Studies on the Purification and Properties of Polyphenol oxidase form cucumbers

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ABSTRACT

Polyphenol oxidase (PPO, EC 1.14.18.1) was extracted from cucumber using 0.1 M phosphate buffer, pH 5.0. Purification of the enzyme was achieved by a combination of ammonium sulfate precipitation (20-80% saturation) and DEAE Sepharose CL-6B chromatography. Overall the specific activity of the purified fraction increased 124.9 fold with a yield of 71.4%. The purified preparation gave one protein band on both gel filtration and SDS-PAGE with respective molecular weight of the enzyme being estimated to be about 148 KDa and 76 KDa, indicating a dimeric structure with identical monomers. The optimum temperature and pH for maximum PPO activity occurred at 40 ℃ and 7, respectively with catechol as substrate. The enzyme was satble (＞ 80% max. activity) for 10 min at temperature less than 50 ℃ or for 1 hr between pH 4 and pH 8. As to substrate specificity, a maximum activity was associated with pyrogallol, followed by 4-methyl catechol and catechol. The results indicated that the cucumber PPO was more active toward o-diphenols. The cucumber polyphenol oxidase was inhibited by copper com plexing agents and by reducing agents. Complete (100%) inhibition was noted in the presence of 10 mM of potassium cyanide, L-ascorbic acid, glutathione or sodium metabisulfite. Values calculated from Lineweaver-Burk graphs showed Km values were 1.15 mM for 4-methyl catechol, 2.076 mM for catechol and 6.520 mM for pyrogallol. From these results, We suggest cucumber PPO has more affinity for 4-methyl catecchol than for the other substrates. The 4-methyl catecchol used as for substrate sodium metabisulfite and potassium cyanide exhibited uncompetitive inhibition upon the cucumber PPO, with Ki values are 0.0177 mM and 0.0218 mM,respectively. L-ascorbic acid showed competitive in hibition upon the cucumber PPO, with Ki values is 0.0153 mM. The catecchol used as for substrate sodium metabisulfite and potassium cyanide exhibited the same uncompetitive inhibition upon the cucumber PPO, with Ki values are 0.0833 mM and 0.0820 mM, respectively. L-ascorbic acid showed noncompetitive inhibition upon the cucumber PPO, with Ki values is 0.0659 mM. The pyrogallol used as for substrate L-ascorbic acid and potassium cyanide exhibited noncompetitive inhibition upon the cucumber PPO, with Ki valu es are 0.1840 mM and 0.010 mM, respectively. sodium metabisulfite showed competitive inhibition upon the cucumber PPO, with Ki values is 0.2380 mM. Of inhibitors tested, cucumber PPO were markedly inhibited by metal enzyme inhibitor such as potassium cyanide, L-ascorbic acid, glutathione or sodium metabisulfite and L-cysteine. Althought potassium cyanide was strongly inhibited PPO activity but toxic. On the other hand L-ascorbic acid and L-cysteine were both effectiveness inhibitor of cucumber PPO. They are naturally occurring and nontoxi. We suggested it may be useful in preventing enzymatic browning of cuccumber products.

Keywords : enzymic browning ; borwn melanins

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