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Inhibition of the Catecholase Activity of Mushroom Tyrosinase by Carbon Monoxide

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The catecholase activity of tyrosinase extracted from freeze-dried mushroom powder was found to be reversibly inhibited by carbon monoxide. Dissociation of the carbon monoxide–enzyme complex was followed at various pHs between 4.0 and 8.0. The minimum value of the first-order rate constant for this reaction coincides with the pH of the maximum catecholase activity pH 6.5. Carbon monoxide treatment of the enzyme extracts prevented the self-inactivation of the enzyme.

Tyrosinase (monophenol, dihydroxyphenol oxidoreductase EC 1.14.18.1) is a copper-containing enzyme that catalyzes two reactions in which molecular oxygen is the hydrogen receptor, phenol is the hydrogen donor, and AH_2 represents the hydrogen donor:

$$\text{monophenol} + O_2 + AH_2 \rightleftharpoons o\text{-dihydroxyphenol} + H_2O + A$$

$$o\text{-dihydroxyphenol} + \tfrac{1}{2}O_2 \rightleftharpoons o\text{-quinone} + H_2O$$

A number of substances are known to inhibit this enzyme. They can be conveniently divided into three types (Robb, 1985): (1) general chelating agents for copper, (2) noncompetitive inhibitors with respect to the phenolic substrate, and (3) analogues of phenols, although a clear distinction is not always possible. Carbon monoxide a known inhibitor of many copper-containing oxidases and can be expected to act as an inhibitor of type 2 above.

Although carbon monoxide has been suggested as an inhibitor for tyrosinase, there are no detailed data on the subject. A gaseous inhibitor for tyrosinase could have an important role in maintaining the quality of fresh mushrooms and other vegetables during storage. The objective of this work was therefore to investigate the effect of carbon monoxide on mushroom tyrosinase activity.

RESULTS AND DISCUSSION

The pH dependence of the catecholase activity of the extracted tyrosinase was investigated over the range pH 4.5–9 using tyrosinase citrate–phosphate buffers. The specific activity of the extracted tyrosinase at pH 4.0 was 0.2 (μmoles/minute per milligram of protein), which increased to a maximum of 1.7 at pH 6.5 (Figure 1). This activity profile is similar to those previously reported (McCord and Kilara, 1983). The tyrosinase activity of the carbon monoxide treated extract was considerably reduced compared to the control, 0.034 and 0.55, respectively, while the nitrogen-treated aliquot was only slightly reduced 0.45. Since the activity of the nitrogen-treated extract was considerably higher than the carbon monoxide treated extract, the effect of the carbon monoxide is not simply due to reducing the dissolved oxygen concentration. Carbon monoxide has been shown to be a inhibitor for many copper-containing oxygenases. The effect of carbon monoxide on tyrosinase was found to be reversible; the activity of the carbon monoxide treated extract was restored to that of the control after bubbling through air. The kinetics of the tyrosinase reactivation was studied by following the enzyme activity when a carbon monoxide saturated extract was mixed with buffer saturated with air in the absence of substrate. Various pHs in the range 4.0–8.0 were investigated. In each case, after an initial rapid increase the activity approached asymtopically the maximum value (Figure 2). If the reactivation is assumed to follow first-order kinetics, then it can be described by the equation $A_t = A_m(1 - e^{-kt})$, where A_t is the activity at time t, A_m is the initial activity, and k is the reaction rate constant. A linear relationship was found between ln $(1 - A_t/A_m)$ and t for the reactivation at all pHs, from which the reaction rate constants were calculated (Figure 1). It can be seen that the smallest value of k coincides with the pH maximum activity, pH 6.5. Tyrosinase has been shown to be subject to substrate-induced inactivation. The transformed substrate becomes covalently attached to the enzyme blocking the entry of fresh substrate (Schwimmer, 1981). Our experiments show that this self-inactivation can be prevented by carbon monoxide. Both carbon monoxide treated and untreated enzyme preparations were incubated in the presence of catechol. Aliquots were taken and mixed with fresh substrate and the activity assayed. The carbon monoxide treated solution retained its activity until at 17 min excess air was introduced by vigorous stirring and thus carbon monoxide removed (Figure 3). With the untreated enzyme extract, a steady drop in ac-

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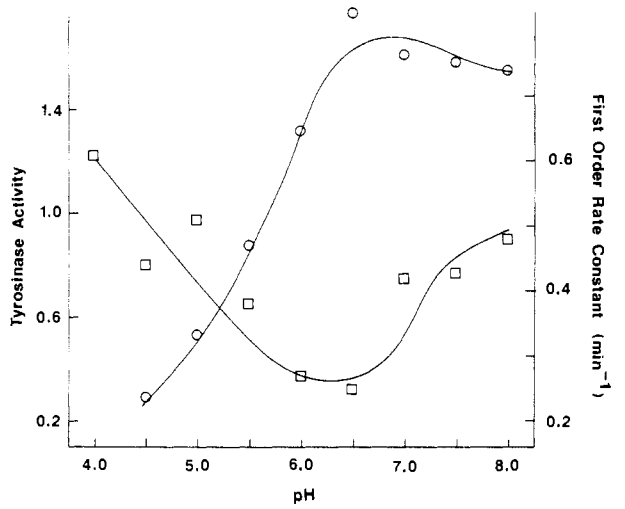

Figure 1. Tyrosinase activity (O) (micromoles of quinone produced/minute per milligram of protein) and the first-order rate constant (□) (reciprocal minutes) for the dissociation of the tyrosinase–carbon monoxide complex both as a function of pH.

