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SPRING GREENS PEROXIDASE AND ITS APPLICATIONS

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ABSTRACT

An extract of Thai vegetable, spring greens (Brassica alboglabra), with high peroxidase activity, was partially purified for peroxidase. By using a CM-cellulose column, 959-fold purification, 8.9% yield of bound fraction I of spring greens was achieved. All fractions showed peroxidatic activity corresponding to molecules with a molecular weight range of 40,000-41,000 as determined by Sephadex G-200 column and SDS-PAGE.

The Soret region absorption spectrum of the partially purified enzyme shows a maximum at 402 nm and has the R_z value 0.53. It was inhibited by typical hemoprotein inhibitors KCN and NaN₃. It was also inhibited by high concentration of H₂O₂ as horseradish peroxidase (HRP).

Partially purified peroxidase from spring greens had at least 7 isozymes when isoelectric-focusing was performed. The pI was in the range 4.6 through 8.4. K_m values for diamino-benzidine of the partially purified spring greens peroxidase are 5.5x10⁻⁴ M for unbound fraction from CM-cellulose column, 5.7x10⁻⁴ M for bound fraction I, 6.7 x10⁻⁴ M for bound fraction II and 1.5x10⁻⁴ M for bound fraction III. K_m for H₂O₂ of the enzyme is 2.7x10⁻⁵ M. The present study points out that partially purified peroxidase from spring greens catalyzes the peroxidation of iodide and iodination of tyrosine.

The enzyme was relatively stable at 4 °C in the range of pH 4 to 9, and could be preserved by 1 mg/ml BSA and 1% Triton X-100.

For application, partially purified peroxidase (bound fraction I from CM-cellulose) can conjugate with IgG as with HRP, precipitate phenols and anilines from water similarly to HRP and choysum peroxidase.

