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Determination of the functions of the putative metal-binding domain of the SCV helicase

Key Messages

1. The inhibition of SARS coronavirus (SCV) helicase by bismuth compounds is mediated via the abilities of bismuth ions to displace (essentially required) zinc ions that are bound to cysteine residues located within the metal-binding domain.
2. Several novel bismuth compounds targeting SCV helicase have been synthesised. These compounds can inhibit SCV helicase activity and viral growth and potentially target other viruses.

Introduction

Severe acute respiratory syndrome (SARS) was first recognised in late 2002 and was shown to be caused by the SARS coronavirus (SCV).[1,2] We have reported the purification and characterisation of the SCV helicase,[3] and identified a number of lead compounds able to inhibit SCV helicase activities and viral growth.[4,5] One of these contained bismuth—ranitidine bismuth citrate (RBC). To reveal the mechanisms underlying RBC action, we investigated the interaction between zinc, bismuth, and the helicase protein. Mutational analysis was used to pinpoint the key amino acid residues necessary for the helicase function. Several novel bismuth compounds with SCV inhibiting activity were synthesised. This study was conducted from February 2005 to January 2007.

Characterisation of the Zn^{2+}- SARS coronavirus helicase interaction

The N-terminal of the SCV helicase consisted of a cysteine rich domain: the metal-binding domain (MBD). Sequence alignment suggested that this entailed Zn^{2+} binding. To determine how helicase binds to Zn^{2+} ions, we used 4-(2-pyridylazo) resorcinol / *p*-hydroxymercuriphenylsulfonic acid assay to demonstrate that zinc did indeed bind to the MBD of SCV helicase via the cysteine residues (Fig 1).

To study the effect on the structure of MBD upon zinc binding, we measured the circular dichroism of MBD (Fig 2). The zinc-bound form of MBD showed the characteristics expected of high helix content. However, addition of excess ethylenediaminetetraacetic acid to remove the zinc ions led to a decrease in ultraviolet light absorption, suggesting that the protein changed to a random structure in the absence of Zn^{2+}.

Mutational analysis of the SARS coronavirus helicase metal-binding domain

To define the precise role of individual amino acids within the SCV helicase MBD, we individually mutated several key cysteine residues within the MBD. The mutant proteins were then studied by measuring their ATPase and unwinding activities. All the mutant proteins lost their activities.

Determination of the mechanism of ranitidine bismuth citrate–mediated inhibition of SARS coronavirus helicase activity

Bi^{3+} is known to have a high affinity for thiolate sulphur.[6] We therefore measured the ultraviolet light absorbance spectrum of the SCV helicase, both in the absence and presence of RBC. The cysteine-rich MBD was the target of the bismuth ion. Addition of Bi^{3+} to the Zn^{2+}-bound form of MBD led to an increase in ultraviolet light absorbance, indicative of replacement of Zn^{2+} by Bi^{3+}.[7]

Synthesis of bismuth complexes against SARS coronavirus

Bismuth compounds effectively inhibited SCV growth in cell culture. The

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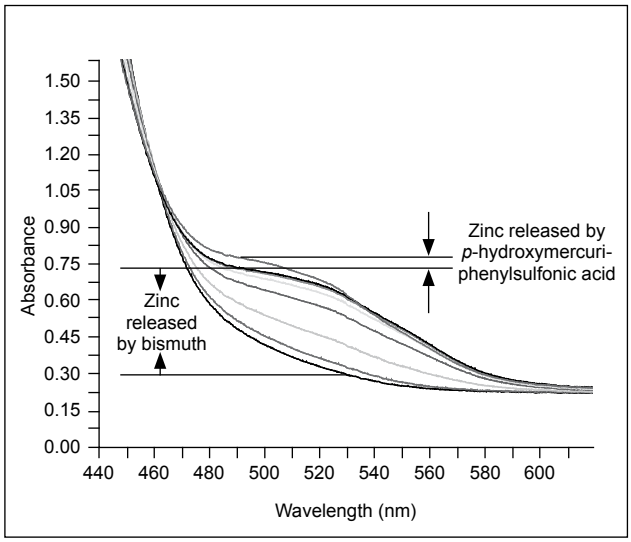

Fig 1. Zinc that binds to the metal-binding domain can be replaced by bismuth

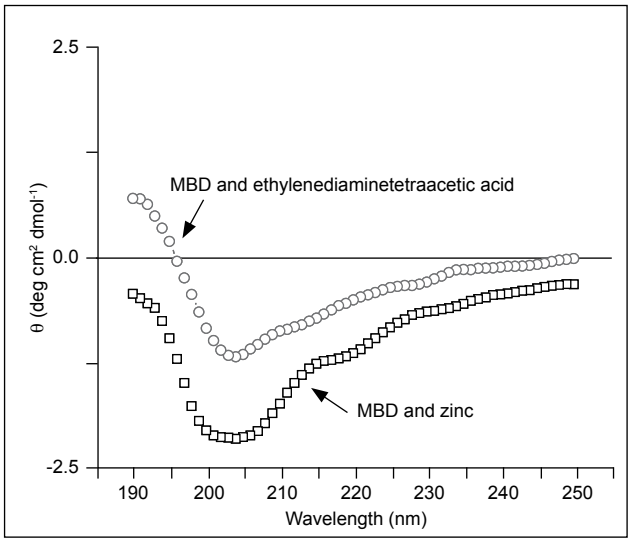

Fig 2. Circular dichroism of metal-binding domain (MBD) with and without zinc binding

mechanism was through replacement of zinc bound to the MBD. We then designed and synthesised a series of bismuth complexes, including complexes of: bismuth porphyrin, bismuth macrocyclin, bismuth 12-crown-4, bismuth bipyridine, bismuth phenanthroline, bismuth nitrilotriacetate, bismuth ethylenediaminetetraacetic acid, and bismuth acetohydroxamate.[3] The newly synthesised compounds were tested for their activity against SARS helicase. The two bismuth porphyrin complexes and RBC exhibited the the most significant inhibition activities as revealed by in vitro experiments.[8]

Conclusion

We determined the interaction between zinc, bismuth, and the helicase protein, enabling us to construct mechanistic models for bismuth-mediated inhibition of the SCV helicase functions. The zinc ions bound to the MBD of SCV helicase were replaced by bismuth ions upon addition of RBC and other bismuth compounds, resulting in dysfunction of the helicase. These compounds may also be used to inhibit other viruses.

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