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Multinuclear NMR Study on Inhibitory Mechanisms by Chloride Ions of Polyphenol Oxidases Derived from Higher Plants

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Summary

Polyphenol oxidase (PPO) is the enzyme that catalyzes the oxidation of polyphenol to quinone. PPO's physiological significance has not been yet definite. It produces the coloring that is beneficial for food products such as coffee and tea. On the other hand, it is also the enzyme that causes the discoloration in fruits and vegetables when damaged during shipping, storage, as well as other manufacturing processes. Therefore, the regulation of PPO activity is an important study in the food industry.

In our research using mushroom PPO and catechol as a substrate, the reaction rate was decreased in the presence of higher levels of substrate. In addition, halides inhibited PPO activity at lower concentration range of catechol, where substrate inhibition was not perceived, whereas halides inhibited the activity at a lower level in the presence of higher levels of catechol. These findings lead us to propose the existence of a second binding site for catechol in addition to the substrate-binding active site (site A). Substrate binding to this second site (site B) causes the inhibition of the enzyme's activity. Further, chloride binds to site B and can also cause enzyme inhibition. Our mathematical model that take into account the binding sites steadily followed the measured activity, confirming the justification of the model.

Kojic acid, a competitive inhibitor of the enzyme was used to explore the binding of substrate to PPO. Isothermal titration calorimetry (ITC) and saturation transfer difference (STD) spectrometry were undertaken to probe the binding mode. These measurements indicated that although kojic acid binds to PPO, halides did not affect the binding strength of kojic acid.

Active site of PPO is composed by a dicopper site with each copper bound by three His residues. When we abbreviate the site as $[Cu(I)_2]$ and $[Cu(II)_2]$ depending on the oxidation state of Cu, the enzyme reaction is proposed to proceed in such a way that $[Cu(II)_2]$ oxidize catechol to produce $[Cu(I)_2]$ and quinone (step 1), dioxygen oxidize $[Cu(I)_2]$ to produce $[Cu(II)_2]$ and peroxide ions (step2), and then the peroxide oxidize catechol, leaving $[Cu(II)_2]$. Based on a HSAB principle, which indicates Cu(II) preferentially binds to F^-, while Cu(I) does to I^-, it is most likely that F^- binds $[Cu(II)_2]$ and inhibits step 1. I^- ion, on the other hand, binds $[Cu(I)_2]$ and interfere with the process of step 2. The findings that inhibition PPO activity was most severe for F^- followed by I^- with similar inhibitory effect of Cl^- and Br^- and that ITC experiment showed I^- bind PPO at the reduced form alone support the inhibitory model.