JOURNAL OF PROTEOMICS 75 (2011) 517–531

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot

Mass spectrometrical analysis of cuticular proteins from the wing of *Hebemoia glaucippe* (Linnaeus, 1758) (Lepidoptera: Pieridae)

Narkhyun Bae[a], *Martin Lödl*[b], *Arnold Pollak*[a], *Gert Lubec*[a,*]

[a]*Department of Pediatrics, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria*
[b]*Naturhistorisches Museum Wien, Burgring 7, 1010 Vienna, Austria*

ARTICLE INFO

Article history:
Received 24 May 2011
Accepted 22 August 2011
Available online 28 August 2011

Keywords:
Cuticular proteins
Butterfly wings
Hebemoia glaucippe
Mass spectrometry
Posttranslational modifications

ABSTRACT

Although several insect cuticular genes and proteins are annotated and an arthropod cuticular database is available, mass spectrometrical data on cuticular proteins and their post-translational modifications are limited.

Wings from *Hebemoia glaucippe* were analyzed by scanning electron microscopy or homogenized, proteins were extracted and run on 2DE.

In-gel digestion was carried out by using trypsin, chymotrypsin and Asp-N and subsequently the resulting peptides and post-translational modifications were identified by ion trap tandem mass spectrometry (nano-LC-ESI-MS/MS; HCT).

A complex wing skeleton and the cuticle of *H. glaucippe* were demonstrated. Cuticle protein 18.6, isoform A, pupal cuticle protein, cuticular protein CPR59A and two putative proteins, putative cuticular protein B2DBJ and putative cuticle protein CPG31 with two expression forms were identified. Two phosphorylation sites on the same peptide, T213 and S214, were identified on putative cuticle protein CPG31, quinone formation was observed at Y76 on cuticular protein CPR59A probably indicating the presence of post-translational modifications.

The results may be relevant for the interpretation of mechanoelastic and physical properties of these proteins. Along with the extraordinary architecture the proteinaceous matrix is probably representing or allowing the unusual aerodynamic function of the butterfly wing. Moreover, the results may be important for mechanisms of insecticide and drought resistance.

1. Introduction

In a recent publication, structural cuticular proteins from arthropods annotation, nomenclature and sequence characteristics are reviewed [1] and an arthropod database is publicly available [2]. As to insect cuticular proteins Andersen et al. [3] have given a most useful introduction into cuticular proteins (CP) and reviewed structure and function. In Lepidoptera a series of cuticular protein families are known and consist of CPR with the R&R consensus sequence, CPF/CPFL, Tweedle (TWDL), CPLCP, CPG, CPAP3, BcNCP1 orthologs, 18 aa motif, CP with less than 3 AAP and dumpy family members. Description of these families is provided in the above mentioned review and it must be stated that most informa-

* *Corresponding author at:* Medical University of Vienna, Department of Pediatrics, Waehringer Guertel 18, A-1090 Vienna, Austria. Tel.: +43 1 404003215; fax: +43 1 404006065.
E-mail address: gert.lubec@meduniwien.ac.at (G. Lubec).

1874-3919/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jprot.2011.08.017

tion is given at the nucleic acid/genomic level rather than at the protein level.

Homology structural models of the R&R consensus region from a lepidopteran RR-1 protein and from a composite of numerous RR-2 proteins were constructed [4,5] and presented a half barrel structure with an opening nicely sized to hold a chitin chain [1] containing an aromatic residues thought to be essential for chitin binding [6]. *Bombyx mori* (Linnaeus, 1758) (Fam. Bombycidae) has 89 RR-2 genes [7] and the authors propose that the large number of genes serves the possibility of divergence of scale structures to adapt to the environment in lepidopteran species [8]. Chitin-binding was also shown for the *B. mori* TWDL family as direct measurement of the binding of a recombinant CPT1 protein to chitin beads was observed [9]. CPLCP proteins were detected in *B. mori* [10] but no function could be assigned to these proteins. Several CPG genes have been identified in *B. mori* and are characterized by their high glycine content [11] mostly with the repeats GGYGG or GGxGG. In *P. xuthus* (Linnaeus, 1767) (Fam. Papilionidae) a lepidopteran specific CPG with GGY motifs was identified and indeed, the CPG family seems to be restricted to Lepidoptera.

