

Axillary gland secretions contribute to the stress-induced discharge of a bactericidal substance in *Corydoras sterbai* (Callichthyidae, Siluriformes)

Sekrete der Axillardrüsen von *Corydoras sterbai* (Callichthyidae, Siluriformes) sind Bestandteil einer bei Stress abgegebenen bakteriziden Substanz

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Zusammenfassung: Das von mehreren *Corydoras sterbai*-Individuen während eines Transports ins Wasser abgegebene Sekret wirkt hemmend auf die Lichtemission von *Vibrio fischeri*. In einer SDS-PAGE dieser Sekrete und der isolierten Axillardrüse finden sich übereinstimmende Banden mit einer molekularen Masse von etwa 72,000; 26,000; und 21,700 D. Dies zeigt, dass die Welse bei Beunruhigung auch Axillardrüsensekret ins Wasser abgeben. In welchem Masse dieses an der nachgewiesenen Bakterizidie beteiligt ist, bleibt zu untersuchen.

Schlüsselwörter: Axillardrüsen, Callichthyidae, *Corydoras*, bakterizide Eigenschaften

Summary: Secretions discharged in the water during transport by some individuals of *Corydoras sterbai* reduce light emission of the bacterium *Vibrio fischeri*. SDS-PAGE of these secretions and of the isolated axillary-gland shows three identical lanes with molecular masses of approx. 71 000, 26 000 and 21 700 D. This suggests that during stress also secretions of the axillary glands were exuded into the water. The contribution of axillary gland secretions to the bactericidal properties remains to be shown.

Key words: axillary glands, Callichthyidae, *Corydoras*, bakterizidial properties

1. Introduction

Corydoras spp. under stress, e.g. during transport or after catching in the wild or in the aquarium, obviously discharge substances that are harmful to conspecifics and other fishes; this was repeatedly described by aquarists and tradesmen (e.g. Evers 2002, Schäfer 2003). In addition, severe pain is reported by people when injured by pectoral spines of *Corydoras* spp. Generally, in Siluriformes toxic secretions arise (1) from unicellular glands in the epidermis called club cells (see Whitear and

Mittal 1983; unpublished observations), (2) from gland cell aggregations surrounding the first pectoral fin ray and/or (3) from axillary glands that occur in some taxa. Toxic properties of the epidermal glands of catfish have been thoroughly studied, but information of axillary glands is poor (for review see Perrière and Goudey-Perrière 2003).

Recently we described the presence of large axillary glands in the catfish *Corydoras aeneus* (Callichthyidae) (Greven et al. 2006). In the present note we give some evidence that secretions from these glands in the re-

lated *C. sterbai* contribute to the substances discharged in the water under stress.

2. Material and methods

Six specimens of *Corydoras sterbai* (fig. 1) were bought from a commercial dealer. During transport in a plastic bag (2.5 l), they

discharged substances that make the water cloudy.

A 250 ml sample of this water was frozen in liquid nitrogen and freeze-dried. The resulting “raw-toxin” and for comparison axillary glands excised from one catfish were homogenized in distilled water. Protein concentration of the raw toxin was



Fig. 1: *Corydoras sterbai*.

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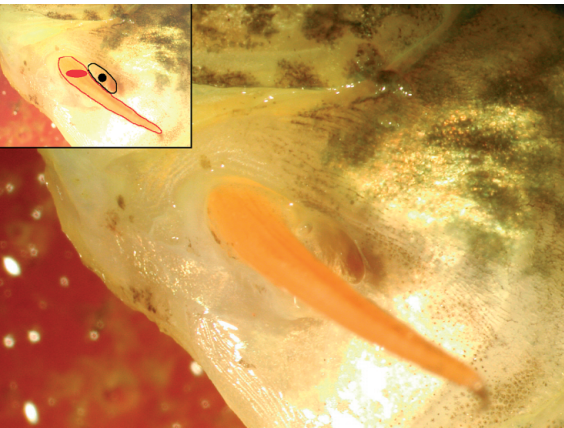


Fig. 2: Opening of the axillary gland (circle) immediately near the first pectoral spine (ellipse).

Abb. 2: Öffnung der Axillardrüse (Kreis) nahe des ersten Brustflossenstrahls (Ellipse).

