LOCALIZATION OF THE GLYCOPROTEIN Cb42 IN LARVAE OF THE SCREWWORM FLY, *CHRYSOMYA BEZZIANA* (DIPTERA: CALLIPHORIDAE)

CRAIG EISEMANN[1] and SRI MUHARSINI[2]

[1] *120 Taringa Parade, Indooroopilly, Queensland 4068 Australia*

[2] *Research Institute for Veterinary Science*
Jalan R.E. Martadinata 30, P.O. Box 151, Bogor 16114, Indonesia

ABSTRAK

EISEMANN, CRAIG dan SRI MUHARSINI. 2000. Lokalisasi glikoprotein Cb42 pada jaringan larva lalat *screwworm Chrysomya bezziana* (Diptera : Calliphoridae). *Jurnal Ilmu Ternak dan Veteriner (Edisi Khusus)* 5(3): 197-200.

Letak Cb-peritrofin-42 pada jaringan larva stadium tiga dari lalat *Chrysomya bezziana* telah diidentifikasi dengan menggunakan antibodi primer yang diproduksi terhadap protein tersebut melalui proses rekombinan bakterial yang tidak berikatan glikosilat. Dua teknik yaitu antibodi imunofluoresen pada jaringan yang tidak difiksasi dan teknik *immunogold electron microscopy* pada jaringan yang dipotong sangat tipis dan difiksasi, telah digunakan dalam penelitian ini. Hasilnya menunjukkan bahwa Cb42 terdapat di seluruh permukaan dan seluruh lapisan membran peritrofik. Dengan teknik *immunogold* dapat diketahui bahwa Cb42 terdapat pada sel-sel sekresi dari membran peritrofik pada kardia, dan pada organ khusus pembuat membran peritrofik yang terletak pada perbatasan antara usus bagian depan dan tengah. Terjadinya reaksi Cb42 pada lapisan membran peritrofik yang masih utuh dengan antibodi primer yang digunakan dalam uji antibodi imunofluoresen menunjukkan bahwa glikoprotein tersebut merupakan target molekular potensial dalam vaksinasi induk semang terhadap larva *Chrysomya bezziana*.

Kata kunci: Cb-peritrofin-42, Cb42, glikoprotein, membran peritrofik, imunolokalisasi

ABSTRACT

EISEMANN, CRAIG and SRI MUHARSINI. 2000. Localization of the glycoprotein Cb42 in larvae of the screwworm fly *Chrysomya bezziana* (Diptera: Calliphoridae). *Jurnal Ilmu Ternak dan Veteriner (Edisi Khusus)* 5 (3): 197-200.

The glycoprotein Cb-peritrophin-42 was localized in third instar larvae of *Chrysomya bezziana* using primary antibodies raised against a non-glycosylated bacterial recombinant form of this protein. Both immunofluorescent antibody techniques on unfixed whole mounts of gut tissues and immunogold electron microscopy techniques on ultra-thin sections of fixed and embedded tissues were employed. The protein was shown to be exposed over the whole of both surfaces of the peritrophic membrane and to occur throughout its thickness. Immunogold labelling indicated that Cb-peritrophin-42 was expressed in the peritrophic membrane-secreting cells of the cardia, a specialized peritrophic membrane-forming organ situated at the junction of the foregut and midgut. The accessibility of Cb-peritrophin-42 present in intact peritrophic membrane to the primary antibodies used in the immunofluorescent antibody localization indicates that this glycoprotein is a potential molecular target for vaccination of host animals against larvae of *Chrysomya bezziana*.

Key words: Cb-peritrophin-42, Cb42, glycoprotein, peritrophic membrane, immunolocalization

INTRODUCTION

During a study of the feasibility of vaccinating host livestock against larvae of the Old World Screwworm fly *C. bezziana*, the glycoprotein designated Cb-peritrophin-42 (Cb42) was isolated from a crude preparation of peritrophic membrane (PM) collected from laboratory cultures of *C. bezziana* larvae (RIDING *et al*., 2000). The PM is a semi-permeable sheath enclosing ingested food material in the midgut and hindgut of many species of insects and other taxa. It is composed largely of glycoproteins, proteoglycans and polysaccharides and acts as a selective filter for digestive enzymes and nutrient molecules in the gut lumen (PETERS, 1992). Studies with larvae of the sheep blowfly *L. cuprina* have shown that antibodies raised against components of the PM can exert deleterious effects on larvae ingesting them, apparently by binding to, and partly blocking pores in the PM (*e.g.* CASU *et al*., 1997).

