UNIVERSITY OF CALIFORNIA
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Extraction, Partial Purification, and Characterization of the Abalone Shell Peptides

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Biology

by

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June 1985

ABSTRACT

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Abalone shell peptides were extracted from adult, juvenile, and post-larval shells of the red abalone, Haliotis rufescens, by decalcification with EDTA. The extracted peptides were fractionated by gel-filtration chromatography.

Shell protein extracted from the adult abalone was shown to contain calcium. Complete removal of the bound calcium was not possible even after several desalting steps.

Gel-filtration column chromatography performed in bicarbonate buffers allowed the separation of different constituent shell peptides that would otherwise elute from columns as a single, high molecular weight aggregation complex. This effect of bicarbonate buffers proved to be useful, since shell peptides thus could be partially purified in one single fractionation step.

The cationic carbocyanine dye, Stains-all, was shown to interact with shell peptides and stain them blue. The

dye-peptide complexes absorbed maximally at 572 nm, reflecting a state that resulted from the binding of individual dye molecules at the negatively charged aspartic acid residues. Stains-all staining of shell peptides was much more effective than previously used methods of detection with Coomassie Blue or silver staining. This method proved useful for the rapid detection of shell peptides in chromatographic eluates and in nondenaturing acrylamide gels.

Soluble shell peptides from adult and juvenile shells appeared to be heterogeneous, with molecular weights ranging from 21,000 - 57,000 d. A majority species, corresponding to a 43,000 d peptide was isolated from both adult and juvenile shells. Amino acid analyses showed that 35% of the total amino acid residues were aspartic acid, 28% glycine, and 10% serine. A 54,000 d peptide was unique to the juvenile shell and was different from the other shell peptides in that it stained pink with Stains-all rather than blue. Furthermore, amino acid analysis of the 54,000 d peptide indicated that 13% of the total amino acid residues were aspartic acid, 70% glycine, and 14% serine. A similar 54,000 d shell peptide was isolated from the post-larval

shells. The heterogeneity of the shell protein constituents has been shown to increase with the age of the shell. The juvenile shell peptide population appeared as a link between adult and post-larval shell peptides, since it shared components from each of the other two populations. The possibility that these shell peptides might represent products of different genes is discussed.

This work is a result of research sponsored in part by NOAA, National Sea Grant College Program, Dept. of Commerce, under grant number NA80AA-D-00120, project number R/A-51, through the California Sea Grant College Program, and in part by the California State Resources Agency. The U.S. Government is authorized to reproduce and distribute for governmental purposes.