PREPARATION OF NANOCERIA CONJUGATES FOR BIOANALYTICAL ASSAYS

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ABSTRACT

Enzymes, such as phosphatase, peroxidase and galactosidase, are used extensively as reporters in bioanalytical assays, especially immunoassays and nucleic acid hybridization assays, due to the signal amplification introduced through substrate turnover. The aim of the present work is to exploit the oxidase-like catalytic properties of cerium oxide nanoparticles for the preparation of reporters for protein and DNA assays. Because the biotin-avidin interaction is of fundamental importance for the development of antibody- and nucleic acid-based assays we prepared nanoceria-streptavidin conjugates that maintain both the oxidase-like activity of nanoceria and the high affinity of streptavidin for biotinylated molecules. We investigated various approaches of synthesis and surface modification of cerium nanoparticles with –COOH groups starting from aqueous solutions of $Ce(NO_3)_3$. One synthetic approach involved controlled alkaline precipitation in a 25% NH_3 solution in the presence of ethylene glycol (at 60 ^oC) followed by sodium citrate treatment. The optimum oxidase activity of nanoceria was at a Ce^{3+}: citrate molar ratio of 6:5. The average diameter of nanoceria was found, by transmission electron microscopy (TEM), to be 2.4 nm. In another synthetic approach we mixed Ce^{3+} with polyacrylic acid (PAA, Mr = 1800) followed by controlled alkaline precipitation. The optimum Ce^{3+}:PAA ratio was 10:1 and the average size of nanoceria was 1.5 nm. The oxidase activity of nanoceria was assessed by using 0.5 mM 3,3,5,5'-tetramethylbenzidine (TMB) as a chromogenic substrate at an optimum pH of 4.5. The synthesized nanoceria was conjugated to 12 mg/mL streptavidin using 0.24 g/mL N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride. The conjugate was purified from the free streptavidin and free nanoceria by size exclusion chromatography on a Sephacryl S200 column. The functionality of the conjugate was evaluated by using microtiter wells coated with biotinylated albumin. The absorbance was measured at 450 nm by a microplate photometer.

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