SHORT COMMUNICATION
HIGH CONCENTRATIONS OF ZINC IN THE FANGS AND MANGANESE IN THE TEETH OF SPIDERS

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Various organisms have recently been shown to contain significant amounts of zinc and manganese in mechanical structures such as mandibular teeth. These accumulations are different from those in the bones and teeth of vertebrates and the shells and teeth of some mollusks in that the elements are both less common and are not obviously incorporated in biominerals. High concentrations of zinc (about 1% of dry mass) were reported in the jaws of polychaete worms by Bryan & Gibbs (1979), using wet-ash analysis. These authors suggested that the zinc influences the mechanical properties of the jaw in a fashion analogous to the role of biominerals in the teeth of many organisms. Hillerton & Vincent (1982) detected zinc and manganese in the cutting edge of the mandibles of herbivorous insects from several orders using an electron microprobe. They determined the zinc concentration, averaged over the whole mandible, with wet chemical analysis, finding about 0.5% in a locust species. Hillerton et al. (1984) found either manganese or zinc in large quantities in the mandibles of separate species of beetles. Hillerton & Vincent (1982) went on to suggest that zinc may increase the density and fracture toughness of the mandibular teeth by increasing the number of secondary bonds in the cuticle. The hardening hypothesis has been accepted by Bone et al. (1983), who studied zinc in chaetognath teeth and spines, and by Perry et al. (1988), who studied zinc in copepod mandibles.

We have recently surveyed such accumulations with an MeV-ion microprobe technique that yields the approximate element concentration averaged over specimen thickness. Because this technique is not confined to surface measurements, unlike techniques that use electrons, it is useful for surveying unsectioned specimens. It can be used, with decreasing accuracy, on samples whose areal (i.e. projected) density reaches up to 20 mg cm$^{-2}$. We have described the use of the ion microprobe for measuring concentrations of minor elements in biological specimens, and have reported the concentration of zinc (about 4%) and manganese (about 0.4%) in the mandibular teeth of ants (Lefevre et al. 1987; Schofield et al. 1988). In the course of these surveys, we have found that a variety of spiders contain surprisingly high concentrations of zinc in a thin surface layer on their fangs. Their cheliceral teeth and their pedipalp and leg claws, in contrast, are rich

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Fig. 1. STIM and PIXE images showing the large fang and smaller marginal teeth on the chelicera of a garden spider. (A) STIM image in which lighter shades indicate larger areal densities. (B) PIXE image, showing that zinc X-rays originate on the fang. (C) PIXE image, showing the same region as B in manganese X-rays. Manganese appears to be concentrated on the tips of the marginal teeth. Scale bar, 100 μm.

in manganese but do not contain zinc. We are unaware of other reported cases of organisms with separate, but similar, mechanical structures that are highly enriched with different cations.

Fig. 1A is a scanning transmission ion microscopy (STIM) image obtained from measured energy losses of 4 MeV protons (Lefevre et al. 1987). It shows the fang and the smaller marginal teeth on the chelicera of a garden spider Araneus diadematus (Eisenbeis & Wichard, 1987). Lighter shading indicates larger proton energy losses and, therefore, larger areal density. The areas which were opaque to 4 MeV protons appear speckled, as only noise was recorded.

Fig. 1B was made using proton-induced X-ray emission (PIXE) and shows the yield of zinc X-rays from the same field as Fig. 1A. Fig. 1C is also a PIXE image of this region, but in manganese X-rays. Lighter shades in the PIXE images correspond to greater X-ray yields and thus greater quantities of these elements. The data show that zinc is found only in the fang, and that manganese is found mainly near the tips of the marginal teeth.

Approximate element concentrations can be derived from the data used to construct the PIXE and STIM images (Lefevre et al. 1987; Schofield et al. 1988). The areal density of an element for each point in the image is obtained from the X-ray data and the total areal density for each point is calculated from the proton energy loss data. We estimated that errors introduced by the approximations used in these calculations together with systematic errors amounted to about 30% of
Fig. 2. The concentrations of zinc and manganese in the region shown in Fig. 1. (A) A rise of one vertical division indicates a dry mass concentration of 5% zinc. (B) One vertical division indicates 1% manganese. These concentrations were calculated from the proton-energy-loss and X-ray yield data used, respectively, for Fig. 1A, B and C.