The work described here was undertaken to confirm the presence of Cb42 in the PM of larval *C. bezziana* and to obtain information on its distribution within the PM and on its probable site of expression. The glycoprotein Cb-peritrophin-48 has previously been localized to PM in third instar larvae of *C. bezziana* using immunofluorescence assay (IFA) techniques (VUOCOLO *et al*., 2000) and both IFA and immunogold

techniques have been employed successfully to demonstrate the occurrence and distribution of native glycoproteins in PM in larvae of *L. cuprina* (ELVIN *et al*., 1996; SCHORDERET *et al*., 1998). In higher Diptera, including calliphorids, PM is generally considered to be formed entirely in the cardia (PETERS, 1992). Investigations on the site of origin of Cb42 were therefore focussed mainly on this organ.

Cb42 has been expressed in bacteria as a recombinant protein and used to vaccinate sheep and rabbits (WIJFFELS *et al*., 2000). Consequently, antibodies to a non-glycosylated form of the protein were available for localization studies. These antibodies, being directed exclusively against the polypeptide part of the native glycoprotein, would not cross-react with oligosaccharide epitopes present in other glycoproteins or proteoglycans present and should therefore provide a reliable indication of the presence of native Cb42.

MATERIALS AND METHODS

Insects

Third instar larvae of *C. bezziana*, still feeding, were removed from a laboratory colony maintained at Balai Penelitian Veteriner, Bogor, Indonesia (SUKARSIH *et al*., 2000). They were then rinsed in water and immediately frozen in liquid nitrogen before being transported on dry ice to CSIRO Long Pocket Laboratories in Brisbane, Australia, where they were stored at –70°C until used.

Immunofluorescent Antibody Technique (IFA)

Newly thawed larvae of *C. bezziana*, previously handled as described above, were dissected in 0.1 M phosphate-buffered saline (PBS) and the cardia, together with an attached length of anterior midgut, was removed from each. This attached midgut was split open and trimmed away, leaving a length of exposed PM still attached to the cardia. Each preparation was agitated gently in the PBS to remove gut contents from the PM. Some preparations were then incubated overnight at 4°C in either affinity purified rabbit anti-recombinant Cb42 antibody (WIJFFELS *et al*., 2000) diluted to 25 μg ml^{-1} in PBS or in a control irrelevant antibody solution (affinity-purified anti-bovine leptin) at the same concentration. The tissue preparations were then washed in PBS (4 × 15 min) and incubated in an FITC-labelled sheep anti-rabbit immunoglobulin antibody (180 μg ml^{-1}) in PBS for 2 h at room temperature. After further washing as before, the preparations were mounted on glass slides in PBS under a coverslip, and immediately examined in a fluorescence microscope and photographed on Kodak Ektachrome 400 transparency film using an exposure for 120 s.

Immunogold localization

Third instar larvae of *C. bezziana* which had been handled as described above were dissected in PBS and the cardia removed from each. They were then immediately fixed in 4% paraformaldehyde (TAAB, Reading, Berkshire, U.K.) in PBS for 1 h at room temperature. The cardiae were then washed in PBS, dehydrated through an ethanol series (30%, 50%, 70% and 90%) and embedded in medium grade LR White resin (London Resin Co., Reading, Berkshire, U.K.) which was then polymerized overnight at 50°C in airtight gelatin capsules. Ultra-thin sections were cut longitudinally through embedded cardiae on an LKB Ultrotome Nova ultramicrotome and the sections were taken up on butvar-coated copper grids (ProSciTech, Thuringowa, Qld, Australia). Sections on grids were processed by transferring them between drops of PBS containing 0.1% Tween 20 and 0.5% (w/v) ovalbumin. The drops also contained, in succession, (a) 10% normal goat serum, (b) 100 μg/ml of either the rabbit anti-Cb42 or the control antibody preparation described above, and (c) a 1:100 dilution of a goat anti-rabbit immunoglobulin antibody conjugated to 10 nm diameter colloidal gold particles (British Biocell International, Cardiff, U.K.). Sections were washed 5 times on drops of diluting buffer after steps (b) and (c) and finally on distilled water. They were then stained successively in 2% aqueous uranyl acetate and 0.1 M lead citrate (5 min each) and examined and photographed in a JEOL 1010 transmission electron microscope.

