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Inhibitory Effect of Ammonium Tetrathiotungstate on Tyrosinase and Its Kinetic Mechanism

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Tyrosinase requires copper ion at the active site to oxidize phenols to catechols. Here, with a strategy to detect an effective inhibitor by chelating coppers, inhibitory actions of a copper-chelating ammonium tetrathiotungstate (ATTT), known as a drug for treating Wilson's disease, was elucidated. Treatment with ATTT on mushroom tyrosinase completely inactivated enzyme activity in a dose-dependent manner. Progress-of-substrate reaction kinetics using the two-step kinetic pathway and melanin producing cell-based assay revealed that ATTT acts as a kinetically competitive inhibitor *in vitro* and decreases melanin content by inhibiting the tyrosinase activity *in vivo*. Progress-of-substrate reaction kinetics with increasing ATTT concentrations and activity restoration with an increase of substrate indicated that the copper-chelating ATTTmay bind slowly to the active site by competing with substrate, and the enzyme-ATTT complex subsequently undergoes reversible conformational change, leading to complete inactivation of the tyrosinase activity. Thus, inhibition by ATTT on tyrosinase could be categorized as competitive and complexing type of inhibition with a slow and reversible binding. Our results may provide useful information regarding effective inhibitor of tyrosinase as whitening agents in the cosmetic industry.

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