

## Original article

P71

**Leucine aminopeptidase of *Fasciola gigantica*: functional characterization and evaluation as a vaccine in animal fasciolosis**

Wansika Phadungsil\*, Vithoon Viyanant, Rudi Grams

Graduate Program in Biomedical Sciences, Faculty of Allied Health Sciences, Thammasat University, Pathumthani, Thailand.

\*Presenting Author

**Abstract**

The infection of livestock animals with the parasite *Fasciola gigantica* causes economic loss due to mortality, weight loss, reduced productivity and poor milk production. An effective vaccine to overcome these problems would be a viable alternative to the currently used drugs. Leucine aminopeptidase (LAP) plays an important role in the parasite's biology, such as in processing, maturation, activation or degradation of substrates and is, therefore, considered as a candidate for development of a vaccine. In the presented work the protein coding fragment of a *FgLAP* cDNA was subcloned into the pThioHisB bacterial expression vector. Recombinant *FgLAP* was expressed and purified from *Escherichia coli* TOP10. The purified *rFgLAP* has and will be used in further studies such as proteolytic activity assays, immunohistochemistry and vaccine development. *FgLAP* protein- and DNA-based immunization trials have been performed in mice to test the antigen's vaccine potential. In addition to the recombinant *FgLAP*, a complex of synthetic *FgLAP* peptides (sp*FgLAP*) was designed from the active site of *FgLAP* and has also been used for protein-based immunization. DNA-based immunization has been performed with the pSecTag2A mammalian expression vector containing the *FgLAP* coding DNA.

**Keywords:** *Fasciola gigantica*, leucine aminopeptidase, vaccine, molecular cloning

**Introduction**

Fasciolosis is a helminth disease caused by trematodes in the genus *Fasciola* (*F. hepatica* and *F. gigantica*) and belongs to the plant-borne zoonoses. *F. gigantica* is the common *Fasciola* species infecting ruminants in the tropical parts of Asia. The infection of livestock animals with the parasite causes economic loss due to, weight loss, reduced productivity and poor milk production. Triclabendazole is the standard drug for treatment of animal fasciolosis, but the cost of treatment with this drug is a problem in developing countries. An effective vaccine to overcome these problems would be a viable alternative to Triclabendazole and other currently used drugs.

**Methods**

In the present study a cDNA encoding leucine aminopeptidase from *Fasciola gigantica* (*FgLAP*) was synthesized and isolated from total RNA using reverse transcription and polymerase chain reaction with oligonucleotide primers specific to the leucine aminopeptidase DNA sequence from *F. hepatica*. The sequence of the obtained *FgLAP* cDNA was determined by Sanger dideoxy sequencing. The coding DNA fragment of the *FgLAP* cDNA was then inserted into the pThioHisB bacterial expression vector and *Escherichia coli* TOP10 was transformed with the plasmid for production of recombinant *FgLAP* (*rFgLAP*) fused to bacterial thioredoxin encoded by the plasmid. Expression of *rFgLAP* was induced by addition of 1 mM IPTG to the medium.

Both protein- and DNA-based immunization trials have been started to test the vaccine potential in mice. Recombinant *FgLAP* and synthetic peptides (sp*FgLAP*) which

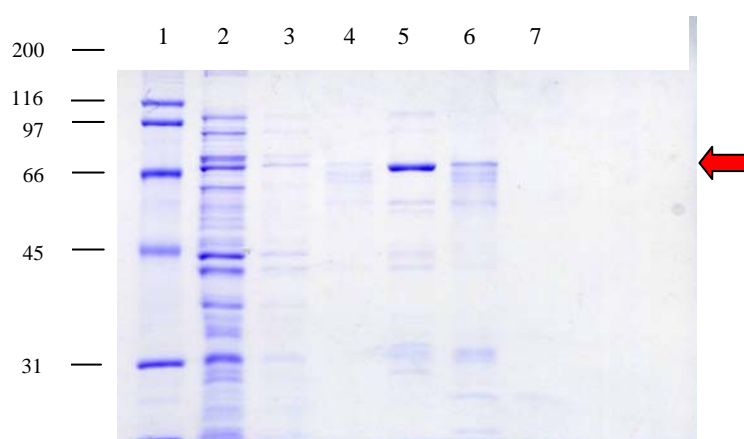
cover parts of the active site of FgLAP have been used in protein-based immunization. DNA-based immunization has been performed with mammalian expression vectors carrying the FgLAP encoding sequence with and without additional signal peptide encoding sequences.

## Results

The deduced amino acid sequence of FgLAP is closely related to the homologous leucine aminopeptidase of *F. hepatica*. Both proteins contain 523 amino acid residues and have 98% sequence identity while LAP from *Clonorchis sinensis* has only 70% identity (Fig. 1). The bacterially expressed and purified rFgLAP-thioredoxin fusion protein was soluble and migrated as a single band at a molecular mass of 66 kDa on SDS-PAGE (Fig. 2).

