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Chitin in the epidermal cuticle of a vertebrate (*Paralipophrys trigloides*, Blenniidae, Teleostei)

G. P. Wagner[a]*, J. Lo[b], R. Laine[b] and M. Almeder[c]

[a]*Department of Biology, Yale University, P.O. Box 6666, New Haven (Connecticut 06511, USA)* [b]*Department of Biochemistry, Louisiana State University, Baton Rouge (Louisiana 70803-1806, USA),* [c]*Department of Zoology, University of Vienna, Althanstraße 14, A-1090 Vienna (Austria)*
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Abstract. Lectin binding, endo-chitinase binding and enzymatic degradation studies show that the epidermal cuticle of the bony fish *Paralipophrys trigloides* (Blenniidae) is chitinous. This is the first evidence that a vertebrate species possesses a chitinous tissue. Recently a *Xenopus* gene has been identified which has significant sequence similarity to the catalytic domain of yeast chitin synthase III, a chitin producing enzyme[1,2]. Taken together these two findings imply that chitin synthesis capability may be a basic vertebrate feature.
Key words. Chitin; cuticle; evolution; vertebrates; bony fish; Blenniidae; *Paralipophrys trigoides.*

Chitin is the β 1,4 glycosidic polysaccharide of the amino sugar N-acetyl glucosamine[3]. While chitin is widely found in the cuticles of acoelomatic and protostome animals, it is unknown from vertebrates[4,5]. It has been long known that some teleost fishes produce a fibrous cuticle on parts of their body surface[6-8]. The best examined example of a fish cuticle is the surface secretion on the pectoral fins of blennies[9]. Here we report histochemical and biochemical evidence for the presence of chitin in the blenny cuticle.

The conclusion that chitin is present in the blenny cuticle is based on lectin binding, the resistance of the lectin binding structures to hydrolysis and proteolysis, endo-chitinase binding to the cuticle (see table), and enzymatic degradation by chitinase.

We found that FITC conjugated wheat germ agglutinin (WGA, Sigma L 4895) bound strongly at the cuticle and the distal cytoplasmatic segments of the superficial epithelia cells (SEC) fig., B). These cells are known to produce the cuticle[6,7]. The WGA binding sites in the cuticle and the SEC's underlying the cuticle are resistant to trypsin treatment and prolonged alkali treatment (0.2 n NaOH for two hours at 37 °C; fig., C and D). WGA binding has been shown to be specific, since it can be completely suppressed by incubating WGA with N, N′, N″ tri-acetylchitotriose (Sigma T 2144, TACT), which blocks the binding sites of the lectin. The same results where obtained with *Phytolacca americana* mitogen (poke wheat mitogen, PWM). These results led to the hypothesis that the cuticle contains chitin. This hypothesis was tested in two ways: 1) by a biochemical assay for chitin[10] and 2) histochemical localization of endo-chitinase binding sites on paraffin sections of blenny pectoral fins.

For the biochemical assay complete fin hooks were hydrolyzed with NaOH and the pellet treated with *Serratia marcescens* chitinase[10]. In the liquid phase the presence of soluble amino sugars was detected[11]. No reaction was obtained from fin parts without cuticle or from control experiments without the chitinase.

Cloned chitinase from *Vibrio parahaemolyticus* was used to study chitinase binding to the cuticle of *Paralipophrys trigloides*. On paraffin sections of pectoral fin hooks, indirect enzyme linked immunoassay using anti-chitinase antibody and direct enzyme linked assay with

Table. Summary of histochemical results showing the presence of chitin in the cuticle of *Paralipophrys trigloides*. All reactions where done on 5 µ paraffin sections of pectoral fin hooks. The lectin staining was done according to the one step method described by Leathem and Atkins[16]

