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# Cadmium-Binding Proteins in the Scallop *Pecten maximus*

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**Scallops, *Pecten maximus*, accumulate cadmium naturally in the digestive gland to a level of approximately 100 ppm wet weight. Of this cadmium, 60% was soluble and was composed of three weight classes as judged by Sephadex G-100 chromatography. Of the soluble cadmium, 60% was in the 55,000 molecular weight range and 20% each in an excluded fraction and a 10,000 molecular weight fraction.**

**The 55,000 molecular weight fraction, after further purification, showed a maximum cadmium concentration of 1.4% by weight. The cadmium was thiolate bound but not as strongly bound as in the case of metallothionein.**

**The 10,000 molecular weight fraction was a metallothionein-like protein.**

## Introduction

The scallop, *Pecten maximus*, was probably the first living organism in which cadmium was detected. Fox and Ramage (*1*), using a spectrographic method, reported the astonishing values of 500 to 2000 ppm dry weight of cadmium in the digestive gland. Several other studies have confirmed that high levels of cadmium are indeed present in the digestive gland of scallops which were not apparently associated with any metal pollution (*2–4*). Although Pecten is caught commercially for human consumption, it probably does not represent a potential health hazard since the large adductor muscle and the gonad (and possibly the kidney which lies between these) are the only parts generally eaten. Cadmium levels in the muscle and gonad are low. In the kidney a value of about 20 ppm wet weight has been reported by Bryan (*4*); however, the organ is small and cannot represent a significant cadmium source.

There appears to have been no report to date on the identification of the cadmium-binding component(s) in the scallop digestive gland. In this paper we present in outline our progress in characterizing the components involved.

## Materials and Methods

### Animals

Scallops, *Pecten maximus*, were purchased from the Marine Biological Station, Millport, Isle of Cumbrae, Scotland, and were from the Clyde sea area.

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### Homogenization and Chromatography

Each 10 g of tissue was homogenized with 20 mL of 0.1 M Tris HCl, pH 8.0, containing 10 mM PMSF (phenylmethanesulfonyl fluoride) and centrifuged at 100,000gfor 1 hr. The supernatant (cytosol) was used for further purification. Chromatographic separations were carried out at 4°C using the conditions given in the legends to the figures.

### Amino Acid Analysis

Samples were oxidized before analysis with performic acid (*5*).

### Metal Analysis

Tissue was analyzed after wet ashing and chromatography fractions were determined by direct aspiration (*6*).

### Protein and Carbohydrate Analysis

Protein was determined by the method of Sedmak and Grossberg (*7*) and total carbohydrate by the method of Dubois et al. (*8*) using galactose as standard.

## Results and Discussion

The scallops were found to contain approximately 100 ppm (wet weight) of cadmium in the digestive gland, a level which showed little individual or seasonal variation. The tissue also contained relatively high concentrations of zinc [218 ppm wet weight ± 106 (SD)] and copper [71 ppm wet weight ± 17 (SD)].

Figure 1 is a Sephadex G-100 chromatogram of the cytosol. It can be seen that cadmium is present in three weight classes: molecular weights > 150,000 (i.e., above

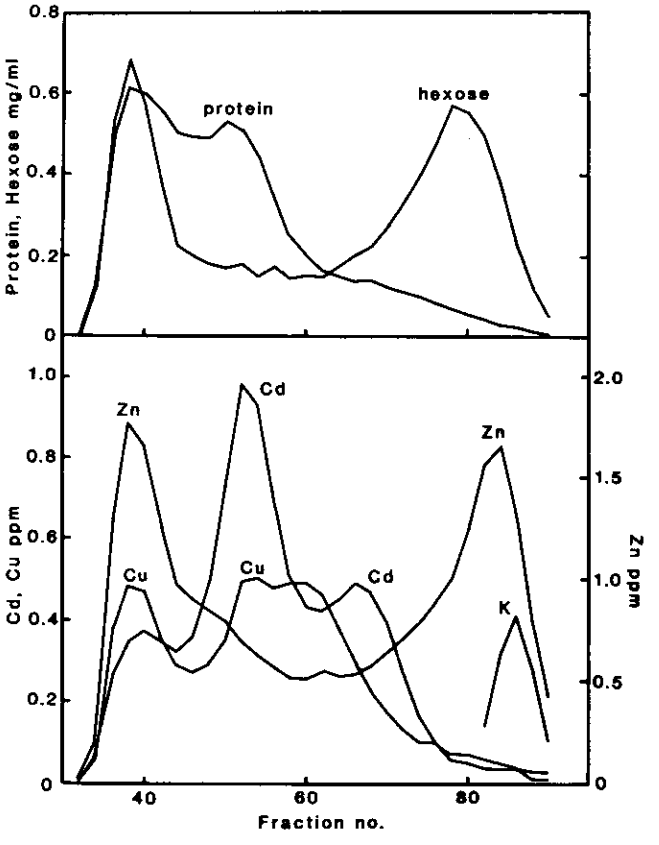


FIGURE 1. Sephadex G-100 chromatography of digestive gland cytosol. Cytosol (4.6 mL) was applied to a Sephadex G-100 column (1.6 cm diameter × 92 cm) equilibrated with 0.1 M NaCl, 10 mM Tris HCl, pH 8.6 and eluted with the same buffer. Flow rate: 25 mL/hr. Fraction size: 2.1 mL.

