An expanded and functionally diverse family of Kunitz inhibitors from a metazoan parasite

MARTÍN FLÓ[1,2], BEATRIZ ALVAREZ[2], ENRIQUE SOTO[3], CECILIA FERNÁNDEZ[1]

FACULTIES OF [1]CHEMISTRY AND [2]SCIENCES, UNIVERSIDAD DE LA REPÚBLICA, URUGUAY; [3]INSTITUTE OF PHYSIOLOGY, BENEMÉRITA UNIVERSIDAD AUTÓNOMA DE PUEBLA, MÉXICO

We are characterising a multigene family of secreted monodomain Kunitz proteins from the flatworm *Echinococcus granulosus* (*Eg*KU-1–*Eg*KU-8) acting at the interface with the dog definitive host. Typically, these proteins are inhibitors of serine peptidases that tightly bind to the active site through an antipeptidase loop mimicking a substrate, yet they are cleaved extremely slowly; in addition, some members of Kunitz families from animal venoms act as cation channel blockers. Our studies show that the parasite family contains molecules with either activity. On the one hand, the close paralogs *Eg*KU-3 (Leu at the antipeptidase P1 site) and *Eg*KU-8 (Arg in P1) behaved as typical slow tight binding inhibitors of chymotrypsins and trypsins, respectively (K_i 10^{-11} M). In turn, *Eg*KU-7 (Arg in P1), which carries an extension at the C-terminus of the Kunitz domain, also inhibited trypsin, but the inhibition differed from the one observed for *Eg*KU-3 and *Eg*KU-8. Indeed, it interacted with the enzyme through two independent sites, the antipeptidase loop and the C-terminal extension, that showed a different pattern of interaction with bovine and canine isoforms. Both sites were susceptible to hydrolysis by bovine trypsin; in contrast, the antipeptidase loop showed preference for canine anionic trypsin (K_I^a 10^{-12} M) over other trypsins (K_I^a 10^{-11} M), whereas the second site preferred canine cationic trypsin (K_I^b 10^{-11} M) over other trypsins (K_I^b 10^{-10} M), thus indicating that the canine enzymes could be the physiological targets of *Eg*KU-7. On the other hand, the close paralogs *Eg*KU-1 and *Eg*KU-4, which do not inhibit peptidases, blocked voltage dependent potassium channels (K_v; IC_{50} 10^{-7} M); and also acid-sensing ion channels (ASICs; IC_{50} 10^{-9} M), an activity we recently described for Kunitz proteins. Taken together, our results suggest that Kunitz families in animal venoms and parasite secretions expand and diversify through similar mechanisms.