ABSTRACT

Heme sensor proteins perform a variety of important functions in both prokaryotic and eukaryotic organisms. Heme-regulated inhibitor (HRI) is an example of a eukaryotic heme-sensor protein, which catalyzes the phosphorylation of the α subunit of the eukaryotic initiation factor 2 (eIF2α). In this bachelor thesis, the pET-21c(+)/eIF2α plasmid was amplified and its authenticity for the eIF2α expression was verified with the use of two independent methods. Next, HRI and eIF2α were produced using the recombinant expression in *E. coli* BL-21(DE3) cells transformed with the pET-21c(+)/eIF2α and pET-21c(+)/HRI plasmid, respectively. Both proteins were then isolated from the cells and purified with the use of affinity chromatography and gel permeation chromatography. eIF2α was obtained in sufficient yield (560 μg out of 1 l of TB medium) and purity (90%). A lower yield (25 μg out of 1 l of TB medium) and purity (20%) was reached in the case of HRI. On the other hand, the authenticity of the HRI product was confirmed using spectrophotometric characterization and its enzyme activity was verified as well. Pilot experiments showed that GTP may replace ATP in the process of eIF2α phosphorylation, while UTP and CTP may not.