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Vermipharmaceuticals and active proteins isolated from earthworms

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ABSTRACT

The earthworm is one of the typical saprophagous organisms that has been successfully used to convert organic waste into biomass. Indeed, vermicomposting occurs throughout the world. More recently, research on pharmaceuticals derived from earthworms, known as green biomedicine, has been increasing. As a result, earthworms have become an international medicine, even though their original utilization in traditional medicine has been known for thousands of years. With the development of biomedicine, scientists have rediscovered the medicinal value of earthworms related to many chemical components, including (1) earthworm proteases (lumbrokinase, collagenase, superoxide dismutase, cholinesterase, catalases, glycosidases); (2) metal-binding protein (metallothionein, calmodulin-binding protein); (3) other active proteins including those with proliferative improving activity like lysenin, eiseniapore, antitumor proteins, and glycoprotein; (4) active peptides (gut mobility regulation peptide, antibacterial peptide); (5) earthworm metabolites (carbamidine, lumbrinin, lumbrofobrin, terrestrolumbrolysin); (6) special organic acids (succinic acid, lauric acid, and unsaturated fatty acid) and (7) other components such as purin, vitamin B, tyrosine and Se. In this paper, we mainly describe earthworm fibrinolytic enzymes and antibacterial peptides in particular.

Introduction

Earthworms live in an environment filled with various kinds of pathogens. Physiologically and evolutionally speaking, earthworm survival in such an environment must have favoured the development of efficient defense mechanisms against various environmental pathogens during the course of evolution, including the production of certain anti-microbiological substances, especially active proteins and enzymes. Early interest in earthworm enzymes, at the end of the 19th century, focused on digesting functions, with Frédéricq's discovery that an enzyme secreted from the alimentary tract of earthworms had proteolytic activity (Frédéricq 1878). Other earthworm-derived enzymes continued to be found thereafter (Tracey 1951).

The medicinal properties of earthworms are known and have been used in many different countries and cultures (Stephenson 1930; Bristowe 1932; Reynolds and Reynolds 1972). Carr (1951) described how the Cherokee Indians of the Great Smokey Mountains used earthworm poultices to draw out thorns. Scientific interest in earthworm pharmaceutical use began in the 1980s. Mihara et al. (1983) first isolated a group of proteases from the earthworm *Lumbricus rubellus* and studied their fibrinolytic activity. Subsequently, isolation and purification methods have been developed, including gel filtration, affinity chromatography, ion exchange chromatography and high-pressure liquid chromatography (HPLC) that simplify such studies. Many active proteins/proteases, such as earthworm fibrinolytic enzyme, earthworm-tissue plasminogen activator, and earthworm plasminogen activator have been isolated from different species; some have already been used as drugs to treat clotting diseases.

Use of earthworms in traditional medicine has a long history. Five hundred years ago, Shizhen Li compiled the famous medical book *Compendium of Material*, in which the earthworm (Earth dragon) was recorded as a drug prescribed for antipyretic and diuretic purposes in the form of a dried powder. This remedy is still used in Chinese folk medicine. In order to meet the increasing demand for green natural medicine in recent years, bioactive components with medicinal value from earthworms have already provoked increased attention in Asia and elsewhere in the world. Therefore, further research into the nature and function of medicinal compounds derived from earthworms is needed.

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Earthworm enzymes

Fibrinolytic enzymes

Earthworm fibrinolytic enzyme (EFE) has been analyzed rather extensively. Qiao et al. (2001) and Wu et al. (2002) reported certain features of intestinal absorption and effects of intact fibrinolytic enzyme III-1 from *L. rubellus*. They first revealed α2-macroglobulin (α2M) bound to the enzyme in an equal mole-to-mole ratio. The intrinsic fluorescence of α2M was enhanced with an observable blue shift in emission maxima, suggesting that α2M was one of the several important inhibitors of EFEs that is absorbed into blood. In order to investigate whether earthworm fibrinolytic enzyme III-1 (EFE-III-1) isolated from *L. rubellus* is capable of transport into blood through the intestinal epithelium and can retain its biological function while in circulation, Wu et al. (2002) produced an antibody against EFE-III-1. The immunological results showed that 10–15% of intact EFE-III-1 was absorbed by the intestinal epithelium using the incubation chamber method. The enzyme could be detected in the intestinal epithelial cells by immunohistochemistry. Furthermore, immuno-reactive intact EFE-III-1 was found in either the serum or plasma after intraperitoneal injection of rats. There, approximately 10% of the total enzyme could be transported through the intestinal epithelium. The maximum remaining activity in blood was assayed around 60 min after the intraperitoneal injection.

