, V., et al., (2003) Mechanisms and enzymes involved in SARS coronavirus genome expression, *J. Gen. Virol.*, **84(9)**, 2305-2315.
- 2) Cai, Q.C., et al., (2003) Putative caveolin-binding sites in SARS-CoV proteins, *Acta Pharmacol Sin*, **24(10)**, 1051-9.