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Review

Glycolipoprotein extract of *Eisenia foetida* (G-90): A source of biological active molecules

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Abstract

The G-90 is a macromolecular mixture with glycolipoprotein characteristics obtained from tissue homogenate of the earthworm *Eisenia foetida*. This macromolecular mixture exhibits numerous biological activities such as mitogenicity, anticoagulation, fibrinolysis, bacteriostasis and antioxidation *in vitro* and *in vivo* conditions. It is neither mutagenic nor cancerogenic. In our investigation G-90 did not contain the growth factors (FGF and EGF), but it stimulated their synthesis in cell cultures, which caused increased cell proliferation. It is likely that some pleiotropic activity of G-90 could be associated with the insulin-like proteins, tyrosine like serine peptidases and the immunoglobulin-like molecules. Some factors in G-90 recognised sera of patients with tumors, as well as those suffering from cardiopathies. Accordingly, G-90 is a valuable mixture of biologically active macromolecules, which could be very useful in human and veterinary medicine.

Keywords: *Eisenia foetida*; Glycolipoprotein extract; G-90; Biological activities

1. Introduction

Many details of the cell division and differentiation mechanisms are not completely known. The enzymes, growth factors, cyclins and other signal molecules are activated into the cycles of mitosis, and these cycles are repeated during the evolution. More and more factors and pathways included in these processes are being investigated. Studies showed that the position on phylogenetic tree and the level of ontogenesis determine a capability of specific differentiation during cell cycle. Phylogenetic lower organisms (invertebrates) and even certain vertebrates (Amphibia) very successfully recover lost part of the body. It is proposed that they possess the macromolecules with special activities. The ability of complete regeneration of a part of the body is based on genetic code. During the evolution the code has been lost or inactivated. For the above mentioned reason it would be very interesting to discover and investigate the macromolecules from that pathway. Macromolecules with regenerative ability could help in tissue regeneration and maintaining homeostasis as well. Many investigators consider that the earthworms are appropriate organisms for investigation of tissue regeneration phenomena.

The earthworm (phylum Annelida, family Lumbricidae) is one of the first organisms in the evolution of any phylogenetic tree that possesses immunological recognition and memory. They regenerate amputated parts of their body if the nervous system is intact [18]. The segmented earthworm's body cavity is filled up with coelomic fluid, which shows numerous biological

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activities such as the haemolytic and agglutinative activities [8,25,36], a mitogen [38], the bacteriostatic and bacteriolytic activities [9,19]. The coelomocytes, cells that are present within the coelomic fluid, are thought to be responsible for such effects, as they secrete proteins and glycoproteins which act as opsonins, agglutinins, lysine and a certain factor which resembles macrophage migration inhibition factor [8,35].

2. Characterization of tissue homogenate of *Eisenia foetida* (G-90)

The separation of coelomic fluid is a time consuming process that yields a low quantitative effect. Therefore, our study was focused on earthworm tissue extracts. The preparation from tissue homogenate, named G-90 (40 μg proteins/mg of lyophilized powder), was separated on 17 fractions. In double gel diffusion on 1% agar it interacted with human and animal, normal and pathological sera. All fractions showed the presence of amino groups, sugars and glycolipids. The phosphoric and sialic acids were not detected. G-90 stimulated the proliferation of cell cultures, depending on the concentration of G-90 and the type of the cells. Ames test showed neither mutagen nor carcinogen presence [22]. The properties of G-90 are summarized in Table 1.

3. Mitogen effect of insulin-like proteins from G-90

The anciently known mitogens belong to insulin superfamily, which is carried out by one basic genetic code for the insulin-like macromolecules. Since insulin-like molecules have already been detected in tissue of various invertebrates, it is possible that similar structures are included in G-90 as well. Therefore, we searched G-90 for the molecules, immunologically similar to vertebrate insulin. Also we examined circumstances under such insulin-like molecules might affect mammalian normal and tumor cell growth *in vitro* [22].

Table 1
The properties of G-90

Yield	About 20 mg powder/1 g wet earthworms
Protein content	40 μg/mg lyophilized powder
Thin-layer chromatography	Amino groups, sugars, glucolipids
Haemagglutination assay	*N*-acetyl-galactoseamin, β-galactose, ∀-L-fucose, D-mannose, *N*-acetyl-glucosamine
IR spectral analysis	795/cm (CO, C–CO groups), 1250/cm (sulphate esters groups) 1640–1660/cm (*N*-acetylhexosamine, sphingolipid, NH_2 groups), 2940/cm (CH and fatty acids) 3300–3400/cm (OH, carbohydrates)
SDS-PAGE	17 fractions (Mw 20–70 kDa)
Isoelectric focusing	10 bands in range pH 2.5–5.2, 1 band at pH 8.5
Immunological interaction	Human normal and pathological sera
Ames test	Not mutagen nor carcinogen

Besides metabolic function, insulin-related system obviously has some additional functions. Several reports clearly point out that vertebrate insulin could act ontogenetically, before the appearance of pancreatic β-cells, as a paracrine hormone extremely important for normal development [17]. Furthermore, the appearance of ''high'' insulin-like growth factors and other not yet completely identified insulin-like molecules, in the circulation of patients with malignoma, and related hypoglycemia, is very often reported [26]. After injury, regenerating nerve, muscle or endothelial cells produce more insulin-like factors than normally [16]. Therefore, insulin and related molecules could be viewed as highly important not only in regulation of normal cell growth and differentiation, but also in tumorogenesis, and not only for vertebrates, but also for invertebrate organisms [3].

The presence of insulin-like structures in G-90 was shown by radioimmunoassay. After purification on Sephacryl S-200HR, the 7 fractions, molecular masses from 97.7 kDa to 19.9 kDa, were detected [20]. All fractions have shown antigen similarity with insulin, and stimulated the proliferation of normal and malignant cells in serum-free condition (melanoma B16, HeLa). So far, insulin-related molecules have not yet been identified in earthworm tissue. Also, it has not been shown whether insulin-related molecules, found in some other invertebrate sources, could affect cell growth *in vitro*. A strong stimulating effect of cell proliferation detected in serum-free conditions, which is completely abolish in serum supplemented medium, suggested some kind of competition among serum and earthworm factors for cell receptors, or a kind of interactions.

Furthermore, it is possible that mammalian cell possesses receptor(s) for an ''evolutionary old'' form insulin-like molecule. Since the intensity of cell proliferation caused by such molecules varies, the quantity of proposed receptor(s) on various cell types must also differ. However, insulin-like properties and mitogenicity of isolated molecules are not ultimately connected.

4. Adhesins of immunoglobulin-like superfamily from G-90

The evolution of genetic code of the immunoglobulin superfamily is rather known. During the evolution the basic code has been mutated to give different