UNIVERSITY OF CALIFORNIA
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Purification and Characterization of Glucose-6-phosphate Dehydrogenase from the Abalone *Haliotis rufescens*

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

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in

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by

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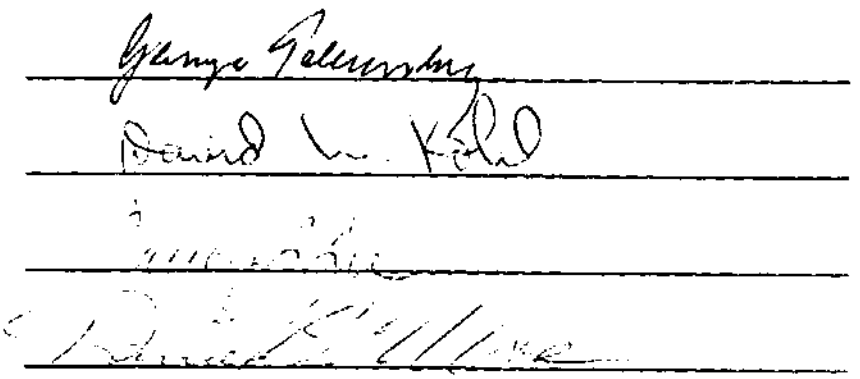

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ABSTRACT

Purification and Characterization of Glucose-6-phosphate Dehydrogenase from the Abalone Haliotis rufescens

by

Irwin Miguel Vivas

Glucose-6-phosphate dehydrogenase was isolated and purified to near homogeneity from the gill of the abalone Haliotis rufescens. The purification procedure involved the sequential application of affinity chromatography in 2',5'-ADP-Sepharose and gel filtration in Ultrogel AcA 34. The H. rufescens enzyme was purified to a specific activity of 270 units/mg protein.

The molecular weight of the Haliotis rufescens glucose-6-phosphate dehydrogenase subunit was found to be 56,000 ± 3,000 Da, by SDS-polyacrylamide gel electrophoresis. The molecular weight of the native enzyme, determined by gel filtration, was dependent on the concentration of NADP in the elution buffer. Dimer, tetramer and octamer forms of the protein, with approximate molecular weights of 98,000, 190,000 and 420,000 ± 10,000 Da, were detected when the coenzyme concentrations in the elution buffer were 0, 0.02 and 0.4 mM, respectively.

Haliotis rufescens glucose-6-phosphate dehydrogenase

oxidized glucose-6-phosphate at a maximal rate; 2-deoxy-glucose-6-phosphate, galactosamine-6-phosphate and glucose-6-sulfate also were oxidized, but less efficiently. The apparent K_m for glucose-6-phosphate was 1.5×10^{-5} M. NADP was the only coenzyme used by the Haliotis rufescens enzyme and the apparent K_m for this substrate was 5×10^{-5} M. Inhibition by NADPH was competitive with respect to NADP and noncompetitive with respect to glucose-6-phosphate. The apparent K_i for NADPH was 0.1×10^{-6} M. The optimum temperature for the enzyme-catalyzed reaction was 50 ^{o}C and the activation energy was 1.04 kJmol^{-1}. NADP protected the Haliotis rufescens glucose-6-phosphate dehydrogenase against thermal inactivation, whereas glucose-6-phosphate caused an increase in its temperature lability.

The amino acid composition of the partially renatured enzyme after SDS-gel electrophoresis, showed a high ratio of basic amino acids to acidic amino acids. The significance of the role played by the glucose-6-phosphate dehydrogenase in the Haliotis rufescens gill, and the high specificity of the enzyme found in this tissue, are discussed.