STUDIES OF ZINC (II) UPTAKE USING RAT LIVER PLASMA MEMBRANE VESICLES

A THESIS

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by

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Abstract

Zinc, like other trace elements, is often protein-bound and its metabolism involves intestinal absorption, bloodstream transport, and liver metabolism. The studies of zinc metabolism using hepatocyte cultures are difficult to interpret.

In this thesis, divalent zinc transport has been studied using purified rat liver plasma membrane vesicles and a chromophoric chelator which monitors the movement of zinc across the membrane. Rat liver plasma membranes were isolated from rat liver via differential and gradient centrifugation. The purity of membrane was assessed by "marker enzyme" activities. The chromophoric dye (AAIII) or (APIII) was incorporated into the vesicles by dehydration/ hydration. The free dye was separated from the encapsulated dye using a Sephadex G-50 column. Transport kinetics were measured spectrophotometrically. Kinetic studies using this technique demonstrate that the zinc uptake is saturable with a Km = 11.34$\pm$9.02 μM and V_{max} = 0.867$\pm$0.25. The kinetics imply a facilitated zinc (II) transport process which is comparable to those in the more complex hepatocyte cell culture studies.

Kinetics studies using free APIII and zinc showed a decrease of APIII-Zinc complex spectra with time and this slow change was dependent on zinc and APIII concentrations, perhaps implying the presence of contaminant dye which was able to react with APIII or APIII-Zn complex resulting in the decreasing of absorbance.