

Abstract

Proteins containing Kunitz domain are mostly inhibitors of serine proteases. Their general characteristic is the presence of three disulfide bonds and small sizes around 6–10 kDa, although sometimes they consist of several Kunitz domains or they are part of more complex proteins. Their function is usually related to the regulation of physiological and proteolytic processes, but also to an interaction with pathogens or other defense mechanisms, such as being part of the sea anemone mucus or the venom of snakes and other invertebrates.

We focused on Kunitz proteins in *Eudiplozoon nipponicum*, a helminth of the class Monogenea parasiting on gills of common carp (*Cyprinus carpio*). In the transcriptome of this parasite, several sequences with Kunitz domain have been identified based on similarities with the one already described Kunitz protein, *EnKT1*, suggesting that this parasite, like other bloodfeeding parasites, uses a whole set of these serine protease inhibitors with other specific functions.

Several sequences with the Kunitz domain found in the transcriptome were verified by PCR and optionally supplemented by RACE-PCR. One protein, called *EnKC1*, was subsequently produced by recombinant expression in *E. coli* cells of SHuffle™ and Rosetta Gami B strains.

Recombinant protein with the Kunitz domain was found to have inhibitory activity against chymotrypsin (in accordance with the specificity determining aminoacid at the active site of the domain) and factor Xa. In contrast, no effect was observed on thrombin, kallikrein and plasmin, nor on the function of fish or human complement in the lysis of rabbit erythrocytes.

The newly characterized protein from the parasite *E. nipponicum* has a different specificity compared to the previously described *EnKT1*. It is so-called chymotrypsin-like, ie it inhibits chymotrypsin, and furthermore it does not have such a wide spectrum of action like *EnKT1*. However, it could contribute to the ability of blood intake when sucking on carp gills or processing it in the intestine of the parasite.