

Prace oryginalne

Computational analysis of *Ancylostoma ceylanicum* cysteine proteinase

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ABSTRACT. The potential tertiary structure of *Ancylostoma ceylanicum* cysteine proteinase was obtained by Automatic Program 3D-JIGSAW and used for finding homologues of known structure by VAST program. The results of computational analysis showed the presence of domains recognizing host immunoglobulins. Based on this analysis we suggest that this protein is involved in cleaving of host antibodies and therefore it may be promising vaccine candidate. In this paper we present the computational analysis of parasitic antigen which is very helpful in evaluation of the potential role of this protein.

Key words: *Ancylostoma ceylanicum*, computational analysis, cysteine proteinase, tertiary structure

Introduction

Hookworms are very important blood sucking nematode parasites of man and domestic animals. Humans are permissive hosts for three hookworm species: *Necator americanus*, *Ancylostoma duodenale*, and *Ancylostoma ceylanicum* [1]. Ancylostomosis together with other soil transmitted parasitic diseases is the major health problem in the developing countries [2]. It causes blood loss and iron-deficiency anemia of approximately 730 millions of peoples [3]. Infections with hookworms are very common in tropical regions of the earth [3, 4]. The work on construction of vaccine against hookworm infections has being continued for many years, but without success so far. Research is focused on a number of bioactive molecules produced by larval and adult stages of the parasite, which are associated with larval skin penetration, intestinal tissue invasion, immune evasion, digestion of haemoglobin and/or other macromolecular substrates. McKerrow [5] suggested that cysteine proteinases could be used as the most promising vaccine candidates against hookworm infections. These enzymes play a very important role in the host-parasite interaction. They are involved in parasite feeding, tissue migration, and neutralization of host immunological response. Kofta et al. [6] obtained 74–100% reduction in the fluke burden when *Fasciola hepatica* cysteine proteinase cDNA was used to vaccinate rats against fasciolosis.

The aim of this paper is to present a novel bioinformatic tool for evaluation of biological role and 3D structure of potential vaccine antigens of helminth parasites.

Material and methods

ACEY-1 cDNA sequence described by Mieszczanek et al. [7] was translated to aminoacid sequence using Translate program (http://www.expasy.ch/tools/dna.html).

Cysteine proteinase aminoacid sequence was used to obtain the potential tertiary structure by Automatic Program 3D-JIGSAW (http://www bmm.icnet.uk/servers/3djigsaw/) [8–10]. This struc-

ture was compared to proteins with solved 3D structure deposited in the MMDB/PDB database using the Vast program (http://www.ncbi.nlm.nih.gov/Structure/VAST/vastsearch.html).