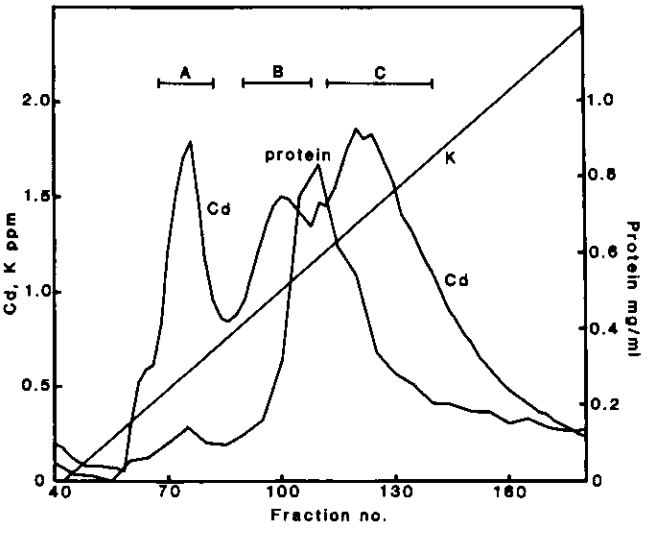


FIGURE 2. DEAE–cellulose chromatography of 55,000 molecular weight. Sephadex G-100 fraction. The Sephadex G-100 product in 10 mM Na borate, pH 8.6, was applied to a DEAE–cellulose column (4.4 cm diameter × 6.0 cm) and eluted with a salt gradient: 10 mM Na borate, pH 8.6, to 0. 15 M NaCl, 3.20 ppm K, 10 mM Na borate, pH 8.6 (1L + 1L). Flow rate: 150 mL/hr. Fraction size: 11.4 mL.

the exclusion limit of the gel), 55,000, and 10,000.

Of the soluble cadmium, approximately 60% was associated with the 55,000 molecular weight component. PMSF inhibited approximately 55% of the total protease, but most of the PMSF-insensitive protease also eluted in the region corresponding to 55,000 molecular weight. These endogenous proteases caused problems in the subsequent purification attempts and were manifest in poor reproducibility of the DEAE–cellulose chromatograms. Figure 2 illustrates a chromatogram type which was encountered frequently. The amino acid composition of the pooled fractions under the bars marked A, B, and C are given in Table 1. It can be seen that fraction A, which contains the highest cadmium (1.1% by weight), also has the highest Cys content. Gel electrophoresis under both denaturing and nondenaturing conditions gave very poor resolution. This was probably the result of proteolysis.

The main cadmium peak (from a different DEAE–cellulose chromatography experiment) was used to study the cadmium binding. Sephadex G-25 experiments as a function of pH showed that at pH 5 half of the cadmium was lost. This indicates that the binding was weaker than that in the case of cadmium metallothionein where the pH had to be lowered to pH 3.5 in order to displace half of the cadmium (*10*). The cadmium could be displaced by the following sulfydryl reagents: *p*-hydroxymercuribenzoate, *N*-ethylmaleimide, 2,2′-dithiopyridine and tetrathionate. This indicates that the metal was thiolate bound. This conclusion is in accord with the ultraviolet spectra of fractions A (from Fig. 2). At neutral pH there is a shoulder at 250 nm (as well as a peak at 270 nm), and this absorption at 250 nm was reduced to 47% of the absorbance at neutral pH on acidification to pH 2. The cadmium thiolate chromophore is known to absorb at 250 nm and the cadmium is displaced by protons at low pH which causes loss of this absorption (*11*).

The component of molecular weight 10,000 showed a single sharp peak of cadmium on ion-exchange chromatography (Fig. 3). The main cadmium containing fractions were pooled and contained the metals cadmium, zinc, and copper in the following molar ratios 1:0.81:0.18. The total sulfydryl, determined by using 5,5′-dithiobis(2-nitrobenzoic acid) (*12*), to metal (Cd + Zn + Cu) ratio was 2.0. The material showed many of the properties typical of metallothionein. The molecular weight determined by SDS–polyacrylamide gel electrophoresis was 8000. The cadmium was retained during Sephacryl S-300 chromatography at pH 5.5. The ultraviolet absorption showed a high $A_{250}/A_{280}$ ratio (5.0) at neutral pH and on acidification the absorbance at 250 nm was reduced to 29% of the value at neutral pH.

