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Engineering DUB-deficient Viral Proteases from FIPV and PEDV Coronaviruses

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ABSTRACT

Coronaviruses form a class of viral pathogens lethal to humans and livestock. This issue is compounded by a lack of commercially available treatments or vaccines. In 2014, porcine epidemic diarrhea virus (PEDV) emerged in the United States and accounted for an estimated 7 million porcine deaths. Deaths of humans, companion animals, and livestock caused by coronaviruses highlight the need for therapeutic strategies to combat this devastating disease. One strategy involves engineering papain-like protease 2 (PLP2), an enzyme conserved among coronavirus species that is critical for virus replication and pathogenesis. PLP2's de-ubiquitinating (DUB) activity aids in the suppression of the host's innate antiviral immune response. By targeting and disrupting ubiquitin binding in PLP2 and thus its DUB activity, the virus would no longer be able to antagonize the innate immune response. To this end, we introduced informed single-point mutations in PEDV and in feline infectious peritonitis virus (FIPV) PLP2s using structure-guided mutagenesis. We then characterized the kinetic activity of the resulting mutants in vitro using fluorescent peptide and ubiquitin substrates. Through these studies, we were able to evaluate the relationship between PLP2-ubiquitin binding and DUB activity. Preliminary data analysis suggests that residues outside the active site of PLP2 and within the ubiquitin-binding interface are necessary for DUB activity; these residues can be selectively disrupted to abolish DUB activity relative to the wild-type. These results describe a series of DUB-deficient PLP2 mutants that can be leveraged as tools for use in future coronavirus research. Such tools will allow creation of an attenuated virus strain that could aid in vaccine and drug design.

KEYWORDS

Coronaviruses, FIPV, PEDV, papain-like protease, viral proteases, immune response, ubiquitination