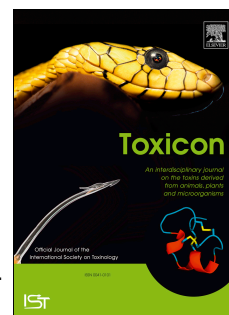


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Recombinant expression and predicted structure of parborlysin, a cytolytic protein from the Antarctic heteronemertine *Parborlasia corrugatus*

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1 **Recombinant expression and predicted structure of parborlysin, a cytolytic protein from** 2 **the Antarctic heteronemertine *Parborlasia corrugatus***

3
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13 14 **Abstract**

15 The heteronemertine *Parborlasia corrugatus* contains a cytolytic protein, parborlysin, which
 16 after extensive purification was found by Edman sequencing to be a mixture of several
 17 homologs. To investigate this microheterogeneity and enable the analysis of single toxins, we
 18 have obtained seven parborlysin isoform genes from *P. corrugatus* collected in Antarctica.
 19 Total RNA was isolated from the homogenized head region and parborlysin genes were
 20 identified from a cDNA library using degenerate primers. The translated sequences reveal that
 21 the isoforms are ~10 kDa basic (pI~10) proteins of which all but one harbour six cysteine
 22 residues. We generated a model of the three dimensional structure of parborlysins, which
 23 suggests that they are composed of five alpha-helical segments that include large, exposed
 24 hydrophobic surfaces. Finally, we constructed plasmids and inserted them into *E. coli* to
 25 obtain overexpressed amino- or carboxy-terminal polyhistidine-tagged parborlysin isoforms
 26 fused to the third domain of the *E. coli* periplasmic-protein TolA to facilitate toxin isolation.
 27 One of the isoforms adversely affected growth in the *E. coli* expressing it. Although we
 28 succeeded in isolating one of the recombinant parborlysin constructs, it lacked haemolytic
 29 activity.

30
 31 **Key words:** Cytolysin; Nemertean; Nemertine, Nucleotide sequence; *Parborlasia corrugatus*;
 32 Parborlysin; Toxin

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