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Sensitive and selective colorimetric assay of alkaline phosphatase activity with Cu(II)-phenanthroline complex

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Abstract

Alkaline phosphatase (ALP) plays a vital role in dephosphorylation- and phosphorylation-related cellular regulation and signaling processes. Accordingly, the development of efficient methods for ALP activity assay is of significant importance in clinical diagnosis. In this work, a simple and practical method is reported for the first time for the sensitive and selective colorimetric assay of ALP activity by exploiting a water-soluble Cu(II)-phenanthroline complex as the probe, on the basis of the distinctive metal-to-ligand charge-transfer (MLCT) properties. This method is simply built on a two-step chromogenic reaction: the enzymatic hydrolysis of the substrate ascorbic acid 2-phosphate to ascorbic acid (AA), followed by the reduction of the colorimetric probe $Cu(BPDS)_2^{2-}$ (BPDS = bathophenanthroline disulfonate) by AA to its cuprous form. The latter process triggers a turn-on spectral absorption at 424 nm and a striking color change of the solution from colorless to blackish-green. Needless of complicated protocols and instrumentation, this method allows a sensitive readout of ALP activity within a wide linear range of 0−200 mU mL^{-1}, with a detection limit down to 1.25 mU mL^{-1}. Results also reveal that it is highly selective and holds great potential in ALP inhibitor efficiency evaluation. In addition, quantitative analysis of ALP activity in spiked serum samples has been realized successfully in the linear range of 0−200 mU mL^{-1}, with a detection limit of 1.75 mU mL^{-1}. Advantages of simplicity, wide linear range, high sensitivity and selectivity, low cost, and little background interference render this method great potential in practical applications.

Keywords: Colorimetric; alkaline phosphatase; bathophenanthroline disulfonate; Cu(II)-phenanthroline