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Missing parvalbumin: Implications in diagnostic testing for tuna allergy

To the Editor:

Parvalbumin is a 12-kd protein found in abundance in the white muscle of lower vertebrates. It is considered a major fish panallergen.¹ However, there have been conflicting reports regarding parvalbumin in tuna. Two previous studies reported the absence of parvalbumin in this fish.^{2,3} This was also our experience with our initial studies on tuna. However, others have reported the presence of parvalbumin in tuna, and it was also found to be allergenic.⁴ This study set out to evaluate the reasons for these inconsistent findings.

A fresh whole tuna, *Thunnus tonggol* (Bleeker) of about 400-mm fork length (FL) was skinned and cut transversely into sections along the length, beginning about 20 mm posterior to the edge of the operculum, with each section

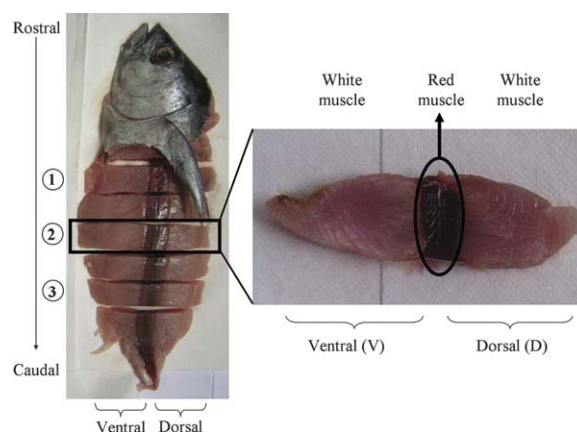


FIG 1. Dissection of *Thunnus tonggol* (Bleeker) for evaluation of parvalbumin. The fish was skinned and torso sectioned transversely into 6 portions (left). Three of these sections, the (1) rostral, (2) mid, and (3) caudal portions, were further divided into 3 parts, dorsal (D) and ventral (V) white muscle and middle red meat (right).

measuring about 25 mm in thickness (Fig 1). Three sections (at about 0.4 FL, 0.6 FL, and 0.8 FL) were chosen for analysis. For each section, the ventral and dorsal portions of the white muscle were separated from the red muscle (Fig 1), and extracts made in PBS (pH 7.4) containing 1 mmol/L phenylmethylsulfonylfluoride. Each extract (5 μ g) was separated in 8% TRIS-tricine gel and electroblotted onto Hybond-C Extra nitrocellulose membranes (Amersham, Uppsala, Sweden). Purified Gad c 1 (cod parvalbumin; kind gift from Pharmacia Diagnostics, Uppsala, Sweden) was used as a positive control. The membranes were blocked with 5% skimmed milk powder in 0.05% PBS-Tween 20 for 1 hour at room temperature

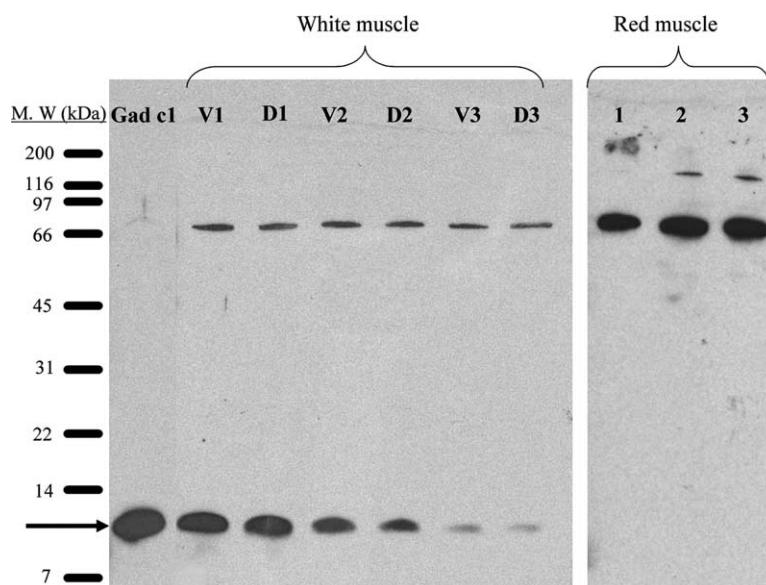


FIG 2. Immunoblot detection of parvalbumin in extracts of *Thunnus tonggol* (Bleeker). Left panel lanes, Gad c 1 (cod parvalbumin), ventral (V), and dorsal (D) white meat portions of sections 1, 2, and 3 (see Fig 1). Right panel lanes, Red meat portions of sections 1, 2, and 3. The 12-kd specific parvalbumin band is shown with an arrow. M. W., Molecular weight.

and incubated with mouse monoclonal antiparvalbumin antibody clone PARV-19 (1:500; Sigma-Aldrich, St Louis, Mo) overnight at 4°C, followed by biotin-labeled goat antimouse IgG (1:10,000 dilution; Sigma-Aldrich), and then ExtrAvidin peroxidase conjugate (1:5000 dilution; Sigma-Aldrich), each for 1 hour at room temperature. The bands were visualized by the addition of SuperSignal WestPico Chemiluminescent Substrate (Pierce, Rockford, Ill) for 5 minutes and exposure with Kodak Biomax MS film (Rochester, NY). Membranes were washed between incubations with 0.05% PBS-Tween 20, which was also used as the diluent for the antibodies.

Parvalbumin was found in the white muscle of tuna and was absent in the red (Fig 2). It was also unequally distributed, with ventral white muscle containing slightly more parvalbumin than the dorsal white muscle. These findings were replicated in similar experiments on 2 other fish of the same species. The amount of parvalbumin decreased from the rostral region to the caudal region. This observation has also been reported for cod.⁵ The bands seen at 66 and 116 kd (for red meat; Fig 2) were non-specific, because these were also present when a mouse antitrinitrophenol mAb (BD Biosciences Pharmingen, San Jose, Calif) was used as a negative control antibody (data not shown).

The findings of this study provide an explanation for the inconsistencies in the earlier reports of parvalbumin in tuna. It is likely that different parts of the fish were sampled, and those reporting absence of parvalbumin were likely to have used a substantial portion of the red muscle. This observation is likely to be unique to fish with large portions of red muscle in sections of its torso.

Although white meat of tuna is favored when eaten raw (as in sashimi), both white and red meat of the fish are consumed in the cooked form. Therefore, these findings have significant implications when preparing tuna fish diagnostic allergen extracts from raw material. The white muscle at the ventral portion of the rostral end of the fish would be the most suitable for studies on parvalbumin of this fish.

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