RESULTS AND DISCUSSION

Immunofluorescent Antibody Localization

PM incubated successively in anti-Cb42 antibody and FITC-labeled secondary antibody showed a strong uniform fluorescence over its entire surface, indicating the widespread occurrence of native Cb42 in this structure (Figure 1a). Other structures and tissues, such as gut cells, cuticle and basal laminae, did not exhibit any similarly strong fluorescence after this treatment (data not shown). PM and other structures incubated in control antibody showed no significant fluorescence (Figure 1b), confirming the specificity of the localization. Cb42 appears therefore to be confined largely to the PM.

Immunogold localization

Sections of larval cardiae incubated in anti-Cb42 primary antibody showed moderately heavy colloidal

gold labeling after subsequent incubation with the goat anti-rabbit immunoglobulin conjugate. The labelling occurred largely throughout the developing PM itself (Figure 2a), in the cytoplasm of the PM-secreting midgut cells in the anterior half of the cardia and amongst the microvilli of these cells, adjacent to the PM. Sections incubated in control primary antibody in place of the anti-Cb42 showed only the expected light, apparently random labeling (Figure 2b). These results provide further evidence that Cb42 is an intrinsic component of PM in this species and indicate that, along with other PM components (PETERS, 1992), it is expressed in and exported from the PM-secreting cells of the anterior cardia. Within the PM, Cb42 appears to be distributed approximately evenly throughout the thickness of the membrane, from one surface to the other.

With the freezing and fixation regime used, preservation of the membranes and cytoplasm of the cardia cells was insufficient for details of the organelles and other structures implicated in the synthesis, transport and secretion of Cb42 to be resolved. However, some label was found in large vacuoles in the distal region of the PM forming cells, and it is possible that these are involved in the process of secreting the glycoprotein. In future work, the inclusion of a low concentration of glutaraldehyde (e.g. 0.1 - 0.5%) in the fixative may increase ultrastructural preservation to allow a more detailed study of these events, as has recently been achieved for another PM glycoprotein in *L. cuprina* (WIJFFELS *et al*., in preparation).

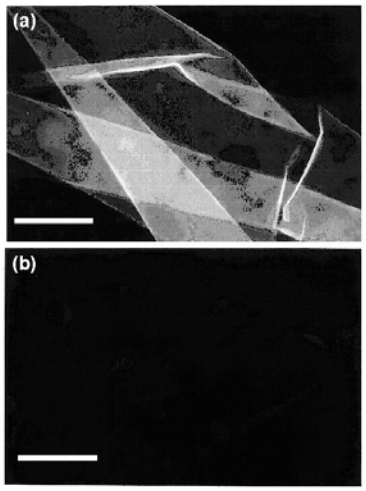

Figure 1. Localization of Cb42 on peritrophic membrane of third instar larvae of *C. bezziana* using the immunofluorescent antibody technique (IFA): (a) with rabbit anti-Cb42 antibodies, (b) with control antibodies. Scale: 50 μm

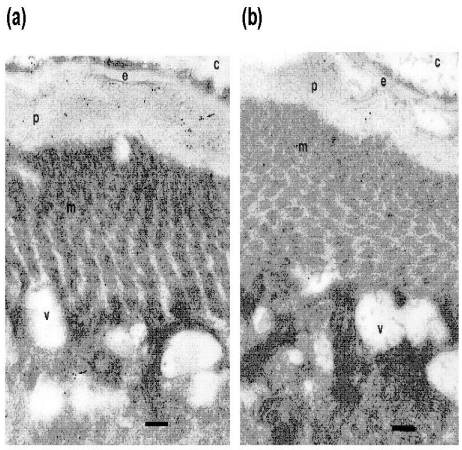

Figure 2. Localization of Cb42 in cardia of *C. bezziana* using the immunogold technique on ultrathin sections through the peritrophic membrane-forming zone: (a) with rabbit anti-Cb42 antibodies, (b) with control antibodies. Some of the colloidal gold particles are arrowed. p, developing peritrophic membrane; c, cuticle of cardia foregut cell; e, epicuticle of foregut cell; m, microvilli of PM forming cell; v, vacuole in PM forming cell. Scale: 200 nm

