INVESTIGATION OF HALOTAG FUSION PROTEIN FUNCTION IN *E. COLI* AND *PLASMODIUM*

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ABSTRACT

HaloTag (HT) is a modified bacterial haloalkane dehalogenase enzyme that has been developed as a multi-purpose tool for studying protein functions, including protein purification, intracellular protein localization, time-dependent labeling, and control of intracellular protein levels. Ligand-mediated control of intracellular protein is an attractive tool for drug target validation. The function of HT was initially tested in *E. coli*. EGFP-HT fusion protein was functional when expressed in *E. coli*, although the expression was markedly lower than expected. Another reporter protein, dihydrofolate reductase thymidylate synthase (DHFR-TS) was used as a fusion partner to HT and its function was tested in *E. coli*. It was observed that the fusion of HT to DHFR-TS protein lowered the expression and intracellular activity of DHFR-TS compared with a 6x His tagged DHFR-TS. The DHFRTS-HT fusions had correspondingly lower DHFR activity to complement a DHFR-TS *E. coli* mutant. The expression of the EGFP-HT fusion protein was also tested in the blood stages of the *Plasmodium berghei* rodent malaria parasite. Correct integrants were validated by PCR. EGFP-HT mRNA expression was detected by RT-PCR in transgenic EGFP-HT parasite lines. However, no EGFP-HT fusion protein was detected by Western blot. In conclusion, HT can be expressed as a fusion protein, but the low levels of protein activity suggest that HT interferes with fusion protein expression and/or stability in *E. coli* and *Plasmodium sp*.

KEY WORDS: HALOTAG / REVERSE TRANSCRIPTION PCR (RT-PCR) / PROTEIN EXPRESSION/ FUSION PROTEIN

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