

# An immunodominant antigen of *Brugia malayi* is an asparaginyl-tRNA synthetase

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**Abstract** Lymphatic filariasis is caused by infection with the filarial nematodes *Brugia malayi*, *Brugia timori*, *Wuchereria bancrofti* and *Onchocerca volvulus* which collectively infect about 200 million persons throughout the world. Protein sequence homology analysis of a major nematode antigen suggested that it was a class II aminoacyl-tRNA synthetase. The overproduction, purification and verification that the major *B. malayi* antigen is an asparaginyl-tRNA synthetase is described.

**Key words:** Antigen; Asparagine; Aminoacyl-tRNA synthetase; Filariasis; *Brugia malayi*

## 1. Introduction

Nematodes are one of the three groups of helminths that parasitize man. According to the World Health Organization nematode diseases affect more than one billion persons in various geographic areas throughout the world and the filarial nematodes *Brugia malayi*, *Brugia timori*, *Wuchereria bancrofti* and *Onchocerca volvulus*, which cause lymphatic filariasis (elephantiasis) or onchocerciasis (river blindness), affect more than 200 million persons [1].

In the course of immunological studies of filarial parasite antigens, the gene encoding an immunodominant 63 kDa peptide in *B. malayi* was also found within the genome of another major human filarial parasite, *O. volvulus* the organism which causes onchocerciasis [2,3]. When originally described, the derived amino acid sequence of the *B. malayi* gene product was thought to have no similarity with previously known proteins. Subsequent to recognition of a second class of aminoacyl-tRNA synthetases in 1990 [4,5], reanalysis of the *B. malayi* antigen sequence revealed the presence of one, and possibly two, allowing for a frame-shift error, structural motifs characteristic of the class II aminoacyl-tRNA synthetases which suggested that the antigen was an asparaginyl-tRNA synthetase [6]. Previously, only a truncated 55 kDa portion of the *B. malayi* peptide had been expressed for immunological studies. The missing 8 kDa peptide sequence contained a terminal motif 3 region characteristic of the class II enzymes required for active site formation and aminoacylation activity. We describe here the cloning and overexpression of *B. malayi* antigen as a fusion protein with maltose binding protein and the demonstration that it has enzymatic activity characteristic of an asparaginyl-tRNA synthetase.

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## 2. Materials and methods

### 2.1. Plasmids, expression and purification

PolyA enriched RNA was extracted from adult female *B. malayi* parasites using standard techniques and 50ng was used to synthesize cDNA using reverse transcriptase (Timesaver cDNA Synthesis, Pharmacia, Inc.) The resulting cDNA was used as template for amplification of *B. malayi* antigen gene using two gene specific oligonucleotide primers:

5': ATGACTGTTTATATTTGTCCAGAACTGGAGAT 3' and

5': TTGAATCTTATGGGACACATCGACCAACAAAGCGAGG 3'

with 30 cycles of amplification using the following thermocycler parameters: hot start at 92°C for 60 s, denaturation: 95°C for 30 s, annealing: 55°C for 30 s, primer extension: 72°C for 90 s, terminal extension: 5 min at 72°C. PCR products were subcloned using the TA cloning system (In Vitrogen, Inc.) and cDNA inserts of the correct size and orientation were identified in the TA cloning vector by restriction fragment length polymorphism and DNA sequence analysis.

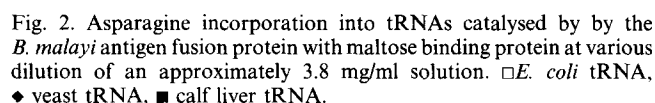
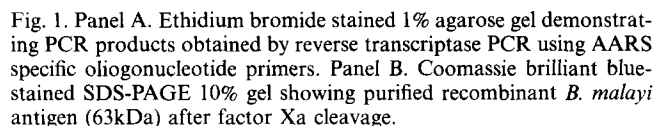
A full-length cDNA was subcloned into the plasmid pMALc (New England Biolabs) for large scale overproduction of the recombinant protein as a fusion protein with the 42 kDa maltose binding protein. Growth media in a 20 litre fermenter was seeded with cultures of the *E. coli* InvαF', a strain harboring the plasmid containing the filarial antigen cDNA, and grown at 30°C. From this culture, induced with IPTG, 40 grams of bacterial cell paste was obtained. A series of purification steps which included anion-exchange chromatography (DEAE-Sepharose CL-6B, Pharmacia), affinity chromatography (amylose resin), preparative Factor Xa (New England Biolabs) cleavage of the maltose binding protein fusion product, followed by anion exchange chromatography by FPLC on Mono Q (Pharmacia) to isolate the recombinant antigen protein (63 kDa).

