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SILKOMICS - A BIOCHEMICAL VIEW OF THE SPIDER SILK PROTEINS

José Roberto Aparecido dos Santos-Pinto[1,2], Helen Andrade Arcuri[1], Franciele Grego Esteves[1], Gert Lübec[2] and Mario Sergio Palma[1]

[1]Center of Study of Social Insects, Department of Biology, Institute of Biosciences of Rio Claro, São Paulo State University - UNESP, Rio Claro, SP, Brazil; [2]Department of Pediatrics, Medical University of Vienna, Vienna, Austria. jrbio04@rc.unesp.br

The silk produced by spiders is characterized by diversity of its chemical composition, structure and function. The silk proteins have interesting relationships between their 3-D structures and mechanical properties of these protein fibers, with an exceptional combination of tensile strength and extensibility. Thus, these proteins have attracted the interest in their exploration and bioprospecting applications, offering a great potential in biomedical applications and textile industry. The spinning of silk involves complex interactions between specialized silk-producing glands that secrete different types of silk fibers, which are subsequently assembled to construct a functional web [1-3]. Herein, we used a proteomic approach (bottom-up gel-based and a shotgun proteomic approach) to identify proteins from the silk-producing glands. The proteomic analysis of six silk glands identified 125 proteins in the major ampullate, 101 in the flagelliform, 77 in the aggregate, 75 in the tubuliform, 68 in the minor ampullate and 23 in aciniform glands. Taken together and based on the functional classification using Gene Ontology, these proteins were organized into seven different groups according to their general function: (i) web silk proteins - the spidroins, (ii) proteins related to the folding/conformation of the spidroins, (iii) proteins that protect the silk proteins from oxidative stress, (iv) proteins involved in fibrillar preservation of silks in the web, (v) proteins related to ion transport into and out of the glands during silk fiber spinning, (vi) proteins involved in prey capture and pre-digestion, and (vii) housekeeping proteins from all of the glands. An experimental approach was developed combining 2-DE with *in-gel* protein digestion by different proteolytic enzymes, followed by mass spectrometry analysis under collision-induced dissociation (CID) and electron-transfer dissociation (ETD) conditions for protein sequencing and assignment of post-translational modifications (PTMs). This strategy permitted to assign about 100% of protein sequences, as well the identification of 15 and 16 phosphorylation sites in spidroin-1A and -1B, respectively. The 3-D structure of these proteins were developed using molecular modeling and molecular dynamics. Thus, we propose a general mechanism of action for the identified proteins in the silk-producing glands from the *Nephila clavipes* spider, since that these proteins may be involved in the production, secretion, storage, transport, silk protection, and conformational changes of spidroins throughout the spinning process. These findings may be valuable for understanding the physicochemical properties of the silk proteins and moreover, future designs of recombinantly produced spider silk proteins.

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