Probing Adsorbed Ferritin Conformation with Monoclonal Antibodies

Donald C. Zapien
Department of Chemistry
University of Colorado at Denver
Denver, CO 80217

Ferritin adsorbed at indium-tin oxide (ITO) electrodes exhibits unusual electrochemical behavior. A cathodic peak at negative potentials indicates that immobilized ferritin undergoes electrochemical reduction. However, the original cathodic peak is not present in the second cycle suggesting that adsorbed ferritin's electroactivity was lost upon reduction. Among other changes, such as those in orientation and protein composition, conformational changes may lead to changes in electroactivity. In this study, we are exploiting the specificity of antibodies to conformational epitopes on the surface of ferritin to probe whether adsorbed ferritin undergoes conformational changes at different potentials.

Balb c mice were immunized with horse spleen ferritin (4 mg/ml in phosphate-saline buffer). At the end of the immunization period, mouse splenocytes were fused to myeloma cells, and the production of ferritin-specific antibodies by the resulting hybridoma cells was verified by ELISA. Seventy two of the cell lines producing antibodies exhibiting the highest ferritin binding were selected for further screening.

Indium-tin oxide material was cut to fit into the wells of a standard flat-bottomed 24-well plate. Ferritin layers were formed on the indium oxide electrodes, and ELISA was used to screen for those producing antibodies specific to ferritin in the adsorbed state.

Time-resolved fluorescence immunoassay (TRFIA) gives sensitivities rivaling those of the more traditional radioimmunoassay. Experiments using TRFIA to determine relative binding at different potentials will be discussed.