There are motifs or short stretches of amino acids that are commonly observed in CPs with unknown function [3]: The 18 aa amino acid motif was described in CPs of *B. mori* by Nakato et al. [12]. Finally, there are 34 proteins in *Bombyx* considered CPH for hypothetical cuticular proteins, because there is no evidence for participation in cuticle formation [1]. Although assignment of lepidopteran proteins into twelve CP families was shown, there is enormous variation of sequences hampering final annotation.

Studies on cuticular proteins may be important for the areas of insecticide resistance [13,14], drought resistance [15] or resistance against heavy metals [16] and for butterfly aerodynamics [17] to name a few.

Studying *Hebemoia glaucippe* wing proteins systematically, we aimed to demonstrate soluble CPs primary structure, cross-linking amino acids and post-translational modifications by a gel-based mass spectrometrical technique.

2. Materials and methods

Focus of the investigation was the Great Orange Tip (*H. glaucippe*) (Fig. 1A) which belongs to the butterfly family Yellows and Whites (Fam. Pieridae). *H.glaucippe* is a mostly common and widespread Indo-Australian species ranging with numerous subspecies from northern India to the Maluku Islands. We used a sample of specimens from West Malaysia (Cameron Highlands region).

The diversity of wing scales can be remarkable in butterflies, but the major part of the surface of *H. glaucippe* is covered by average, flat scales with a common basic structure. The scales are flattened and exhibit a stalk with which the scale is inserted in the wing surface. The 3d-structure is characterized by ridges of overlapping plates (r) (Fig. 1B) connected by cross-bridges (cb) (Fig. 1B). These cross-bridges

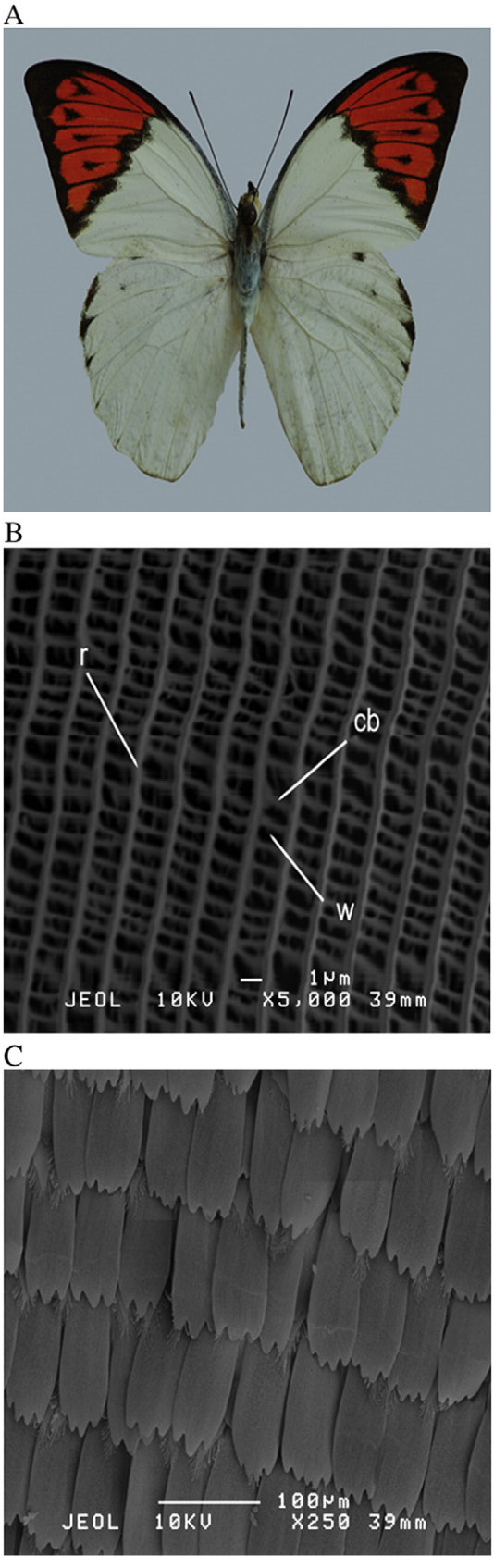

Fig. 1 – (A) *Hebemoia glaucippe*. (B), (C) Scanning electron microscopy analysis of *Hebemoia glaucippe*: scales are flattened and exhibit a stalk with which the scale is inserted in the wing surface. The 3d-structure is characterized by ridges of overlapping plates (r) that are connected by cross-bridges (cb). These cross-bridges are forming windows in between (w).