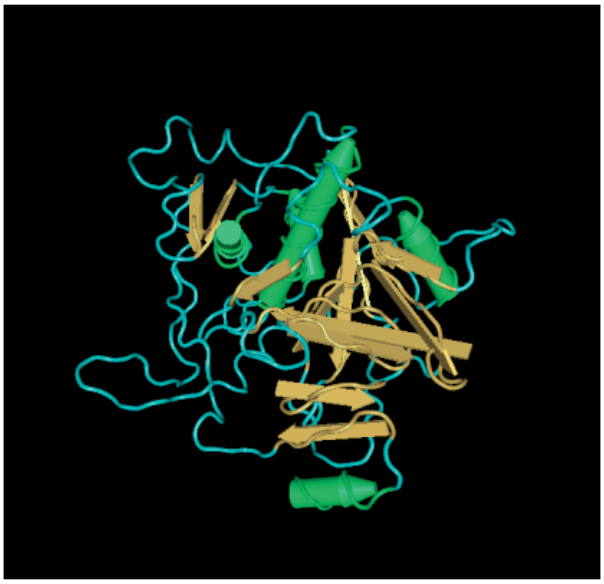


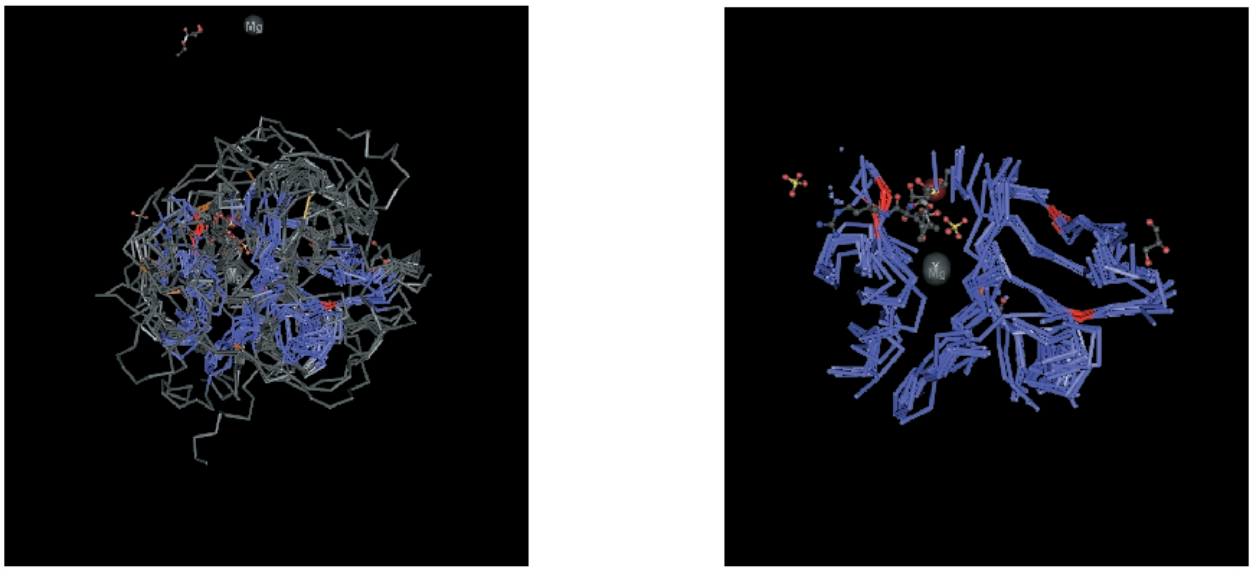
Fig.1. Potential structure of ACEY-1 constructed by Automatic Program 3D-JIGSAW. Green- alpha helix structure, yellow- beta strand structure, blue- coil structure

Results

Automatic Program 3D-JIGSAW designed a tertiary structure of ACEY-1 in *.pdb format (protein data bank format) (Fig. 1). The comparison of 3D structure of ACEY-1 to known proteins revealed homology to *Streptococcus* cysteine proteinase which is known to possess IgG endopeptidase activity [11] and to other proteins (Table 1; Figs. 2, 3).

Discussion

The exact mechanisms of hookworm infection and survival within the mammalian host remain poorly understood. However, recent identification of a number of bioactive molecules released by larval and adult stages of the parasite have shed light on a variety of potential hookworm evolutionary strategies. Acquiring information about molecular structure and biological functions of parasite's antigens play a key role in research focused on the designing vaccines against parasites. Using computational analysis (two programs: Automatic Program 3D-JIGSAW and Vast) we obtained potential tertiary structure of ACEY-1 and found it similar to proteins which biological functions are already known. Especially two of them are very



A B

Fig. 2. A — 3D aligment of overlapping domains of potnetial teritary structure of ACEY-1 and overlapping domains of similar proteins found in MMDB/PDB database. Blue colour- similar overlapping residues, red- identical overlapping residues, orange- disulfide bonds, grey- other aminoacids. B — 3D alignment of overlapping residues of potential structure of ACEY-1 and similar proteins found in MMDB/PDB database. Blue colour — similar residues, red- identical residues, orange- disulfide bonds, other- ligands complexed with analyzed proteins

