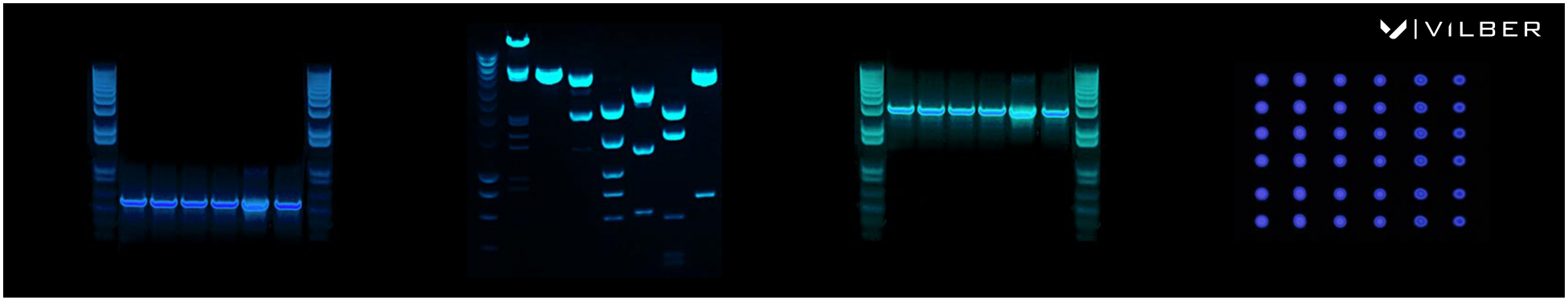

STRUCTURAL AND BIOCHEMICAL CHARACTERIZATION OF THE NSP12-NSP7-NSP8 CORE POLYMERASE COMPLEX FROM SARS-COV-2

AUTHORS

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INTRODUCTION

The ongoing global pandemic of coronavirus disease 2019 (COVID-19) caused by the virus SARS-CoV-2 has caused a huge number of human deaths. SARSCoV- 2 displays the highest similarity in genome sequence to the SARS-CoV emerging in 2002–2003. Both viruses utilize the same host receptor, angiotensin-converting enzyme 2 (ACE2), for cell entry and cause respiratory symptoms that may progress to severe pneumonia and lead to death. However, compared with SARS-CoV, SARS-CoV-2 has a much higher transmission rate and lower mortality. The replication of coronavirus is operated by a set of nonstructural proteins (nsps) encoded by open reading frame 1a (ORF1a) and ORF1ab in its genome. Among them, nsp12, the catalytic subunit with RNA-dependent RNA polymerase (RdRp) activity, conducts the polymerase reaction with extremely low efficiency, whereas the presence of nsp7 and nsp8 cofactors remarkably stimulates its polymerase activity. The nsp12-nsp7-nsp8 subcomplex is thus defined as the minimal core component for mediating coronavirus RNA synthesis. These properties allow SARS-CoV-2 to be transmitted among humans furtively, and to be considered as a highly potent antiviral drug target because of its higher evolutionary stability. Therefore, understanding the structure and function of the SARS-CoV-2 polymerase complex is an essential prerequisite for developing novel therapeutic agents.

OBJECTIVE

This work determined the near-atomic-resolution structure of the SARSCoV-2 nsp12-nsp7-nsp8 core polymerase complex and revealed its reduced polymerase activity and thermostability. This structure highly resembles the counterpart of SARS-CoV with conserved motifs for all viral RNA-dependent RNA polymerases and suggests a mechanism of activation by cofactors.

MATERIAL & METHODS

SARS-CoV-2 Core Polymerase Complex design. To design the experimental model, *Escherichia coli* BL21 (DE3) strain (EC0114), MAX Efficiency DH10Bac Competent cells (10361-012), Sf9 (11496015) and High Five (B85502) cells were exploited. For the SARS-CoV2 nsp12 proteins, the genes were codon-optimized for *Spodoptera frugiperda* (Sf9) and incorporated into the pFastBac-1 plasmid. Proteins were expressed with High Five cells at 27°C for 48 h post infection. As for the nsp7-nsp8 cofactors, they were expressed using *Escherichia coli (E. coli)* expression systems. The three protein subunits were then mixed *in vitro* to constitute the core polymerase complex. The structure of the SARS-CoV-2 nsp12- nsp7-nsp8 complex was determined at 3.7-A° resolution. **In vitro polymerase activity assay.** The activity of SARS-CoV-2 polymerase complex was designed with slight modifications when compared to SARS-CoV. Briefly, a 40-nt template RNA corresponding to the 30 end of the SARS-CoV2 genome was annealed to a complementary 20-nt primer containing a 50-fluorescein label. To perform the primer extension assay, nsp12, nsp7 and nsp8 were incubated with annealed RNA and NTP in a reaction buffer containing Tris-HCl (pH 8.0), KCl, betamercaptoethanol and MgCl2 (freshly added prior usage). The products were denatured by boiling in the presence of formamide and separated by PAGE containing urea run with TBE buffer (see publication for details). Images were taken using a Fusion system (Vilber Lourmat) and quantified with the ImageJ software.

RESULTS

Figure 1. In Vitro Polymerase Activity of nsp12 and Regulatory Effects of Cofactors. (A) Comparison of RNA synthesis activities of the SARS-CoV-2 and SARS-CoV core polymerase complex. Efficient activity of nsp12 polymerase requires the presence of the nsp7 and nsp8 cofactors. Apart from the fully elongated product (green arrowhead), some aberrant termination products were also observed (yellow arrowhead). The excess primer band is indicated by a cyan arrowhead. (B and C) Comparison of the regulatory effects of the nsp7 and nsp8 cofactors in the context of the SARS-CoV-2 (B) and SARS-CoV (C) nsp12 polymerase, respectively. (D) Comparison of the activity of the nsp12 polymerase of different viruses in the same context of cofactors. Polymerase activity was quantified by integrating the intensity of the fully elongated product bands. The results, in histograms, are presented as the means ± SD, where error bars represent SDs.

CONCLUSION

Reduced Activity of the SARS-CoV-2 Core Polymerase Complex. Given the residue substitutions between SARS-CoV-2 and SARS-CoV polymerase subunits but the high degree of overall sequence similarity, this study compared the enzymatic behaviors of the viral polymerases, aiming to analyze their properties in terms of viral replication. Both sets of core polymerase complex could well be visualized with the Fusion from Vilber Lourmat (France) thus allowing to demonstrate that the residue substitutions in nsp12 contributed to the reduction of the polymerase activity, with a similar effect on the variations in the nsp8 cofactor.

Figure 1.