### 2.2. Aminoacylation assays

Enzymatic activity was measured by the incorporation into unfractionated tRNA of <sup>14</sup>C-labelled amino acids. Protein samples (10 μl) were added to 40 μl Tris (64 mM)-HCl buffer pH 7.6 containing: 0.5 mM spermidine, 5 mM disodium ATP, 500 μg unfractionated *E. coli* or brewers yeast tRNA or 250 μg calf liver tRNA and labelled amino acid. These were 0.1 mM L-[<sup>14</sup>C]asparagine (98 cpm/pmol), 0.1 mM L-[<sup>14</sup>C]aspartic acid (100 cpm/pmol) and 1 μCi U-[<sup>14</sup>C]protein hydrolysate. All tRNAs were from Boehringer and all labelled amino acids from Amersham. After incubation for 15 min at 37°C, the reactions were quenched with 50 μl of 5% (w/v) TCA, and the mixture was spotted onto a Whatman GF/A glass fibre filter. The filter was then washed 3 times with 5% TCA, and subsequently with ethanol, ethanol:ether, ether, dried and counted for radioactivity.

## 3. Results

Fig. 1A shows an ethidium bromide stained agarose gel of the product obtained by PCR using *B. malayi* cDNA and the two putative aminoacyl-tRNA synthetase primers. This incorporated into the plasmid pMALc could be used to express a protein of *M<sub>r</sub>* 105K on SDS-PAGE which corresponded to the fusion protein between maltose binding protein and the 63 kDa protein in *E. coli* (InvαF'). After a preliminary fractionation of the bacterial cell extracts by anion-exchange chromatography



SDS-PAGE (Fig. 1B). This material when assayed under similar conditions to that for the fusion protein i.e. a solution of 2.2 mg/ml diluted 1:1000 used in the aminoacylation of calf liver tRNA (see section 2.2) incorporated 35 pmol asparagine which corresponds to a specific activity under the assay conditions of 1.5  $\mu\text{mol/mg}$ .

The new DNA sequence data for the *B. malayi* asparaginyl-tRNA synthetase [3] demonstrated a discrepancy with the previously published sequence [2], which produced a frameshift that obscured a typical class II aminoacyl-tRNA synthetase motif 2 sequence (RAEK....SRTRRH) at amino acids 314–324 [6]. Although the original observation that, allowing for a frame shift error, the sequence of the *B. malayi* antigen was possibly an asparaginyl-tRNA synthetase [6], the sequence of the highly homologous *O. volvulus* antigen suggested that this might be an aspartyl-tRNA synthetase [3]. The results presented above demonstrate that the *B. malayi* antigen is an asparaginyl-tRNA synthetase effectively aminoacylating tRNA from eucaryotic sources and can be purified to a specific activity similar to that found e.g. seryl-tRNA synthetase from bovine liver [7]. Alignment of known asparaginyl-tRNA synthetase sequences with those of both the *B. malayi* antigen demonstrate conservation of both active site motifs and other conserved sequences (Fig. 3) are consistent with this observation.