Method	Cut	SEC	BM	LC-matrix	LC-fibers
WGA	+	+	−	+	−
Tr., WGA	+	+	−	−	+
Tr., NaOH, WGA	+	+	−	−	−
Tr., WGA + TACT	−	−	−	−	−
PWM	+	+	+	−	−
Chitinase	+	+	−	−	−
Tr., Chitinase	+	+	−	−	−

Legend: *BM*: basal membrane of epidermis; *Chitinase*: chitinase (E.C. 3.2.1.14) cloned from *Vibrio parahaemolyticus* and expressed in *E. coli.* was used for indirect enzyme linked immunoassay using anti-chitinase antibody and direct enzyme linked assay with FITC-chitinase conjugate; *SEC*: superficial epidermal cells underlying the cuticle and producing the cuticular matrix; *Cut*: cuticle, extra cellular material on the epidermis of fin hooks[4,5]. *LC*: lepidotrichal chord, a connective tissue pad underlying the cuticular epidermis of the pectoral fin[15]; NaOH: pre-treatment of sections with 0.2 n NaOH for up to two hours at 37 °C; *PWM*: *Phytolacca americana* mitogen (pokeweed mitogen, binds to chitin oligosaccharides and N-linked glycosyl units); *TACT*: preincubation of lectin with N, N′, N″ tri-acetylchitotriose, which blocks the binding sites of the lectin; *Tr.*: pre-treatment of sections with trypsin for 15′ at 37 °C; *WGA*: *Triticum vulgare* agglutinin (wheat germ agglutinin, binds strongly to chitin oligosaccharides and Manβ1,4G1cNacβ1,4GLcNacβ,N-Asn).

