Protein function

Chaperonin turned insect toxin

Antlions are larvae of the Myrmeleontidae family that live on other insects[1] by sucking out the body fluid from their prey, after first paralysing it with a toxin produced by salivary bacteria. Here we show that the paralysing toxin produced by bacterial endosymbionts in the saliva of *Myrmeleon bore* larvae is a homologue of GroEL, a protective heat-shock protein known as a molecular chaperone. The amino-acid residues critical for this protein's toxicity are located away from the regions essential to its protein-folding activity, indicating that the dual function of this GroEL homologue may benefit both the antlion and the endosymbiont.

The saliva of the larvae of *M. bore* (Fig. 1a), a pit-building antlion, contains the endosymbiont *Enterobacter aerogenes* (Fig. 1b), which, when grown up in culture and injected into German cockroaches (*Blattella germanica*), rapidly paralyses them. We purified one of the insecticidal proteins from culture broth and found that it migrated on a denaturing SDS–polyacrylamide gel as a single band at a position corresponding to a relative molecular mass of about 63K (Fig. 1c). Partial amino-acid sequencing of this toxin indicated that it was a GroEL homologue.

GroEL, also known as chaperonin, is a product of the *groE* gene of *Escherichia coli* and was first discovered as a mutant that inhibits bacteriophage growth[2]. GroEL forms a homo-oligomer with a double-toroid structure and, in combination with the protein GroES and ATP, acts as a molecular chaperone to ensure correct folding and assembly of proteins[2–4]. However, the paralytic and insecticidal activities described here cannot be explained in terms of a chaperonin function.

The GroEL homologue from *E. aerogenes* showed no acute toxicity towards mice, but it rapidly paralysed and killed cockroaches when injected at a minimum dose of 2.7 ± 1.6 ng (mean ± s.e.m.; $n = 3$); the recombinant protein encoded by its complementary DNA and expressed in *E. coli* was equally toxic. By contrast, GroEL from *E. coli* did not paralyse the insects even at doses as high as 2 μg.

We cloned the *groE* gene from *E. aerogenes* and found that only 11 residues in the GroEL homologue had alignments different from the residues in GroEL from *E. coli* (Table 1) — except at the carboxy terminus, where the GroEL homologue has a methionine residue and is shorter than GroEL by three residues. The amino-acid residues Val 100 (valine at the 100th residue), Asn 101, Asp 338 and Ala 471 are crucial for toxicity, as shown by the marked reduction in toxicity of mutants carrying the substitutions Ile 100, Thr 101, Glu 338 and Gly 471 (Table 1). The importance of these residues was confirmed by reversing the individual mutations (substituting valine for isoleucine at residue 100, and so on) in *E. coli* GroEL, which conferred toxicity on the protein (Table 1).

