The MF6p/FhHDM-1 major antigen secreted by the trematode parasite fasciola hepatica is a heme-binding protein

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Blood-feeding parasites have developed biochemical mechanisms to control heme intake and detoxification. Here we show that a major antigen secreted by Fasciola hepatica, previously reported as MF6p, of unknown function (gb|CCA61804.1), and as FhHDM-1, considered to be a helminth defense molecule belonging to the family of cathelicidin-like proteins (gb|ADZ24001.1), is in fact a heme-binding protein. The hemebinding nature of the MF6p/FhHDM-1 protein was revealed in two independent experiments: (i) immunopurification of the secreted protein·heme complexes with mAb MF6 and subsequent analysis by C8 reversed-phase HPLC and MS/MS spectrometry and (ii) analysis of the binding ability of the synthetic protein to hemin in vitro. By immunohistochemistry analysis, we have observed that MF6p/FhHDM-1 is produced by parenchymal cells and transported to other tissues (e.g. vitellaria and testis). Interestingly, MF6p/FhHDM-1 is absent both in the intestinal cells and in the lumen of cecum, but it can be released through the tegumental surface to the external medium, where it binds to free heme molecules regurgitated by the parasite after hemoglobin digestion. Proteins that are close analogs of the Fasciola MF6p/FhHDM-1 are present in other trematodes, including Clonorchis, Opistorchis, Paragonimus, Schistosoma, and Dicrocoelium. Using UV-visible spectroscopy and immunoprecipitation techniques, we observed that synthetic MF6p/FhHDM-1 binds to hemin with 1:1 stoichiometry and an apparent Kd of 1.14×10-6M-1. We

also demonstrated that formation of synthetic MF6p/FhHDM-1·hemin complexes inhibited hemin degradation by hydrogen peroxide and hemin peroxidase- like activity in vitro. Our results suggest that MF6p/FhHDM-1 may be involved in heme homeostasis in trematodes.