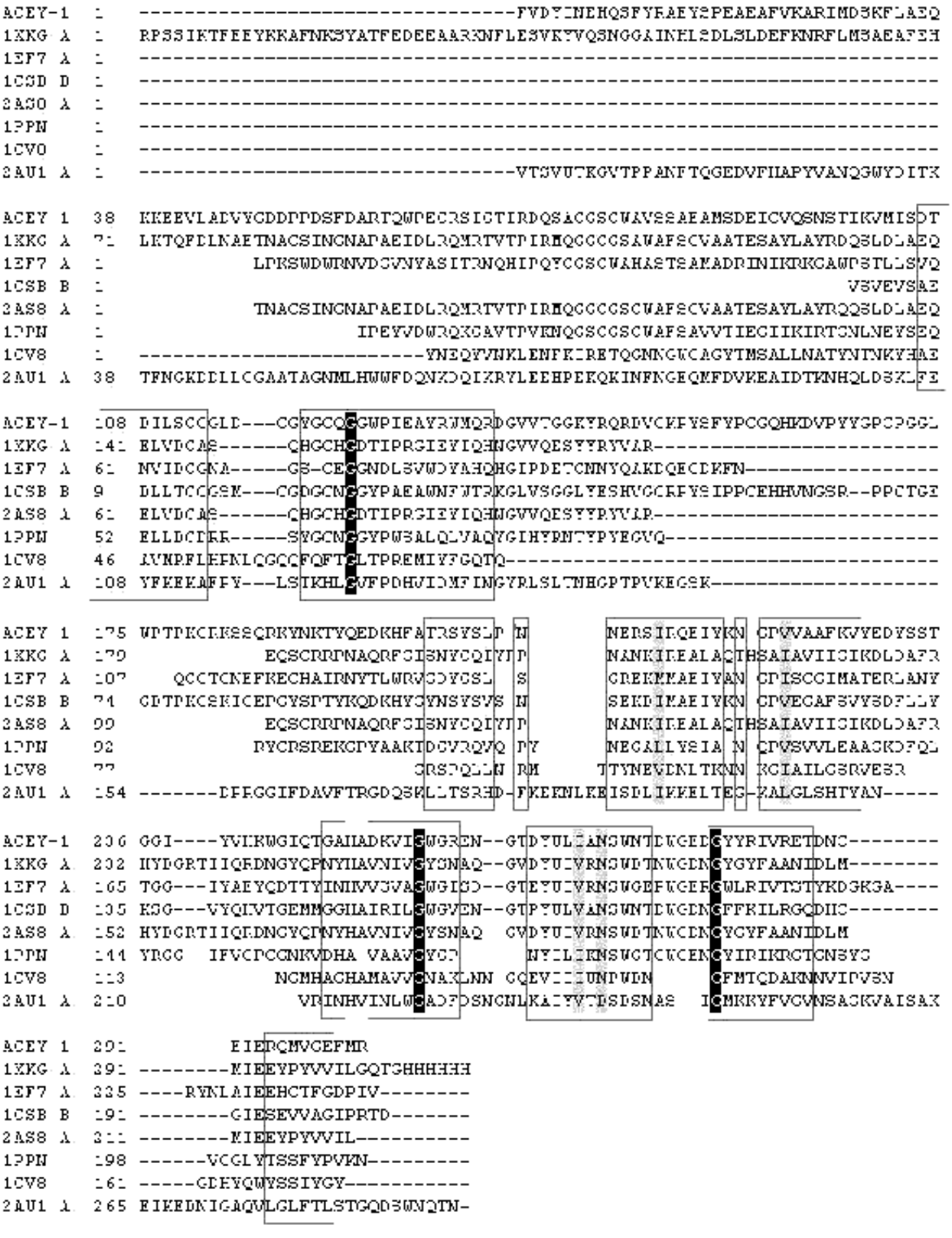


Fig. 3. Aligment of aminoacid sequence of ACEY-1 and proteins found in MMDB/PDB database constructed by VAST program. Similar overlapping residues are grouped in frames. Identical residues are marked in black There are also residues of high similarity in gray marked by the author

Table 1. Proteins found in MMDB/PDB database showing structure similar to ACEY-1

PDB C D	Description	Species	Reference
1XKG A	Crystal Structure of the Major House Dust Mite Allergen Der P 1 in its Pro Form At 1.61 A Resolution	*Dermatophagoides pteronyssinus*	[13]
1EF7 A	Crystal Structure of Human Cathepsin X	*Homo sapiens*	[14]
1CSB B	Papain-Like Lysosomal Dicarboxy-Peptidase Mol_id: 1; Molecule: Cathepsin B; Chain: A, B, C, D, E, F; Ec: 3.4.22.1	*Homo sapiens*	[15]
2AS8 A	Crystal Structure of Mature and Fully Active Der P 1 Allergen˙	*Dermatophagoides pteronyssinus*	[16]
1PPN	Papain Cys-25 with Bound Atom	*Carica papaya*	[17]
1CV8	Staphopain, Cysteine Proteinase From Staphylococcus Aureus V8	*Staphylococcus aureus*	[18]
2AU1 A1	Crystal Structure of Group a Streptococcus Mac-1 Orthorhombic	*Streptococcus pyogenes*	[12]

important from our point of view: Der p 1 and Mac-1. Der p 1 is the 25 kDa major allergen with cysteine protease activity from *Dermatophagoides pteronyssinus* [12]. This similarity could suggest that ACEY-1 is not too good vaccine candidate. However, Mac-1 protein is a *Streptococcus* secreted cysteine protease with IgG endopeptidase activity. It blocks phagocytosis and inhibits the production of reactive oxygen species [18]. Numerous publications showed that cysteine proteases may be involved in tissue penetration of the parasite, in its feeding as well as in defence against effector mechanisms of the host's immune response. Berasain et al. [19] described specific cleavage sites on human IgG subclasses by cruzipain, the major cysteine proteinase from *Trypanosoma cruzi*. Also Kumar and Pritchard [20] have shown that extrectory/secretory cysteine proteases of *Necator americanus* cleave human IgG. Papers mentioned above and present computational analysis of ACEY-1 aminoacid sequences allow us to suggest that this enzyme may be involved in cleaving of host's IgG antibodies and therefore may be a promising antigen candidate for vaccination against hookworm infections. Kofta and co-workers [6] observed 100% (males) and 74% (females) reduction of worm burdens in Sprague-Dawley rats immunized with cDNA encoding one of *Fasciola hepatica* cysteine proteinases and then challenged with *F. hepatica* metacercariae.

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