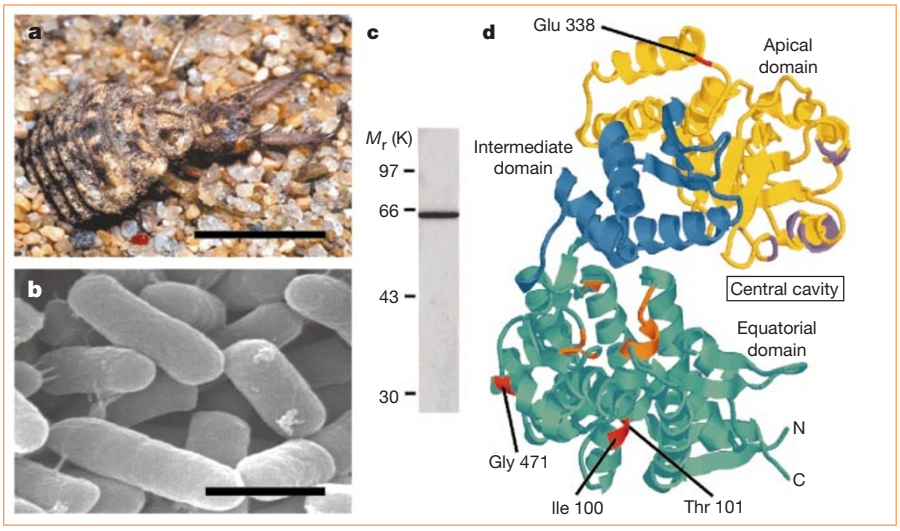

Figure 1 An insect toxin produced by a salivary endosymbiont of antlion larvae is a GroEL homologue. **a,** Larva of *M. bore*. Scale bar, 5 mm. **b,** Scanning electron micrograph of the bacterial endosymbiont *E. aerogenes*. Scale bar, 1 μm. **c,** Electrophoresis on a 10 % SDS–polyacrylamide gel of the toxic protein purified from a culture of *E. aerogenes*. **d,** The three-dimensional structure of a subunit in a 14-mer GroEL molecule from *E. coli* (created using a PDB file,1DER). The apical, intermediate and equatorial domains are coloured yellow, blue and green, respectively. Locations of the residues in which mutation confers toxicity are shown in red. Sites at which mutation blocks the binding of polypeptide and GroES in the apical domain[8] and the residues involved in ATP binding in the equatorial domain[6,7] are shown in purple and orange, respectively.

In the crystal structure of *E. coli* GroEL[5–7], Glu 338 is located in the apical domain, far from the polypeptide- and GroES-binding sites[7,8], whereas Ile 100, Thr 101 and Gly 471 are in the equatorial domain, far from the ATP-binding pocket that faces the central cavity (Fig. 1d)[6,7]. Thus, neither GroES binding nor ATP binding to the GroEL homologue is effective in generating toxicity. Injection of the GroES homologue from *E. aerogenes* together with ATP and the GroEL homologue scarcely influenced the minimum paralysing dose (3.9 ± 0.3 ng; $n = 3$).

Chaperonins from bacterial pathogens can function as cell-signalling molecules, stimulating human monocytes, leukocytes, fibroblasts and epithelial cells to release pro-inflammatory cytokines[9]. The toxicity of the GroEL homologue towards insects can be seen as an effect of a bacterial extracellular chaperonin on eukaryotic cells. The homologue may act on particular receptors in insects to induce paralysis, having evolved this non-chaperone function to establish a mutually beneficial antlion–symbiont relationship. Our finding that insecticidal proteins are produced by endosymbionts to help in capturing prey is likely to extend to many other fluid-feeding carnivorous insects.

Table 1 Paralytic activity of GroELs

E. aerogenes *GroEL homologue*	*MPD (ng per insect)*	*GroEL from* E. coli	*MPD (ng per insect)*
Wild type	3.3 ± 1.1	Wild type	ND
V100 I	ND	I 100V	26.7 ± 3.2
N101T	ND	T101N	61.3 ± 3.7
V125T	53.3 ± 4.1	T125V	ND
D338E	ND	E338D	13.6 ± 1.4
T347A	18.8 ± 2.5	A347T	ND
I 426L	16.0 ± 2.7	L426 I	ND
G428D	46.8 ± 4.1	D428G	ND
K430R	51.2 ± 4.4	R430K	ND
A471G	ND	G471A	14.4 ± 1.3
S527N	25.7 ± 1.7	N527S	ND
P530A	51.5 ± 2.8	A530P	ND

Mutants are represented by the usual notation, in which the wild-type amino-acid residue is given first in single-letter code, then the position in the protein sequence, and finally the substituent amino acid. Minimum paralytic dose (MPD) values are expressed as means ± s.e.m. of minimal GroEL amounts required to paralyse cockroaches within 10 min after injection ($n = 5$). ND, not determined (because of low toxicity: MPD > 1,000). Further details are available from K.M.

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1. Matsura, T. *Ecol. Res.* **1,** 15–24 (1986).
2. Zeilstra-Ryalls, J., Fayet, O. & Georgopoulos, C. *Annu. Rev. Microbiol.* **45,** 301–325 (1991).
3. Sigler, P. B. *et al. Annu. Rev. Biochem.* **67,** 581–608 (1998).
4. Ranson, N. A., White, H. E. & Saibil, H. R. *Biochem. J.* **333,** 233–242 (1998).
5. Braig, K. *et al. Nature* **371,** 578–586 (1994).
6. Boisvert, D. C., Wang, J., Otwinowski, Z., Horwich, A. L. & Sigler, P. B. *Nature Struct. Biol.* **3,** 170–177 (1996).
7. Xu, Z., Horwich, A. L. & Sigler, P. B. *Nature* **388,** 741–750 (1997).
8. Fenton, W. A., Kashi, Y., Furtak, K. & Horwich, A. L. *Nature* **371,** 614–619 (1994).
9. Ranford, J. C., Coates, A. R. M. & Henderson, B. *Exp. Rev. Mol. Med.* http://www-ermm.cbcu.cam.ac.uk/00002015h.htm (2000).