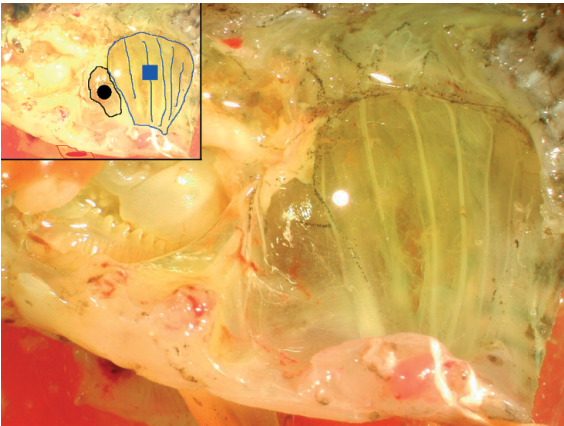


Fig. 3: Exposed axillary gland. Body wall (square); axillary gland (circle).

Abb. 3: Freigelegte Axillardrüse. Körperwand (Viereck); Axillardrüse (Kreis).

estimated by the method of Bradford (1976).

SDS-PAGE of the raw toxin, axillary glands and muscles was performed according to Laemmli (1970). Proteins were stained with Coomassie brilliant blue (Heukeshoven and Dernick 1988).

Toxicity was tested in a Lumistox 300 (Dr. Bruno Lange GmbH) according to DIN/EN/ISO 11348-3 using the luminescent bacterium *Vibrio fischeri*. Toxicity of a substance in this assay is indicated by a concentration dependent decrease of light emission of the bacteria. Freeze dried raw toxin was solved in distilled water and a dilution series (36.3; 48.3; 72.5; 96.7; 145.0; 193.3; 290.0; 386.7, and 580.0 µg/ml) were incubated with *V. fischeri*. The light emitted by the bacteria was measured after 6, 15 and 30 minutes and compared to the light emission of a toxin-free control.

3. Results and discussion

Corydoras sterbai has an axillary gland on each side, which opens immediately below the first, strongly ossified pectoral fin ray (fig. 2) and which can easily be exposed (fig. 3). The position of this gland corresponds to that in *C. aeneus* (Greven et al. 2006) and other Siluriformes (summarized in Perrière and Goudey-Perrière 2003).

Freeze drying of 250 ml water containing the secreted substances resulted in 73.5 mg raw toxin with a protein concentration of 73.6 µg/mg. The SDS-PAGE of entire glands showed various lanes. Comigrating bands in muscle and gland sample indicate a contamination of muscle tissue in the sample of the gland. But some bands found exclusively in the gland sample were seen also in the raw toxin sample (fig. 4).

SDS PAGE showed at least three proteins with

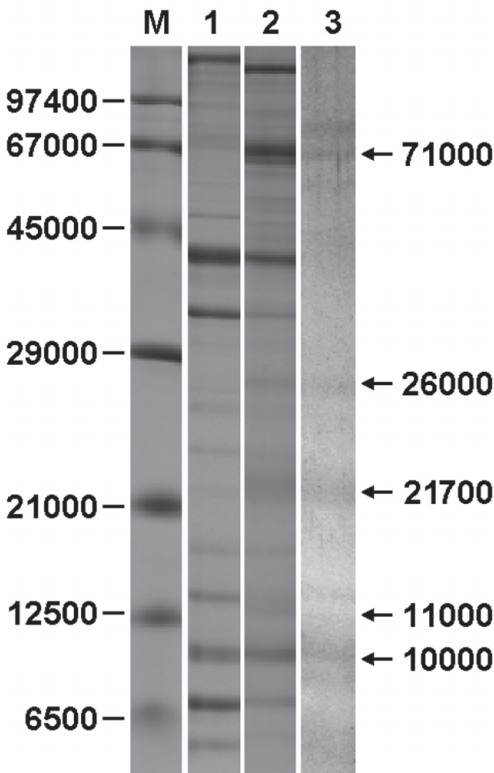


Fig. 4: SDS-PAGE patterns: M molecular mass markers; 1 homogenate of a muscle; 2 homogenate of an axillary gland; 3 freeze dried raw toxin (104 µg raw toxin, ca. 7.7 µg protein); numerals indicate the molecular mass of the corresponding proteins, the arrows mark proteins comigrating in samples of gland and raw toxin.

