Determination of Nanogram Amounts of Copper by Activation of Insolubilized Apo-polyphenol Oxidase

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Summary Chemically-insolubilized apo-polyphenol oxidase is shown to be a very selective, sensitive and stable means of collecting and determining ng amounts of copper(II).

IT has recently been demonstrated that zinc can be reproducibly re-incorporated into apo-alkaline phosphatase, thus providing a means of determining ng amounts of zinc.[1] This enzyme system however, has many disadvantages when applied to trace metal analysis, for example susceptibility to inhibiting and activating metal ions, and the instability and time-consuming preparation of the apo-enzyme.

Insolubilization of a metallo-enzyme is found to eliminate the last two disadvantages, and judicious choice of enzyme can minimize inhibition and activation effects. Polyphenol oxidase (from mushrooms), a copper-containing enzyme, has been covalently linked to the polyacrylamide derivative Enzacryl AA.[2] The copper is readily removed by washing the insolubilized enzyme with a 0·1M cyanide solution, and the insoluble apo-enzyme collected by centrifugation, after washing. This apo-enzyme is reactivated by copper(I) or copper(II); maximal activity is reached after 2 h incubation at 25 °C in pH 7·0 tris maleate buffer solution. After such incubation, the activity of the reactivated enzyme, as measured by its catalysis of the oxidation of catechol, is reproducible and is linearly dependent upon copper(II) concentration over the range 10^{-7}—2×10^{-6}M. This indicates a measurable response to as little as 10 ng of copper(II).

No other metal ions at the 2×10^{-6}M level cause any activation of the insoluble apo-enzyme; activation by a mM concentration of Ni^{2+}, Fe^{2+}, Sr^{2+}, and Mg^{2+} of magnitudes similar to that given by 5×10^{-7}M copper(II), are the only effects observed at this higher level. However, a number of metal ions inhibit the uptake of copper by the apo-enzyme. Large concentrations (mM) of Zn^{2+}, Cd^{2+}, Hg^{II}, Hg^{I}, and Ag^{+} completely prevent incorporation of copper from a 5×10^{-7}M solution; but only Ag^{+} at the

2×10^{-6}M level inhibits copper uptake (by 50%). Polyphenol oxidase was chosen for this study because of its reported freedom from inhibition and activation by metal ions,[3] which is confirmed in the present study. Only vanadium(V) was found to inhibit the insolubilized enzyme (I_{50} *ca.* 3×10^{-3}M).

The apo-enzyme is readily prepared and isolated, and may be stored as a suspension in the pH 7·0 buffer solution for three months at 6 °C without noticeable deterioration. It provides a very sensitive, highly selective means of collecting *and* monitoring traces of copper in aqueous solutions; and it is hoped that it will be applicable to the determination of copper at levels as low as 10^{-12} g ml^{-1} in large volumes of sample.

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