	Motif 1	
Antbm	LGDVFLHLCSSYRAEKSRTTRRHAEYVAHVEAECFFI	341
Nrscsm	LSRCWTLSPSCFRAEKSSTPRHLSEFWMLLEVCMCF	362
Nrsscc	LGDVYTIQESFRAEKSSTRRLHSEVTHIRAEALFL	250
Nrstt	FAKVYTFGPTFRAERSKTRRHLEFWMVPEVAFM	227
Nrsec	LSKIYTFGPTFRAENSNTSRHLAEFWMLEPEVAF	257
Nrshi	LSKIYTFGPTFRAENSNTTRHLAEFWMVPEVAF	268
Antbm	KAFYMQRDAQDNTLTESVDLLMP-GVGEIVGGSMRIWKFDLSKAF	486
Nrscsm	KPFYMKQNSTPDDTVGCFDILLVP-GMGEIIGGSRLREDDYDKLCREM	431
Nrsscc	KSFYMKRCSDDPRTVESVDVLLMP-NVGEITGGSMRIIDMDLMAGF	495
Nrstt	KAFYMEPPDEPDELVLVNDLLRPEGYGIEIGGSQRIHDELLLRKI	375
Nrsec	KAFYMRNLN-EDGKTVAAMDVLAP-GIGEIIIGGSQREERLDVLDERM	407
Nrshi	KAFYMRNLN-DDEKTVAAAMDVLAP-GIGEIIIGGSQREERLEVLDKRM	418
	Motif 2	
Antbm	WYLDQRLYGTCPHGGYGLGLERFICWLTVNTHIRVDCLYPR	538
Nrscsm	WYVSLRKEGSAPHGGFGLGFERPISYLYGNHNKIDAIPIFYR	484
Nrsscc	WFIDQRKYGTCPHGGYIGIGTERILAWLCDRFTVRDCSLYPR	547
Nrstt	WYLDLRRFGSVPHSGFGLGLERTVAMICGLAHVREAIPIFFR	427
Nrsec	WYRDLRRKYGTCPHSGFGLGFERLIAYVTGVQNVDRVPIFFR	458
Nrshi	WYRDLRRKYGSVPHSGFGLGFERLIIVYTVGVQNVDRVPIFFR	470
	Motif 3	
Antbm	WYLDQRLYGTCPHGGYGLGLERFICWLTVNTHIRVDCLYPR	538
Nrscsm	WYVSLRKEGSAPHGGFGLGFERPISYLYGNHNKIDAIPIFYR	484
Nrsscc	WFIDQRKYGTCPHGGYIGIGTERILAWLCDRFTVRDCSLYPR	547
Nrstt	WYLDLRRFGSVPHSGFGLGLERTVAMICGLAHVREAIPIFFR	427
Nrsec	WYRDLRRKYGTCPHSGFGLGFERLIAYVTGVQNVDRVPIFFR	458
Nrshi	WYRDLRRKYGSVPHSGFGLGFERLIIVYTVGVQNVDRVPIFFR	470
Antbm	= antigen of <i>Brugia malayi</i>	
Nrscsm	= <i>Saccharomyces cerevisiae</i> mitochondrial	
Nrsscc	= <i>Saccharomyces cerevisiae</i> cytoplasmic	
Nrstt	= <i>Thermus thermophilus</i> .	
Nrsec	= <i>Escherichia coli</i>	
Nrshi	= <i>Haemophilus influenzae</i>	

Fig. 3. Alignment of asparaginyl-tRNA synthetase conserved sequences of the enzymes from *E. coli* (Nrsec) [13], *Thermus thermophilus* (Nrstt) [14], *Haemophilus influenzae* (Nrshi) [15] and the putative mitochondrial (Nrsscm) [16] and cytoplasmic (Nrssc) [17] from *Saccharomyces cerevisiae* (Nrsscm), with the sequence of the *B. malayi* antigen Antbm. The published DNA sequences for Antbm [2] for motif 2 has been corrected for frame shift errors (M. Kron, unpublished results). Conserved residues for the five proteins are in bold type.

as strongly immunogenic, is understandable in view of what is known about AARS antigenicity in other species. In the group of human autoimmune diseases known as the idiopathic inflammatory myopathies, antibodies have been identified against several human class II aminoacyl-tRNA synthetases; histidyl-, threonyl-, alanyl- and glycyl-tRNA synthetases [8,9]. Anti-AARS antibodies effectively inhibit enzyme aminoacylation activity in vitro, and are able to immunoprecipitate their respective cognate tRNAs [10]. Given the high level of expression of message encoding this enzyme in *B. malayi* [2], it is possible to speculate on the demand for asparaginyl-tRNA synthetase activity in the adult female parasite. Class II aminoacyl-tRNA synthetases also function in the production of adenylated nucleotides, in particular the adenylated nucleotide Ap<sub>4</sub>A which may play a variety of biological roles as signal molecules, regulating both gene expression and enzyme activity [11,12]. Since considerable metabolic effort of the adult female parasite is devoted to nutrition and production of viable embryos and larvae, it is conceivable that the high level of asparaginyl-tRNA synthetase expression reflects unusual metabolic demands associated with larval maturation.

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