Abb. 4: SDS-PAGE Muster: M Marker für die molekulare Masse; 1 Muskelhomogenat; Homogenat der Axillardrüse; 3 gefriergetrocknetes Roh toxin (104 µg Roh toxin, ca. 7,7 µg Protein); die Zahlen geben die molekulare Masse der entsprechenden Proteine an; die Pfeile markieren die übereinstimmenden Proteine in Axillardrüse und Roh toxin.

molecular masses of approximately 71,000; 26,000 und 21,700 D as components of both, the axillary gland and the water, in which the catfishes have discharged substances. Therefore, the axillary glands contribute to the secretions exuded into the water.

The EC50 (effective concentration), i.e. the concentration of a toxic substance, where the light emission is diminished to 50% compared to a toxin-free control, was 515 µg/ml raw toxin at 30 min (fig. 5).

In the catfish *Arius thalassanus* (Ariidae) the secretion of the axillary gland is largely proteinaceous in nature and seems to be less toxic than the gel-like epidermal secretion (Al-Hassan et al. 1987). The relative high protein content of the axillary gland of *C. sterbai* and high amounts of rough endoplasmic reticulum in the gland of *C. aeneus* is evidence for a significant protein component in axillary gland secretions (Greven et al. 2006).

The substances discharged in the water are toxic for the bacterium *Vibrio fischeri*. To our knowledge antimicrobial activity has been unequivocally documented only by epidermal gel-like secretions as yet, discharged after environmental stress at relative high concentrations, e.g. in *Arius thalassinus*, (Al-Hassan et al. 1987), but also in *Ictalurus punctatus* (Ictaluriidae) (Robinette et al. 1998) and which surely are discharged also by *Corydoras* spp. under stress. Currently, our findings show only that axillary secretions are components of the stress-induced discharge of integumental substances, but they do not prove that they are really bactericidal, which property, however, is assumed.

Pharmacological properties of skin secretions and venoms from spine glands in catfishes other than Callichthyidae are well documented (for review see Perrière and Goudey-Perrière 2003) However, their sig-

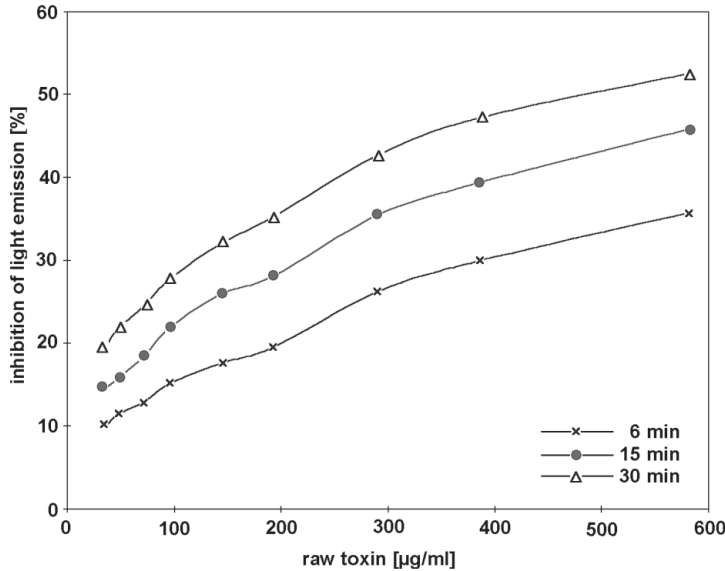


Fig. 5: Luminescent bacteria assay with *Vibrio fischeri* using a dilution series of freeze dried raw toxin. The light emitted by the bacteria was measured after 6, 15 and 30 minutes, compared to the light emission of a toxin-free control, and expressed as percentage of light emission decrease.

Abb. 5: Leuchtbakterientest mit *Vibrio fischeri* anhand einer Verdünnungsreihe von gefriergetrocknetem Rohtoxin. Das von den Bakterien emittierte Licht wurde nach 5, 15 und 30 min gemessen, mit der Lichtemission einer Toxin freien Kontrolle verglichen und als prozentuale Abnahme der Lichtemission dargestellt.

nificance for the fish itself remains to be investigated. Severe pain reported by stung people indicates a role in defence. At least epidermal secretions of *A. thalassinus* were supposed to protect against injuries (due to the enhancement of wound healing) caused by predators or against infestation (Al-Hasan et al. 1987).

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