

## Positive effect of Cu<sup>2+</sup> and Ca<sup>2+</sup> on the Thermostability of Bambara Groundnut Peroxidase A6, and its Catalytic Efficiency Toward the Oxidation of 3,3',5,5'-Tetramethyl Benzidine

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**Abstract :** Improving the catalytic performance of enzymes has been a long-standing theme of analytical biochemistry research. Induction of peroxidase activity by metals is a common reaction in higher plants. We thought that this increase in peroxidase activity may be due, on the one hand, to the stimulation of the gene expression of these enzymes but also to a modification of their chemical reactivity following the binding of some metal ions on their active site. We tested the effect of some metal salts (MgCl<sub>2</sub>, MnCl<sub>2</sub>, ZnCl<sub>2</sub>, CaCl<sub>2</sub> and CuSO<sub>4</sub>) on the activity and thermostability of peroxidase A6, a thermostable peroxidase that we discovered and purified in a previous study. The chromogenic substrate used was 3,3',5,5'-tetramethylbenzidine. Of all the metals tested for their effect on A6, only magnesium and copper had a significant effect on the activity of the enzyme at room temperature. The Mann-Whitney test shows a slight inhibitory effect of activity by the magnesium salt (P = 0.043), while the activity of the enzyme is 5 times higher in the presence of the copper salt (P = 0.002). Moreover, the thermostability of peroxidase A6 is increased when calcium and copper salts are present. The activity in the presence of CaCl<sub>2</sub> is 8 times higher than the residual activity of the enzyme alone after incubation at 80°C for 10 min and 35 times higher in the presence of CuSO<sub>4</sub> under the same conditions. In addition, manganese and zinc salts slightly reduce the thermostability of the enzyme. The activity and structural stability of peroxidase A6 can clearly be activated by Cu<sup>2+</sup>, which therefore enhance the oxidation of 3,3',5,5'-tetramethylbenzidine, which was used in this study as a chromogenic substrate. Ca<sup>2+</sup> likely has a more stabilizing function for the catalytic site.

**Keywords :** peroxidase activity, copper ions, calcium ions, thermostability

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