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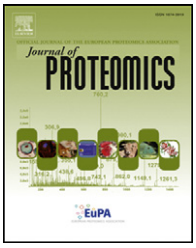

Proteomic analysis of the skin of Chinese giant salamander (*Andrias davidianus*)

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ABSTRACT

The Chinese giant salamander (*Andrias davidianus*), renowned as a living fossil, is the largest and longest-lived amphibian species in the world. Its skin has developed mucous gland which could secrete a large amount of mucus under the scraping and electric stimulation, and the molting is the degraded skin stratum corneum. Although several proteomic studies have focused on functional proteomes of mammalian and frog skin, the skin proteome of Chinese giant salamander has not yet been carefully studied. To establish the functional skin proteome of Chinese giant salamander, two-dimensional gel electrophoresis (2DE) and mass spectrometry (MS) were applied to detect the composition and relative abundance of the proteins in the skin, mucus and molting. Our findings indicated that 249 proteins were identified in the skin, 155 proteins in the mucus, and 97 proteins in the molting. Furthermore, Gene Ontology (GO) analysis showed that these proteins participated in various physiological activities, including extracellular matrix organization, defense, immune response, wound healing, respiration, etc. In conclusion, the proteomic results provide new insight in the aspects of the proteomes in the skin, mucus and the molting of Chinese giant salamander.

Biological significance
This was the first study to examine the protein expression abundance in the skin, mucus and molting of Chinese giant salamander by a proteomics approach. Meantime, the identification of a more global proteome in normal skin may provide a basis for characterizing and comparing the skin proteomes from other amphibian species.

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1. Introduction

The Chinese giant salamander (*Andrias davidianus*), belonging to order Caudata, family Cryptobranchidae, is the largest extant amphibian species. It occurs mostly in southwestern and eastern China and is classified as a class II protected species in China [1] and as one of the top 10 "focal species" by the Evolutionarily Distinct and Globally Endangered (EDGE) project in 2008 (http://www.edgeofexistence.org/). It is renowned as a living fossil, since it has been in existence for more than 350 million years [2,3]. The evolution history of this species likely involves adaptations [4,5], and its ancestral organisms may have represented transition steps from aquatic to terrestrial life, so this species has an important value in scientific research of skin system.

The skin of Chinese giant salamander is dark brown, black or greenish in color and irregularly blotched. It is also rough, wrinkled and porous which facilitates respiration through the skin as this large amphibian lacks gills. The skin is a multi functional organ acting in defense and cutaneous respiration [6], and is covered in mucus which protects their bodies from abrasions and parasites. When irritated or grasped, giant salamanders produce a milky, sticky secretion with heavy and astringency smell owing to its developed mucous gland. The main ingredients of skin mucus are sticky glycoproteins and fiber materials. Generally, the mucous secretions have antibacterial, antioxidation, antitumor, fatigue-resistant, and strengthened immunity [7–9]. Like other amphibians, this salamander has smooth skin that lacks scales, and the molting is the normal metabolism of the skin. Molting is a process of constant renewal of the outer layer of epidermis (stratum corneum) in amphibians, which provides a barrier against injury, pathogens and evaporative water loss [10]. In the private sector, the powder of giant salamander molting mixed with tung oil was utilized to treat burns [11]. In view of these characteristics, the research on Chinese giant salamander skin has important scientific significance. Therefore, it is of great interest to elucidate the proteome of its skin, mucus and molting. On the other hand, there is an urgent need to understand the defense reaction of Chinese giant salamander skin, due to the fact that a dramatic decline in the population has resulted from emerging infectious diseases [12,13].

Despite the high public profile of Chinese giant salamander and their unique life-history characteristics, this species remains poorly characterized at the molecular level. Fortunately, proteomics can provide a global and comprehensive approach to the identification and description of biochemical processes at the protein level. Although several proteomic studies have focused on functional proteome of the human and mouse skin [14–16] and on the skin secretome of the frog [17,18], the comprehensive proteomic profiling of Chinese giant salamander skin, is still unknown. This present study attempts to employ large-scale proteomic analysis to establish the functional proteome in the skin, mucus and molting of Chinese giant salamander. We identified 249 proteins in the skin, 155 proteins in the mucus, and 97 proteins in the molting using two-dimensional gel electrophoresis (2DE) coupled with mass spectrometry (MS). Gene Ontology (GO) analysis showed that these proteins participated in various physiological activities, including extracellular matrix organization, defense, immune response, wound healing, respiration, etc. This study provides some interesting new insights in the proteomes of the skin, mucus and molting of Chinese giant salamander, and may also provide a basis for characterizing and comparing the skin proteomes from other amphibian species.

2. Materials and methods

2.1. Animals

Three or four year old male healthy Chinese giant salamanders with body length of 60–100 cm and weight of about 3 kg, were obtained from a giant salamander breeding base in Wen Quan Zhen, Kaixian Country, Chongqing Municipality, China. The molting was obtained from the breeding pool of Chinese giant salamanders. The mucus was secreted by the dorsal skin of Chinese giant salamanders under the scraping stimulation with a triangle, and was collected in a sterile tube. Subsequently, they were anesthetized and sacrificed by decapitation. The skin tissues were immediately dissected from the giant salamanders, and washed in sterile PBS. The skin, mucus and molting samples were stored in liquid nitrogen for further use. All experiments were performed in strict accordance with the Animal Protection Law of China.

2.2. Histology

The skin tissues mentioned above were fixed in Bouin's solution for 48 h. The fixed tissues were washed in 50% and 70% alcohol in order, and then were dehydrated in a graded series of alcohol, followed by xylene. After that, they were infiltrated overnight with paraffin. The tissues were then sectioned at 8 μm. Sections were stained with hematoxylin and eosin, and photographed on a Leica microscope (LEICA, Germany).

2.3. Protein extraction and two-dimensional gel electrophoresis (2D)

Protein extraction was performed according to the previously described method [19]. Briefly, the skin, mucus and molting samples were respectively grinded into fine powder in liquid nitrogen, and then were suspended in lysis buffer (30 mM Tris, 7 M urea, 2 M thiourea, 4% CHAPS). The suspension was mixed at 4 °C for 1 h by vortex, and then was centrifuged at 20,000 g for 1 h. The supernatant was collected and stored at −80 °C for further use. The concentration of total proteins was determined using a 2D Quantification kit (GE Healthcare, USA).

2D was performed according to the operating manual (GE Healthcare, USA), and each sample was repeated three times. In brief, 1000 μg of samples was dissolved in 450 μl rehydration buffer (7 M urea, 2 M thiourea, 2% CHAPS, 18 mM DTT, 2% IPG buffer 3–10 NL). Then IPG gel strip (pH 3–10 NL, 24 cm) was rehydrated for 12 h at 20 °C. The isoelectric focusing (IEF) was performed in Ettan IPGphor (GE Healthcare, USA) following the conditions: ramped to 250 V in 1 h, held at 1000 V for 3 h, ramped to 10,000 V in 3 h, and held at 10,000 V for 8 h. After IEF, each gel strip was equilibrated for 15 min in 15 ml equilibration buffer (50 mM TrisHCl pH 8.8, 6 M urea, 30% glycerol, 2% SDS, 1% DTT)