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Expression and characterization of M3 protein of Murine gammaherpesvirus 68 prepared in E. coli

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Murine gammaherpesvirus 68 encodes for M3 protein unique among herpesviruses, capable of binding a broad spectrum of chemokines thus modulating the host immune defense against virus infection. It is obvious, that M3 protein has a great potential in the treatment of diseases associated with deregulation of chemokine network, such as diabetes, autoimmune diseases and others. In this work, native recombinant M3 protein (44 kDa) was purified in the amount and purity allowing biological studies on its anti-chemokine activities. M3 protein with or without signal sequence (24 aa) fused with His6Tag anchor at the N- or C- terminal end using pET-26b(+) expression system was prepared by the help of E. coli cells (BL21 (DE3) and Rosetta-gami 2 (DE3) strains). In latter's the co-expression of chaperones and oxidized cytoplasmic environment was utilized to facilitate correct folding of soluble protein in producing cells. Then, the conditions of protein expression and IPTG induction as well as cell disruption and protein purification processes were optimized. All recombinant M3 proteins (full-length and truncated) revealed binding activity against three human chemokines CCL5, CXCL8, and CCL3 tested. The strongest binding activity was found against CCL5 and currently for truncated M3 protein with his tags at N- terminal end co-expressed with chaperones. Generally, the activity against CXCL8 was up to 50 times weaker than against CCL5. The activity against CXCL8 of full-length M3 proteins with his tags at C- and N- terminal end was found comparable. We suggest that E. coli cells might serve as an effective producer of biologically active M3 protein suitable for its further biological studies in vitro and in vivo needed better understand the functions of M3 protein in virus-host interactions.

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