The latter study corroborates the fact that earthworm fibrinolytic enzyme component A (EFEa) showed strong functions in both fibrin-degradation and plasminogen activation. Tang et al. (2002) showed the crystal structure of EFEa using the Multiple Isomorphous Replacement (MIR) method, refined to 2.3 Å resolution. Structure-based inhibitor modeling demonstrated that EFEa's S1 specificity pocket was preferable for elastase-specific small hydrophobic P1 residues, while its accommodation of long and/or bulky P1 residues was also feasible if enhanced binding of the substrate and induced fit of the S1 pocket were achieved. EFEa is thereby endowed with relatively broad substrate specificity, including dual fibrinolysis. The presence of Tyr99 at the S2 subsite indicates a preference for P2-Gly, while an induced fit of Tyr99 was also suggested in accommodating bigger P2 residues. This is the first reported structure for an earthworm fibrinolytic enzyme component and serine protease originating from annelid worms.

Digestive enzymes in the earthworm gut

Earthworms possess a weak but quite complete enzyme system. In the gut, enzymes are capable of degrading the following substrates: heteroside (N-acetylglucosamine), oligosaccharides (maltose laminaribiose) and polysaccharides. Zhang et al. (1993) reported that the strongest enzymatic activities were located in the foregut and midgut. Among the main enzymes found in the gut, cellulases and mannanases were not detected in cultured tissues nor in culture medium, which indicates that these two enzymes were probably produced by microorganisms ingested with the soil. In contrast, oligosaccharidase and heterosidase activities were higher in cultured tissues than in culture medium. Lattaud et al. (1997a) found a wide range of glucosidic substrates characteristic of plant material and used them to reveal the activities of digestive enzymes in the gut (wall and contents) of *Polypheretima elongata* (an endogeic geophagous earthworm). This species consumes some plant substrates but mostly degrades root and fungal substrates, whereas tropical endogeic earthworms feed on litter debris and soils poor in organic matter. The glucosidic activities of *P. elongata* were higher than those found previously in *Pontoscolex corethrurus*. The *in vitro* tissue culture of gut wall enzymes suggested that *P. elongata* can synthesize all of its extra- and intra-cellular enzymes, contrary to *P. corethrurus*, which requires ingested soil microflora in order to hydrolyze some substrates such as cellulose and mannose. It would be interesting to compare cellulases and mannanases of both of these earthworms after extraction and purification, and to identify mechanisms by which *P. corethrurus* enhances microbial activity.

Cellulases

Urbasek (1990) reported cellulase activity in the gut of certain earthworms. Gut cellulase activity occurred in all epigeic and endogeic earthworm species. Activity in the gut contents displayed several pH optima, indicating the presence of numerous enzymes, while only a single pH optimum was detected in gut wall extracts. Higher cellulase activities were found in the gut wall of epigeic than of endogeic earthworms except for the epigeic *L. rubellus*, which showed a significantly lower enzyme activity. Whiston and Seal (1988) had shown that *E. fetida* can produce endogenous carboxymethylcellulase (CMCase). Activity levels indicated that this enzyme was localized in gut tissue.

Glycolytic enzymes

Most endogeic earthworms have a weak enzymatic component, as a result they usually establish mutualistic relationships with soil microorganisms to digest certain organic compounds (Lavelle et al., 1995). Therefore, researchers have cultured intestinal wall tissues *in vitro* to assess the origins of gut glycolytic enzymes. Enzymatic activities were measured in both cultured tissues and culture media. Lattaud et al. (1997b) showed the tropical geophagous earthworm *Millsonia anomala* may use root and fungal substrates available in soils. *M. anomala* cannot produce cellulase and mannanase but instead utilizes the digestive enzymatic capabilities of the ingested microflora, which synthesize extracellular glycolytic enzymes. These enzyme capabilities are similar to those of *P. corethrurus*, but inferior to *P. elongata* that produces cellulase and mannanase. Garvín et al. (2000) reported *Hormogaster elisae* had a wide but not a significantly strong enzyme complement, since all substrates were degraded but most at a low rate. This species cannot produce cellulase and mannanase, therefore, it probably uses the digestive enzymatic capabilities of ingested soil microorganisms to digest these substrates.

Acetylesterases

Engelstad and Stenersen (1991) described acetylesterases (EC 3.1.1.6) in *Eisenia andrei*, *E. fetida* and *Eisenia veneta* by polyacrylamide gel electrophoresis. Four bands were common in *E. andrei* and *E. fetida*, whereas the three bands found in *E. veneta* were unique for this species. Two of the electromorphs were gut enzymes, of which one is unique to *E. andrei* and *E .fetida*. The frequencies of the acetylesterase electromorphs were significantly different in two populations of *E. veneta*, which reflects the wide variability of acetylesterase alleles. Indications of *E. fetida* and *E. veneta* hybridization were also found. Short-term starvation had little influence on the reliability of the method.

Phosphatases

Satchell and Martin (1984) reported phosphatase activity in earthworm faeces and Park et al. (1993) studied activities of phosphomonoesterase and phosphodiesterase from *Lumbricus terrestris*. To do so, the relationship between enzymes that can hydrolyze p-nitrophenylphosphate and p-nitrophenyl phosphate was investigated. In contrast to what occurs with most alkaline phosphatases in other species, the hydrolysis of both substrates was significantly inhibited by dithiothreitol, but not by the thiol-directed inhibitors