FASCIOLA HEPATICA CYSTATINS

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Cystatins are natural protein inhibitors of the papain family of cysteine proteinases that regulate their endogenous activity in eukaryotic cells and tissues. Parasite cystatins are also involved in modulation of host immune responses. Cysteine proteinases play a central role in host invasion and inmune evasion of the parasitic trematode *F. hepatica*; particularly, cathepsin L and B enzymes make up the bulk of adult and juvenile secretion products. We looked for cystatin family members in *F. hepatica* databases. Three cDNA sequences with the QVVAG signature and other cystatin characteristics were identified besides the multy-domain protein already described (AN: AJ312374); we named them FheCyt1 to 3. We cloned the full-length cDNA, obtained by 5' RACE, of the 10 kDa FheCyt1 with 6 cysteines and a predicted leader peptide (AN: AY647146). The coding region, including the putative signal sequence, was subcloned into a yeast expression vector. FheCyt2 and FheCyt3 were identified in the Sanger Institute adult EST library. FheCyt2 is related to cytosolic cystatins and FheCyt3 has a putative leader peptide. FheCyt2 was purified from heat-treated adult fluke somatic extracts, using affinity chromatography on carboxy-methylated papain followed by alkali elution and mass spectrometry. Both recombinant FheCyt1 and native FheCyt2 exhibit potent inhibition of *F. hepatica* cathepsins L1 and L2 and papain.