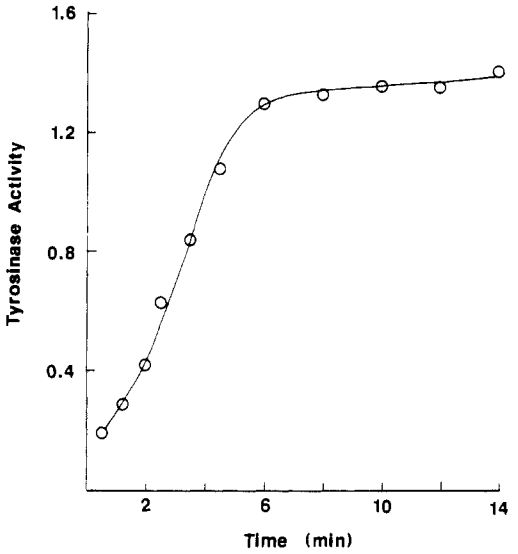

Figure 2. Reactivation of carbon monoxide inhibited tyrosinase in air-saturated 0.2 M citrate–phosphate buffer at pH 6.5. Tyrosinase activity expressed as micromoles of quinone produced/minute per milligram of protein.

tivity was observed (Figure 3) due to self-inactivation.

The results obtained in this work indicate that carbon monoxide is an effective inhibitor of tyrosinase. In the presence of carbon monoxide the activity of this enzyme drops to the zero. However, the inhibition is reversible, and the removal of carbon monoxide leads to the restoration of the initial activity.

The results obtained in this study suggest that carbon monoxide could be an important component in modified atmosphere packing of mushrooms and other vegetables to prevent enzymic browning. Preliminary experiments have shown that mushrooms maintain better color when stored under a carbon monoxide containing atmosphere.

MATERIALS AND METHODS

Fresh mushrooms (*Agaricus bisporus*) were frozen to −18 °C, held at this temperature for 24 h, and then freeze-dried (Vickers-Armstrong, Swindon) with a plate temperature of 10 °C. When dried to a constant weight the mushrooms were ground by a laboratory hammer mill (Christy & Norris, Chelmsford) to pass through a 300-mesh sieve.

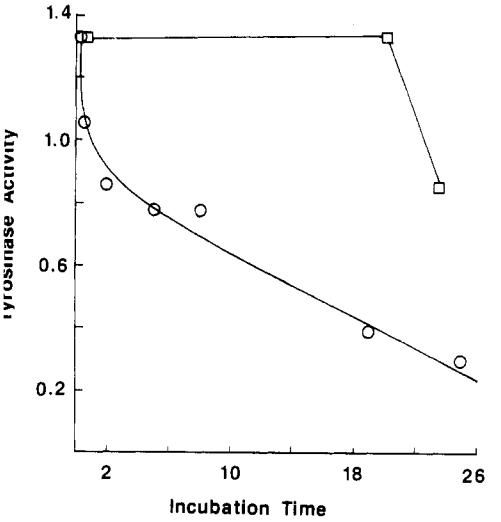

Figure 3. Activity of mushroom tyrosinase in 0.2 M citrate–phosphate buffer at pH 6.5, 6.7 mM catechol and saturated with air (O), or carbon monoxide (□) for 20 min prior to air saturation.

The dried mushroom powder (50 mg) was extracted with 2.5 mL of 0.2 M citrate–phosphate buffer (at the required pH), 1 mL of 1% Triton X-100, 6.5 mL of distilled water, and 500 mg of polyamide CC6 (Macherey Nagel). The mixture was stirred for 1 h 4 °C and then clarified by filtration.

The catecholase activity of the extracts was assayed in a reaction mixture of 3 mL that included 6.7 mM catechol (BDH), 47 mM sodium phosphate buffer (pH 6.5), and 1 mL of extract. The rate of oxidation was followed at 410 nm in a Perkin-Elmer 552 spectrophotometer. A molar extinction coefficient of 1417 M^{-1} cm^{-1} was assumed for the oxidized product (Waite, 1976).

The protein content of the enzyme extracts was determined by the method of Lowry et al. (1951).

Carbon monoxide or nitrogen was passed through the enzyme extract for 20 min at room temperature, aliquots were taken, and the tyrosinase activity was measured. The enzyme activity was also measured in an untreated control.

When the reversibility of the inhibition was studied, the enzyme extract (10 mL) was saturated with carbon monoxide for 15 min after which it was mixed with 0.2 M citrate–phosphate buffer (20 mL) in open flash on a magnetic stirrer. In an control experiment, untreated enzyme extract was used.

Registry No. Tyrosinase, 9002-10-2; carbon monoxide, 630-08-0.

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