These results indicate that Cb42 is confined largely to the PM where, at least in part, it is exposed on the surface of the intact membrane and accessible to antibody molecules. Both IFA and immunogold labelling support this conclusion. Similar conclusions have been reached with regard to other proteins of PM origin in this species (VUOCOLO *et al*., in preparation; WIJFFELS *et al*., in preparation) and in *L. cuprina* (ELVIN *et al*., 1996; SCHORDERET *et al*., 1998). Like these other proteins, Cb42 may therefore be considered a potential molecular target in vaccinating host animals against screwworm larvae, as it is presumably also accessible *in vivo* to host antibodies ingested by feeding larvae. The results also demonstrate that the antibodies raised to a recombinant protein expressed in bacteria are able to react immunologiccally with the native protein in situ in the insect larvae.

CONCLUSION

In vaccination studies, the localization of antigens to the target tissue serves several purposes. It can support the appropriateness of the choice of protein as a potentially protective antigen or, in the case where the antigen is shown experimentally not to protect, it removes one possible explanation for that failure. If the antisera used for the immunolocalization are derived from a recombinant protein, then it also demonstrates that those recombinant proteins are able to elicit an immunological response that recognizes the native antigen in situ. This, again, is important knowledge when the consequences of vaccination are being examined.

ACKNOWLEDGEMENTS

Dra Sukarsih, of Balitvet, Bogor is thanked for providing larvae of *C. bezziana*. This work was undertaken with financial support from the Australian Centre for International Agricultural Research. Sri Muharsini holds a John Allbright Fellowship (ACIAR).

REFERENCES

CASU, R., C. EISEMANN, R. PEARSON, G. RIDING, I. EAST, A. DONALDSON, L. CADOGAN, and R. TELLAM. 1997. Antibody-mediated inhibition of the growth of larvae from an insect causing cutaneous myiasis in a mammalian host. Proc. Natl. Acad. Sci. U S A 94:8939–8944.

ELVIN, C., T. VUOCOLO, R. PEARSON, I. EAST, G. RIDING, C. EISEMANN, and R. TELLAM. 1996. Characterization of a major peritrophic membrane protein, peritrophin-44, from the larvae of Lucilia cuprina. *J. Biol. Chem*. 271:8925 – 8935.

PETERS, W. 1992. Peritrophic membranes. *In: Zoophysiology*. eds Bradshaw D., Burggren W., Heller H., Ishii S., Langer H., Neuweiler G. and Randall D. Vol. 130 pp. 1–238 Springer -Verlag, Berlin.

RIDING, G., S. MUHARSINI, R. PEARSON, SUKARSIH, E. SATRIA, G. WIJFFELS, and P. WILLADSEN. 2000. Fractionation, identification and vaccination efficacy of native antigens from the screwworm fly, *Chrysomya bezziana*. *J. Ilmu Ternak Vet.* (Edisi Khusus) 5(3): 150-159.

SCHORDERET, S., R. PEARSON, T. VUOCOLO, C. EISEMANN, G. RIDING, and R. TELLAM. 1998. cDNA and deduced amino acid sequences of a peritrophic membrane glycoprotein, `peritrophin-48', from the larvae of Lucilia cuprina. *Insect Biochem. Mol. Biol*. 28:99 – 111.

SUKARSIH, S. PARTOUTOMO, R. TOZER, E. SATRIA, G. WIJFFELS, and G. RIDING. 2000. Establishment and maintenance of colony of the Old World Screwworm fly *Chrysomya bezziana* at Balitvet in Bogor, West Java, Indonesia. *J. Ilmu Ternak Vet*. (Edisi Khusus) 5(3): 144-149.

VUOCOLO, T., C.H. EISEMANN, R.D. PEARSON, P. WILLADSEN, and R.L. TELLAM. 2000. Identification and molecular characterization of a peritrophin gene, peritrophin-48, from the myiasis fly *Chrysomya bezziana*. *Insect Biochem. Mol. Biol.* (Submitted).

WIJFFELS, G., T. VUOCOLO, S. MUHARSINI, and F. SUPRIYANTI. 2000. Bacterial expression of larvae peritrophins of *Chrysomya bezziana*. *J. Ilmu Ternak Vet.* (Edisi Khusus) 5(3): 170-176.