	10	20	30	40	50	60	70	80	90	100
FgLAP	M.A.V.V.DL.D.RFD.VIFIND..E..K.AAV.E.L.SFSKINP.L..ELSIVPFP.HPSGRLIYSPTGALNTD.ADIRNVYDAAC..VKRAL.S.G									
FhLAP	M.A.V.V.DL.D.RFD.VIFIND..E..K.AAV.E.L.SFSKINP.L..ELSIVPFP.HPSGRLIYSPTGALNTD.ADIRNVYDAAC..VKRAL.S.G									
CsLAP	M.....V..L.D.RFD...F.ND...E.....A.E.L.SFSK.NP....EL.IVPFP.HPSGRLI.SPTG.LNTD.AD.RNVYDA.C...KRA...G									
	110	120	130	140	150	160	170	180	190	200
FgLAP	...L.YL.S-.R.....WMQRKHLN.LLGAYHA.Y.PLEVREM.P...K..HLGV.....DE.V.RLA.ALEEGRLARDIGSDPERMAAP									
FhLAP	...L.YL.S-.R.....WMQRKHLN.LLGAYHA.Y.PLEVREM.P...K..HLGV.....DE.V.RLA.ALEEGRLARDIGSDPERMAAP									
CsLAP	...L.Y.....WM.R...LLN.LLGAYHA.Y.PLEVREM.P...K..H.G.....D.....A.A.EEGRW.ARDIGSDPERMAAP									
	210	220	230	240	250	260	270	280	290	300
FgLAP	.IV.YLKT.L.G.KGITH.VEKVD.QKYPLMAAVNRAASVVARHGRVHL.Y.PPN...VDTTLYLIGKGITYDTGGADIK.A.GVMAGMHRDKCGAAAI									
FhLAP	.IV.YLKT.L.G.KGITH.VEKVD.QKYPLMAAVNRAASVVARHGRVHL.Y.PPN...VDTTLYLIGKGITYDTGGADIK.A.GVMAGMHRDKCGAAAI									
CsLAP	.V.YL...L.G..GITH.VEKVD..KYPLMAAVNRAASV..RHDGRVHL.Y.PPN...DTTLYLIGKGITYDTGGAD.KA.GVMAGMHRDKCGAAAI									
	310	320	330	340	350	360	370	380	390	400
FgLAP	AG.F.TL..QLQPP.LSVSAALAFVRNSVG.DSVVADEILVARSGQIRIVGNTDAEGRMVM.DLLCEAKEKAV.ATNPFLFTIATLTGHVVRAYKHYTAVM									
FhLAP	AG.F.TL..QLQPP.LSVSAALAFVRNSVG.DSVVADEILVARSGQIRIVGNTDAEGRMVM.DLLCEAKEKAV.ATNPFLFTIATLTGHVVRAYKHYTAVM									
CsLAP	AG.F.TL..QLQPP.LSVSA.LAFVRNS.G.DSVV.DEI.V.R.G.R.GNTDAEGRMVM.DLLCEAKEKAV.ATNPFLFTIATLTGHVVRAYKH.TAVM									
	410	420	430	440	450	460	470	480	490	500
FgLAP	DNGP.RI..VSQ.LQ.AGD.ISDMAEISTVRKEDYEFNRGKTEYED.LQCNLPSSAT.RGHQIPAAFM.LASGLDKHGL.S.KPIPYTHVDVAGSA.EI									
FhLAP	DNGP.RI..VSQ.LQ.AGD.ISDMAEISTVRKEDYEFNRGKTEYED.LQCNLPSSAT.RGHQIPAAFM.LASGLDKHGL.S.KPIPYTHVDVAGSA.EI									
CsLAP	DNGP.R...VSQ.LQ.AGD..SD.AEISTVRKEDYEFN.G.TEYED.LQCNLPSSAT.RGHQ.PAAF...ASGLDKHG..S.KP.PYTHVDVAGSA.EI									
	510	520	530	540	550	560	570	580	590	600
FgLAP	HVLPTAAPLLMFASRYVLPRLGF.									
FhLAP	HVLPTAAPLLMFASRYVLPRLGF.									
CsLAP	HVLPTAAPL.MFASRY.LPR.GF.									

**Figure 1.** The sequence alignment of the *F. gigantica* leucine aminopeptidase (FgLAP) with the LAP sequences from *F. hepatica* (AAV59016) and *Clonorchis sinensis* (ABK91810).



**Figure 2.** SDS-PAGE of rFgLAP: purified under denaturing conditions by Ni-NTA affinity chromatography. Lane 1: flow through, lanes 2, 3: washes, lanes 4-7: elution fractions. The arrow indicates the rFgLAP-thioredoxin fusion protein at 66 kDa.

## Discussion

The purified rFgLAP fused with thioredoxin will be used for further studies such as functional analysis of rFgLAP, production of polyclonal anti-rFgLAP antiserum, analysis of tissue-specific distribution of native LAP, vaccine development approaches. Sera from immunized mice will be used for analysis of specific antibody responses by ELISA and Western blot.

## Conclusion

The FgLAP protein is highly conserved in the genus *Fasciola* (98% identity between the two species) and also shows high conservation to LAP from *C. sinensis* with 70% identity. Recombinant FgLAP could be expressed as a 66 kDa soluble fusion protein in *E. coli*.

## Acknowledgements

This research was financially supported by the Royal Golden Jubilee Program of the Thailand Research Fund (RGJ-TRF), Thammasat University and the Commission on Higher Education of Thailand.

## References

1. Acosta D, Cancela M, Piacenza L, Roche L, Carmona C, Tort JF. *Fasciola hepatica* leucine aminopeptidase, a promising candidate for vaccination against ruminant fasciolosis. *Mol Biochem Parasitol* 2008; 158: 52-64.
2. Piacenza L, Acosta D, Basmadjian I, Dalton JP, Carmona C. Vaccination with cathepsin L proteinases and with leucine aminopeptidase induces high levels of protection against fascioliasis in sheep. *Infect Immune* 1999; 67: 1954-61.
3. Marcilla A, De la Rubia JE, Sotillo J, Bernal D, Carmona C, Villavicencio Z, Acosta D, Tort J, Bornay FJ, Esteban JG, Toledo R. Leucine aminopeptidase is an immunodominant antigen of *Fasciola hepatica* excretory/secretory products in human infections. *Clin Vaccine Immunol* 2008; 15: 95-100.