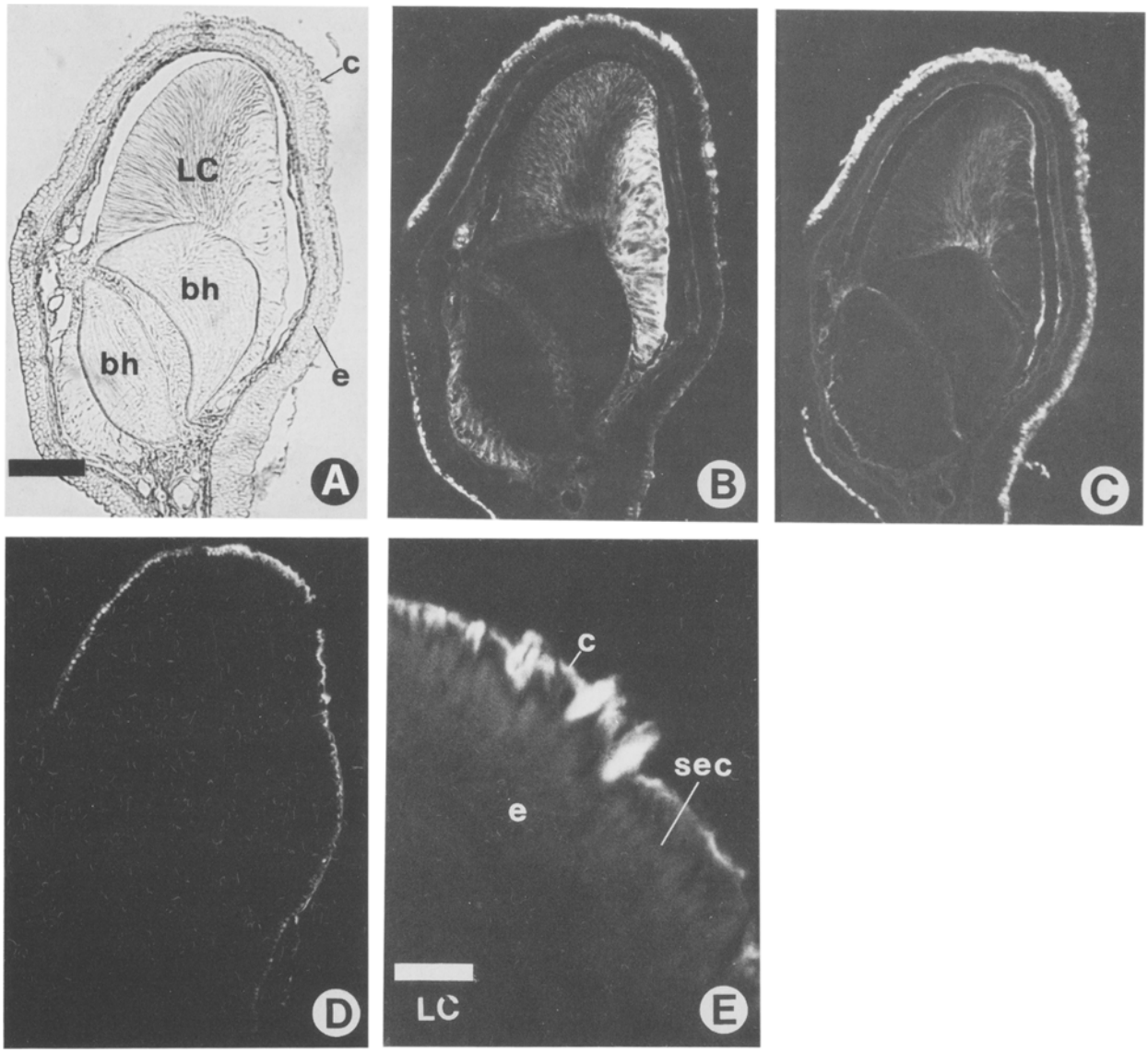

Figure. Sections through a pectoral fin hook of *Paralipophrys trigloides*. *A* Bright field micrograph of an unstained section through a fin hook; bh: bony halve ray of the fin ray, c: cuticle, e: epidermis, LC: lepidotrichal chord, which is a connective tissue pad that is associated with the bony fin ray[15] (bar = 1 mm). *B* epi-fluorescence image of a section stained with FITC conjugated WGA. Note staining in the cuticle and LC. *C* same as *B* but WGA staining was done after treatment with 1% trypsin for 15'. Compare with *B* and note that staining in the LC is largely suppressed by trypsin digestion, indicating that the WGA binding sites in the LC are bound to protein. WGA binding to the cuticle and gets stronger. *D* same as *B* but with combined trypsin and alkali treatment prior to WGA staining. Note that under these conditions WGA binds only to the cuticle and the underlying epidermal cells. Glycoproteins are hydrolyzed under this condition, indicating that the WGA binding sites in the cuticle are not N-linked glycosyl units of glycoproteins. *E* cuticular epidermis at high magnification stained with FITC conjugated endochitinase (bar = 0.25 mm).

FITC-chitinase conjugate indicated that chitinase bound to the cuticle (fig., E). Trypsin pre-treatment of the paraffin sections did not alter the chitinase binding phenomenon which is in agreement with lectin binding results.

Based on the above results we conclude that the cuticle of *Paralipophrys trigloides* is chitinous. We exclude the possibility of a fungal contamination because of the absence of any fungal morphology in the cuticle, and alkali resistant WGA binding sites in the apical protoplasm of the SEC's underlying the cuticle. Given that the cuticle is chitinous we infer that this species possesses a chitin synthase gene. In this context it is interesting to note that a vertebrate gene has been identified, that shows significant sequence similarity to the yeast chitin synthase gene CSD2, the gene DG42 of *Xenopus leavis*[1,2]. Heretofore it has been assumed that the chitin synthase gene was lost prior to the evolution of vertebrates[4,5]. In fact, evidence for the presence of chitin and chitin sulfate in lower chordates, like ascidians[12–14], and lancelets[15], imply that the vertebrates inherited their chitin synthesis capability from their ancestors. The implications for the

development of anti-fungal drugs directed against chitin synthase will have to be reconsidered.

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* To whom correspondence should be addressed.

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