Viral RNA Dependent RNA Polymerase forms Amyloids Like Fibrils via Liquid-Liquid Phase Separation

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Noroviruses (NoV), responsible for severe gastroenteritis, are members of the Caliciviridae family of positive-sense RNA viruses. Currently, human norovirus infection is responsible for ~200,000 deaths annually worldwide, yet there are no effective vaccines/antivirals currently available. RNA-dependent RNA polymerase (RdRP) is considered a promising drug target because of its critical role in genome replication and the synthesis/amplification of sub-genomic RNA. Using light scattering measurements and confocal microscopy, we show that RdRP of human GII.4 NoV forms liquid-liquid phase droplets. With time, these liquid-liquid phase condensates undergo liquid to solid transition resulting in the formation of higher-order oligomers/fibrils. The formation of the higher-order oligomers with increasing temperatures was also confirmed using size exclusion chromatography and analytical ultra-centrifugation. Furthermore, using amyloid-specific dye-based assays such as fluorescence-based Thioflavin-T and Congo red binding in addition to transmission electron microscopy (TEM) analysis, we discovered that RdRP forms amyloid-like fibrils at physiological conditions in vitro. Circular Dichroism (CD) spectroscopy of RdRP with increasing temperatures revealed an increase in the β-sheet content and loss of α-helical content as typically observed in amyloid-forming proteins. Bioinformatics analysis of the RdRP sequence using three independent web-based servers suggests that RdRP has multiple hot spots spread across the sequence that may help in the formation of amyloid-like fibrils corroborating our experimental data. Overexpression of RdRP in Escherichia coli and HEK293T cells also showed the formation of distinct puncta indicating amyloid inclusions. These results set the stage for further investigations to assess the functional role of amyloid-like fibrils in the viral life cycle, and test the hypothesis that liquid-liquid phase condensates formed by RdRP provide a platform for sequestering other non-structural proteins and viral RNA to form replication factories during norovirus infection.