

Properties of Chlorophyllase from *Capsicum annuum* L. Fruits

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The *in vitro* properties of semi-purified chlorophyllase (chlorophyll-chlorophyllido hydrolase, EC 3.1.1.14) from *Capsicum annuum* fruits have been studied. The enzyme showed an optimum of activity at pH 8.5 and 50 °C. Substrate specificity was studied for chlorophyll (Chl) *a*, Chl *b*, pheophytin (Phe) *a* and Phe *b*, with K_m values of 10.70, 4.04, 2.67 and 6.37 μM respectively. Substrate inhibition was found for Phe *b* at concentrations higher than 5 μM . Chlorophyllase action on Chl *a'* and Chl *b'* was also studied but no hydrolysis was observed, suggesting that the mechanism of action depends on the configuration at C-13² in the chlorophyll molecule, with the enzyme acting only on compounds with R13² stereochemistry. The effect of various metals (Mg^{2+} , Hg^{2+} , Cu^{2+} , Zn^{2+} , Co^{2+} , Fe^{2+} and Fe^{3+}) was also investigated, and a general inhibitory effect was found, this being more marked for Hg^{2+} and Fe^{2+} . Functional groups such as -SH and -S-S- seemed to participate in the formation of the enzyme-substrate complex. Chelating ion and the carbonyl group at C3 appeared to be important in substrate recognition by the enzyme. The method for measuring Chlase activity, including HPLC separation of substrate and product, has been optimized.