The protein was heat-stable and could be determined using the cadmium saturation assay for metallothionein (*13*). This component was purified further by preparative gel electrophoresis, and the amino acid analysis of this fraction is included in Table 1. It can be seen that the Cys content was high (16.9 mole-%) but still considerably less than that of horse metallothionein. In this respect it resembles the metallothioneins isolated from the edible mussel *Mytilus edulis* (*14*).

**Table 1. Amino acid composition of preparations of Pecten cadmium binding components and of horse metallothionein.**

| | 55,000 molecular weight component[a] | | | | |
|---|---|---|---|---|---|
| Amino acid | A | B | C | 10,000 component | Horse MT[b] |
| Lys | 8.0 | 4.1 | 4.3 | 14.5 | 11.1 |
| His | 1.7 | 1.4 | 2.0 | 8.2 | — |
| Arg | 2.4 | 5.7 | 5.3 | 3.8 | 2.3 |
| Cys[c] | 8.2 | 3.9 | 4.8 | 16.9 | 33.6 |
| Asp | 11.0 | 11.0 | 11.2 | 11.7 | 5.2 |
| Thr | 8.2 | 6.7 | 7.0 | 4.4 | 3.1 |
| Ser | 6.7 | 4.9 | 6.0 | 6.6 | 11.0 |
| Glu | 7.2 | 10.6 | 10.2 | 7.6 | 4.3 |
| Pro | 6.5 | 8.2 | 8.1 | 3.9 | 4.3 |
| Gly | 8.9 | 7.6 | 7.6 | —[d] | 10.0 |
| Ala | 7.9 | 6.8 | 6.1 | 8.7 | 9.7 |
| Val | 5.5 | 6.2 | 5.9 | 5.9 | 3.1 |
| Met | 1.6 | 2.5 | 2.0 | 1.4 | 1.7 |
| Ile | 3.7 | 4.6 | 4.6 | 1.8 | 0.2 |
| Leu | 6.7 | 4.9 | 5.1 | 2.7 | 0.6 |
| Tyr | 2.5 | 4.2 | 3.8 | 0.8 | — |
| Phe | 3.3 | 6.7 | 6.0 | 1.3 | — |

[a] See Figure 2.
[b] Horse metallothionein. Data of Kägi et al. (*9*).
[c] Determined as cysteic acid.
[d] Not determined (because of contamination).

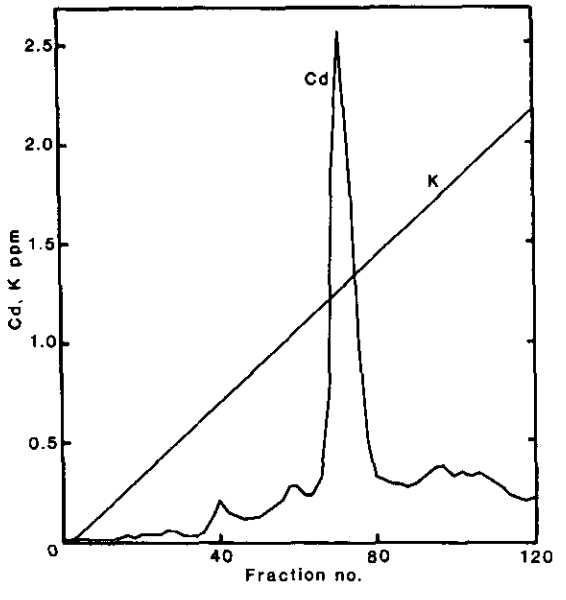


FIGURE 3. DEAE-cellulose chromatography of 10,000 molecular weight Sephadex G-100 fraction. The Sephadex G-100 product in 5 mM 2-mercaptoethanol, 20 mM Tris HCl, pH 8.6, was applied to a DEAE-cellulose column (2.6 cm diameter × 11.0 cm) and eluted with a salt gradient: 5 mM mercaptoethanol, 20 mM Tris HCl, pH 8.6, to 0.15 M NaCl, 2.51 ppm K, 5 mM mercaptoethanol, 20 mM Tris HCl, pH 8.6 (1L +1L). Flow rate: 120 mL/hr; fraction size: 16.5 mL.

Carbohydrate was present with protein in all fractions. The amount of carbohydrate varied from the same weight of protein to approximately 10 times that of the metallothionein fraction from DEAE–cellulose. It is believed that this carbohydrate was not protein-bound.

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