Vitellogenin identification by Western Blot

Immunoblots were performed according to the Licor protocols (https://www.licor.com/bio/applications/quantitative_western_blots/protocol.html) with slight modifications. For immunoblots, size-separated proteins were electrophoretically transferred to nitrocellulose membrane (Amersham™ Protran® Western blotting membranes, 0.45 µm: GE10600002 or 0.2 µm: GE10600004) using either Bjerrum Schafer-Nielsen Buffer with SDS (48 mM Tris, 39 mM glycine, 20% methanol (pH 9.2), 0.1% SDS) with constant current of 140 mA for 1h 30 min (OWL Device) or Pierce 1-Step Transfer Buffer with constant high current of 1.3-2.5 A for 12 min (Pierce™ Power Blotter).To control the transfer efficiency, the membrane was briefly rinsed with water after transfer, and incubated for 5 min in Revert Total Protein Stain, rinsed twice for 30 sec in washing solution (Licor: 926-11015) before acquiring the Revert total stain signal in the 700 nm channel using the Licor Odyssey® CLx Imaging System. The membranes were then incubated in Licor Blocking buffer (927-40100) for 1h at room temperature or overnight at 4°C with agitation. After addition of 0.2% Tween-20, the membranes were incubated with the polyclonal antibody against vitellogenin at a 1:7000 dilution (Davids Biotechnologie Regensburg) for 1 h at room temperature or overnight at 4°C with agitation. After 3 washes with PBS-T (0.2% Tween 20) the membranes were incubated for 45 min at room temperature in secondary antibody solution (IRDye® 800CW Goat anti-Rabbit IgG (H + L), 1:40.000 in Licor blocking buffer diluted 1:1 with PBS-T). After 3 washes with PBS-T (0.2% Tween 20) the vitellogenin specific signals were acquired in the 800 nm channel. The detected fluorescence signals were quantified using NIH software Image J (Christen et al. 2007). The intensity of vitellogenin bands was normalized against the intensity of the uncharacterized *Apis mellifera* protein, which is also detected by the antibody. Although not specified, this protein was well suited as a loading control, due to the fact that this protein is stably expressed in all analysed tissues. Moreover, there was a lack of available commercial antibodies, which are specific for honey bees and antibodies against proteins of other species may lack cross-reactivity.

Data processing and statistical analysis

One-way ANOVA and Bonferroni's multiple comparison test were applied to compare means of exposed and unexposed samples. Data are shown as means ± standard error of means. Statistically significant limits were: one asterisk at $0.05 > p > 0.01$, two asterisks at $0.01 > p > 0.001$ and three asterisks at $0.001 > p > 0.0001$.

RESULTS

Characterisation of vitellogenin antibody

Two rabbits were immunized using custom-synthesized peptides according to the vitellogenin peptide sequences. One peptide was based on the sequence located at the C-terminus and one based at the N-terminus (Fig. 1A). After 63 days of immunization, the final bleed was taken from each rabbit and the polyclonal antibody was purified by affinity purification revealing two polyclonal vitellogenin antibodies. Initially, the binding of the generated antibodies to honey bee vitellogenin from different tissues was analysed by dot blot analysis with a mixture of the two polyclonal vitellogenin antibodies. Figure 1B shows that the vitellogenin antibody mixture clearly detected vitellogenin in the hemolymph, fat body and brain tissue (Fig. 1B).

To further analyse the exact binding properties of the vitellogenin antibody, Western blot analysis was performed. The vitellogenin antibody detected the full length vitellogenin in fat body, hemolymph and brain (Fig. 2). According to our molecular size marker used, the size of the detected band was slightly lower than 180 kDa, representing the full length vitellogenin. In addition, the antibody recognized a lighter vitellogenin protein with a size slightly below 150 kDa in the hemolymph and fat body (Fig. 2A). However, in the brain, this smaller vitellogenin band was only detectable when overexposing the membrane (data not shown). In hemolymph, a band of approximately 75 kDa was detected in addition to the full-length and the lighter vitellogenin. In fat body, hemolymph and brain, an approximately 200 kDa band was detected in addition to 180 kDa vitellogenin (Fig. 2). No signal was detected in honey