The mercury–sulfur bond mediated inhibition mechanism of PCMPS and PCMB on NP exonuclease

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Abstract

According to World Health Organization (WHO) statistics, Lassa fever virus infects about 30-50 thousands of people per year with 1% fatality rate in western Africa, but without any vaccine or effective therapeutic treatments by now. NP exonuclease of Lassa fever virus comprised by N-terminal domain and C-terminal DEDDh exonuclease domain, which are responsible for cap binding and immune evasion, respectively. The two functions of N-terminal domain and C-terminal nuclease domain are essential for viral replication and infection, therefore the inhibitors of NP exonuclease can be use as drugs for antiviral therapies. On the other hand, Sulfhydryl group reactive agents, such asp-hydroxymercuribenzoate (PCMB) and p-chloromercuriphenyl sulfate (PCMPS), belong to organic mercury compounds (organomercurials), which can be use as inhibitors of various enzymes. However, the inhibition mechanisms of PCMB or PCMPS on target enzymes are remain unclear. In this study, we demonstrated PCMB and PCMPS are inhibitors of NP exonuclease by inhibitor coupled nuclease activity assays. We also determined the crystal structures of PCMPS- and PCMB-NP exonuclease complexes. In these structures, PCMB or PCMPS direct interact to cysteine residues of NP exonuclease via a mercury–sulfur bond. Interestingly, the mercury–sulfur bond between PCMPS and NP exonucelase is not located at substrate binding site or active site, and not affect the overall structure of NP exonuclease. In order to reveal why the mercury–sulfur bonds are not occupy the activity site or substrate binding site but affect the activity and substrate binding ability of NP exonuclease, we preform the molecular dynamics (MD) simulation for understanding the dynamic of apo- or ligand bound NP exonuclease. Our MD results demonstrate that the mercury–sulfur bonds formation affect the local interaction networks of NP exonuclease. Perturbation in local molecular interactions altered longer-range protein conformation, substrate binding ability and nuclease activity. In conclusion, our biochemical, structural and MD related studies provide a unique dynamic driven inhibition mechanism of PCMB and PCMPS on NP exonuclease.

Keywords：DEDDh exonuclease, NP exonuclease, antiviral inhibitor, PCMPS, PCMB