Abstract

Following the recent global pandemic caused by the SARS-CoV-2 coronavirus, a new pathogenic virus, monkeypox virus (MPXV), reemerged in early May 2022, raising significant concern. Since the eradication of the deadly smallpox virus in the 1990s, MPXV represents another virus from the Poxviridae family threatening humanity. Initial cases of this virus were recorded in Africa, where occasional small epidemics occur. However, cases have also been reported in Europe and the Americas, including individuals who had not visited affected regions in Africa. As of today, more than 99,000 cases have been recorded in 116 countries. This virus infects not only humans but also dogs, rodents, primates, and other animals. Fortunately, the mortality rate of this virus is not as high as that of smallpox, and symptoms resemble those of influenza, followed by a characteristic rash. MPXV is an enveloped virus with a size of 200–250 nm and a typical "brick" shape, classifying it as one of the larger viruses. Its genome consists of double-stranded DNA (dsDNA) with a size of 200 kb and encodes approximately 200 proteins. Among these proteins is the capping enzyme responsible for synthesizing the 5′ mRNA cap 0 (cap-0). This enzyme comprises two subunits, E1 and E12. The E1 subunit is catalytically active and contains all the catalytic modules necessary for cap formation, including RNA triphosphatase, guanylyltransferase, and methyltransferase. The E12 subunit, while not catalytically active on its own, binds to the C-terminal methyltransferase domain and stimulates its activity.

In the practical part of this study, we focused on characterizing the activity of the capping enzyme from the monkeypox virus. The primary attention was directed towards the methyltransferase domain. The heterodimeric complex E1-E12 was expressed in both a bacterial system (*E. coli*) and insect cells (Sf9 cell line). The formation of the mRNA cap was studied *in vitro* using activity assays with RNA, GTP, and SAM (S-adenosyl-methionine) substrates. Additionally, the inhibition of the methyltransferase domain by the nonspecific inhibitor sinefungin was investigated. The results of this study highlight the activity of the E1 subunit and the role of E12 in the N-7 methylation of the mRNA cap. These findings contribute to a better understanding of cap-0 formation and may support future research and the search for new inhibitors.

Key words: Monkeypox virus, capping enzyme, mRNA cap, E1, E12, inhibition