The reported concentration values. For lower X-ray energies and thicker specimens the errors would be larger.

Fig. 2A shows the approximate concentration of zinc obtained from the STIM and PIXE data. Zinc concentration is proportional to the vertical displacement of each plotted line (5% dry mass per division). Fig. 2B shows the concentration of manganese (1% dry mass per division). The approximate concentrations reported here are average values in cylinders about 15 μm in diameter (the diameter of the PIXE beam) along the beam path. For this reason, the lower three marginal teeth, which are clearly seen in the X-ray image (Fig. 1C), are barely visible in the concentration image (Fig. 2B), because they are seen through the manganese-poor chelicera which dilutes the local concentration. Furthermore, features smaller than 15 μm in diameter will also be diluted. Even so, the 15% concentrations of zinc seen in Fig. 2A are comparable with those that have been found in the choroid of some vertebrate eyes – the highest zinc concentrations that have been reported in normal living tissue (Underwood, 1977).

Fig. 3 shows X-ray spectra obtained at points on the chelicera away from the fang and teeth (Fig. 3A), on the fang (Fig. 3B), and on a marginal tooth (Fig. 3C). These spectra are approximately dose- and mass-normalized and can, therefore, be compared. The X-ray spectrum from the fang exhibits more chlorine and zinc than the chelicera spectrum; the spectrum from the marginal tooth shows relatively more calcium and manganese.

A claw on a leg of this spider and one on a pedipalp were each found to contain manganese at about the same concentration as that observed in the marginal teeth. Like the marginal teeth, they do not contain zinc.
Fig. 3. X-ray spectra obtained from points on the spider chelicera of Fig. 1. The vertical scales have been adjusted for proton dose and for sampled mass (a function of X-ray energy) so that the spectra can be qualitatively compared. (A) On the chelicera away from the teeth and fang; (B) on the fang; (C) on a marginal tooth.

The generality of these zinc and manganese features was investigated by examining several other spiders. A 'tarantula', *Aphonopelma* sp., exhibited a concentration pattern in the fang similar to that in the garden spider, with the exception that the zinc was localized only on the distal one-fifth and on the serrated edges of the fang.

Preliminary surveys of *Alopecosa kochi*, *Araniella displicata*, *Araneus patagiatus* and *Schizocosa minnesotensis* all showed element distribution patterns similar to those of the garden spider. However, concentration measurements were not made on these species. *Achaearanea tepidariorum*, which does not have marginal teeth, did have manganese in its claws.

A transverse section of the tip of the tarantula fang showed that zinc was distributed in a thin layer near the fang surface and that low concentrations of manganese were found in a thicker band beneath the zinc layer. The concentration of zinc near the surface of the fang was about 8%. The concentration pattern shown in Fig. 2A suggests that zinc is also distributed in a thin surface layer on the fang of the garden spider.

The possibility that these zinc and manganese accumulations might be in the form of biologically deposited minerals was investigated using X-ray diffractometry. Neither powder diffraction rings nor discrete reflections were observed from tooth and fang samples. However, owing to the very small sample size, this
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Evidence against crystalline biomineralization must be considered inconclusive. Also, electrode measurements with a sensitivity of 1 mV yielded no electrochemically generated potential difference between the fang and teeth of a live spider.

The presence of these two distinct patterns of high zinc and manganese enrichment in such close proximity causes us to question whether they each simply harden the cuticle. Considering the nature of the structures containing them, it seems likely that these accumulations are associated with the mechanical properties of the cuticle. However, it may be that each enrichment pattern is associated with different mechanical properties in the cuticle, such as hardness, rigidity or resistance to fracture. The manganese-enriched claws and marginal teeth may differ in mechanical properties from the zinc-rich fangs in a manner analogous to the difference between the claws and teeth of mammals. Whatever the properties of the enriched cuticle, the segregation pattern of zinc and manganese makes it unlikely that they both perform the same function.

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References


