Calliphorin, a Protein Involved in the Cuticle Formation of the Blowfly, *Calliphora vicina*

Klaus Scheller, Hans-Peter Zimmermann, and Constantin E. Sekeris

Zoologisches Institut der Universität Würzburg, Deutsches Krebsforschungszentrum, Institut für Zellforschung, Heidelberg, and The National Hellenic Research Foundation, Athens, Greece

Z. Naturforsch. **35 c**, 387–389 (1980); received December 28, 1979/February 14, 1980

Calliphorin, Insect Cuticle, Immunofluorescence, *Calliphora vicina*

Injections of radioactively labelled calliphorin into IIIrd instar larvae of *Calliphora* indicate that the protein is incorporated into the integument. Immunofluorescence techniques show that the endocuticle is the final site of deposition of calliphorin. It is concluded that calliphorin is an essential protein of the tanned cuticle.

Introduction

At a normal insect ecdysis three processes are to be distinguished: 1) the splitting and shedding of the old cuticle 2) the expansion of the new cuticle and 3) the hardening and darkening of the new cuticle [1]. During the metamorphosis of dipteran insects, the soft and usually colourless larval cuticle is transformed into the hard and often darkened cuticle of the puparium, which is followed by the formation of the adult cuticle. The tanning and darkening agents are ultimately derived from the amino acid tyrosine. A key role in the formation of the sclerotized cuticle is played by N-acetyldopamine, which, presumably in its oxidized form, crosslinks the cuticular proteins in a quinone-tanning process [2]. But the characteristics of the protein(s) which is (are) tanned, have remained unknown.

In this study we describe experiments which show that the protein calliphorin is an element of the blowfly cuticle: Radioactively labelled calliphorin injected into larvae is incorporated only in epidermal tissue. Antibodies against calliphorin, coupled to FITC, show a specific fluorescence only in the endocuticle of pupae and adults.

Materials and Methods

Larvae were first washed with tap water and then injected through the anterior part of the body with 1.3 µg [J^{125}]calliphorin-solution. After the desired time the haemolymph was collected and the larvae dissected into the organ fractions. The individual fractions were washed and homogenized at 0 °C in a 0.02 M Na_2H-KH_2 phosphate buffer, pH 6.9, containing 0.02% phenylthiocarbamide, and then lyophilized. Aliquots of the homogenate were used to determine the protein content. The lyophilisate was solubilized and the radioactivity counted. All data were corrected for blank values taken at zero time.

Calliphorin and the antibodies were prepared as described in refs [3, 6]. The purified protein was iodinated after ref. [4].

Immunofluorescence: Pupae were fixed with 4% formaldehyde and 0.5% picric acid in 0.1 M cacodylate buffer, pH 7.2, at room temperature for 90 min. After washing twice in cacodylate buffer the pupae were postfixed with 4% formaldehyde, 0.5% picric acid and 0.25% glutaraldehyde in 0.2 M cacodylate buffer for 180 min. The pupae were dehydrated in graded mixtures of ethanol/methylpropanol, finished by absolute methylpropanol and embedded in dissolved paraplast. After removing the paraplast by treatment with xylol, sections mounted on cover slips were stepwise rehydrated and then treated with rabbit-anti-calliphorin antibodies in Sørensen phosphate buffer, pH 7.2, containing 0.15 mM sodium chloride, for 20 min at 22 °C. After washing five times with Sørensen buffer the sections were incubated with fluoresceine-isothiocyanate-labelled goat-anti-rabbit-γ-globulin (Behringwerke, Marburg) in Sørensen buffer. The sections were photographed on Kodak Tri-X-pan film with epifluorescent illumination and specific interference filters for fluorescein (Leitz microscope).

Results and Discussion

During the postembryonic development of dipteran insects a group of related proteins is synthe-

Requests for reprints and coloured slides should be sent to Dr. Klaus Scheller, Zoologisches Institut, Röntgenring 10, D-8700 Würzburg.

0341-0382/80/0500-0387 $ 01.00/0

sized in the fat body cells [3, 5] and released into the haemolymph. These proteins accumulate and comprise, shortly before pupariation, about half of the total larval salt-soluble proteins. In the blowfly, *Calliphora vicina,* a single protein has been identified, composed of 6–7 identical units of approx. 80000 D each, known as calliphorin [3]. This protein is immunochemically releated to drosophilin [7], lucilin [8] and manducin [9], respectively of *Drosophila, Lucilia* and *Manduca.* It has been demonstrated that the biosynthesis of calliphorin is restricted to 3–5 days old larvae. The translatable calliphorin-mRNA appears only in larvae, being absent in eggs, pupae and adults [10]. Although calliphorin is quantitatively the most prominent protein in developping blowflies, its physiological significance is still unknown. It is generally thought that calliphorin may be a storage protein which functions as a source of nutrients for adult proteins. It may also play a role in providing haemolymph with amino acids to sustain osmotic pressure. Considering the fact that 15% of the amino acid residues in the calliphorin molecule are tyrosine [3], one can suggest that this protein is involved in the process of cuticle formation. As shown by gel electrophoretic and chromatographic techniques, it is recognized that the cuticles of arthropods contain many different proteins.

