Studies of Electrochemically Induced Iron Release of Horse Spleen Ferritin Using Long Optical Path Length Thin-Layer Spectroelectrochemistry

Donald Zapien[1] and Todd Kreutzian[1]

[1]Department of Chemistry, University of Colorado
P.O. Box 173364
Denver, CO 80217
United States

Ferritin is a protein found in most organisms whose principal functions are to sequester excess iron in the cell and to provide iron whenever it is needed. Iron, as Fe (II), enters the protein and is oxidized to Fe (III). Before iron can leave the protein shell, it must first be reduced to Fe (II). Thus, ferritin's functions of iron uptake and release involve redox reactions.

In previous studies, the direct electron transfer of horse spleen ferritin adsorbed at indium-tin oxide (ITO) electrodes has been demonstrated. In addition, the voltammetric data suggested that reduction of the ferritin layer in the presence of an iron-chelating agent induces iron to be released from the protein shell. The objective of this study is to quantitate the amount of iron released using a method that does not rely on having to measure electrolytic charge.

A spectroelectrochemical cell was designed and constructed to allow the direct measurement of the amount of iron released following the application of a negative potential step to an ITO/ferritin electrode. Teflon spacers separate an ITO electrode from the Plexiglas cell wall forming a thin-layer cavity. Visible radiation passes through the thin layer via plastic windows positioned at each end of the cavity. After the cell was aligned in a UV-visible spectrophotometer, the cell cavity was filled with 1,10-phenanthroline in TRIS buffer, and the electrode poised at a potential sustaining ferritin in the oxidized form. The potential was stepped to a reducing value and the absorbance of the iron-phen-anthroline complex measured. The absorbance was projected onto the concentration axis of a curve constructed from the absorbances of series of iron-phenanthroline standards. The amount of iron released was 1.49 E-8 moles. The total moles of iron released as measured by coulometry was 1.25 E-8, assuming one electron transferred per iron atom electrolyzed.

The agreement of 19is induced when ferritin is subjected to a reducing electrode potential, thus supporting the hypothesis that ferritin's principal functions are coupled to electrochemical events. The results also confirm that one electron is transferred for every iron atom that is released. Using the measured amount of iron released, the electrode area, and the average number of iron atoms per ferritin molecule, an experimental packing density is calculated. This value agrees well with theoretical packing density of adsorbed ferritin, indicating that a monolayer of ferritin forms spontaneously on the ITO surface. The suitability of the cell for quantitating the amount of iron released allows for the measurement of the rate constant for iron-release process.

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