Now we present biochemical and cytoimmunochemical evidence indicating that the protein calliphorin is involved in the formation of the cuticle of blowfly pupae and adults. In a first attempt to localize the site of function of calliphorin, 7 days old larvae were injected with radioactively labelled calliphorin and the radioactivity incorporated into various organs measured. As seen from Fig. 1, a high radioactivity is initially observed in the haemolymph. It drops markedly after injection, reaching low levels at 12–24 h. A similar curve of incorporation is seen also in the midgut, perhaps as a result of the high rate of absorption of this organ. The drop in radioactivity there being much more abrupt compared to the haemolymph. The fat bodies, and the Malpighian tubules show only background levels of radioactivity. In the integument, the initially low incorporation increases constantly with time, up to 24 h after injection. Radioactive calliphorin, therefore, is either deposited in the epidermis or is incorporated into the cuticle. It is known, that haemolymph proteins are capable of traversing the epidermis to be incorporated into the cuticle.

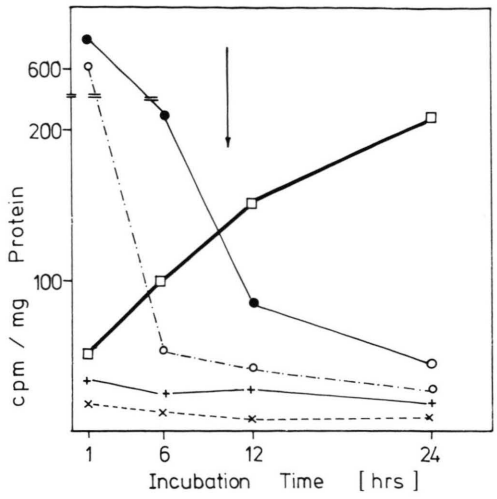

Fig. 1. Incorporation of radioactive calliphorin into: integument □—□; fat body +—+; haemolymph ●—●; midgut ○–·–·–○; Malpighian tubules x----x; of 7 days old larvae of *Calliphora vicina.* The arrow indicates the beginning of puparium formation. Each point represents 20 animals and triplicate readings.

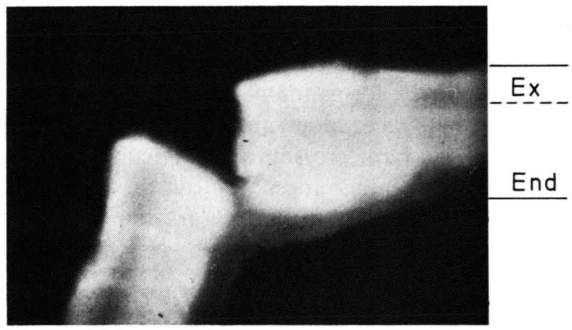

Fig. 2. Specific fluorescence of the binding sites of fluorescine isothiocyanate labelled calliphorin antibodies on a section through the thoracal cuticle of a newly formed adult *Calliphora vicina,* one day before hatching. The exocuticular region (Ex) shows a light (yellow) autofluorescence which can be exactly distinguished from the dark (green) specific FITC-fluorescence in the endocuticular region (End). (Coloured photographs available on request together with reprints.) Magnification 900 ×.

Other experiments with calliphorin antibodies and FITC labelled goat anti-rabbit-γ-globulin were designed to locate in addition to the site of the synthesis, the site of final deposition of calliphorin. In the fat bodies of larvae and of pupae up to 3 days after pupation, calliphorin-specific fluorescence can be detected. Sections of fat bodies of adult blowflies do not show specific fluorescence. These results are

in good agreement with the findings that the protein is synthesized in the fat body until the beginning of the breakdown of the fat body cells and the development of the pharate adult. Serial sections of white puparia, newly formed pupae and adults show specific FITC fluorescence localized in the endocuticular regions of the cuticle, but not in the exocuticle (Fig. 2). This specific fluorescence could not be observed if the sections were incubated with preimmune antibodies instead of calliphorin antibodies (results not shown).

The reason for the absence of specific fluorescence in the exocuticle could either be due to the absence of the protein in this part of the integument or results from modifications of the chemical structures of calliphorin by the tanning process so that antibodies are not longer able to bind.

All the structures investigated which show calliphorin specific fluorescence like cuticles, hairs, bristles, tracheae and compound eyes are of ectodermal origin.

The more detailed examination of the function of calliphorin is under investigation. There is evidence that the role of calliphorin is that of a matrix, into which the different cuticular proteins, which are involved in slerotization, fit.

Acknowledgements

We thank Dr. M. V. Govindan for labelling the calliphorin, Prof. Dietrich Bodenstein and Prof. Werner Emmert for helpful discussions. The Deutsche Forschungsgemeinschaft provided financial aid.

[1] G. Richards, Biochemistry of Insects (M. Rockstein, ed.), Acad. Press, 205–232 (1978).
[2] P. Karlson and C. E. Sekeris, Nature **195,** 183–184 (1962).
[3] E. A. Munn and G. D. Greville, J. Insect Physiol. **15,** 1935–1950 (1969).
[4] A. E. Boulton and W. M. Hunter, Biochem. J. **133,** 529–539 (1973).
[5] E. A. Munn, A. Feinstein, and D. Greville, Biochem. J. **124,** 367–374 (1971).
[6] C. E. Sekeris, R. Perassi, J. Arnemann, A. Ullrich, and K. Scheller, Insect. Biochem. **7,** 5–9 (1977).
[7] D. B. Roberts, J. Wolfe, and M. E. Akam, J. Insect Physiol. **23,** 871–878 (1977).
[8] J. A. Thomson, K. R. Radok, D. C. Shaw, M. J. Whitten, G. C. Forster, and L. M. Birt, Biochem. Genet. **14,** 145–160 (1976).
[9] L. M. Riddiford and K. Scheller, in preparation.
[10] C. E. Sekeris and K. Scheller, Developmental Biology **59,** 12–23 (1977).