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CONVERSION OF CATECHIN AND TANNIC ACID BY AN ENZYME PREPARATION FROM *TRAMETES VERSICOLOR*

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Summary
An extracellular enzyme preparation produced by the white rot fungus *Trametes versicolor* transformed catechin and tannic acid. Optimum conversion of catechin was at 60°C and pH 6.0.

Introduction

Tannins are a group of polymeric phenolic compounds which are widely found in plants. According to their properties, breakdown products and botanical distribution, they can be divided into two broad categories: hydrolysable tannins and condensed tannins [Concon, 1988]. The hydrolysable tannins are polymers with a core molecule of glucose or quinic acid esterified with gallic, digallic and ellagic acids, whereas the condensed or non-hydrolysable tannins are polymers of catechins, leukoanthocyanidins or anthocyanidins [Concon, 1988]. Condensed tannins are the most naturally abundant tannins in plants and, therefore, are frequently found in foods commonly consumed by humans or animals [Griffiths, 1991]. They are potential carcinogens and are believed to reduce the nutritional value of animal feeds [Griffiths, 1991; Smith, 1985]. Thus, suitable methods for a decrease of their content in such commodities are of interest.

Khan and Overend [1990] reported that an enzyme preparation secreted by *Trametes versicolor* transformed catechol (1,2-benzenediol), with the evidence of ring cleavage and the production of hydroxy muconic semialdehyde. This information could be of a particular interest to researchers who study the degradation of tannins, since these compounds contain a 1,2-benzenediol moiety. Considering this, the objective of this work was to test the possible transformation of tannins by this enzyme, examining its ability to transform catechin and tannic acid.

Catechin, or 3,3',4,4',5,7-pentahydroxy-flavan, is used as a standard compound for the determination of the tannin equivalent in foods [Burns, 1971; Maxson and Rooney, 1972]. It belongs to the category of the condensed tannins. Tannic acid, on the other hand, represents the hydrolysable tannins.

Materials and Methods

Enzyme preparation, chemicals: The enzyme was produced in our laboratory as described elsewhere [Lacki and Duvnjak, 1995]. Catechin and tannic acid were from Sigma. The citrate-phosphate buffer (0.05 M) of pH 3.0 to 7.2 was used. All chemicals were of reagent grade.

Assay procedure: The reaction mixture contained 2.7 mL buffer, 0.2 mL catechin solution and 0.05 mL crude enzyme extract. The crude enzyme extract, with the activity of 40 nkat/mL, as measured using ferulic acid [Khan and Overend, 1990], was diluted with distilled water to obtain the range of enzyme concentrations used during experimentation. The reactions were carried out in spectrophotometric quartz cuvettes. The extent of reaction was followed by monitoring the changes in the absorbance at 395 nm. The reactions were carried out for 5 to 15 min., and the absorbance was measured every 5 or 10 s. The linear portion of the progress curve with the maximum slope was used to determine the activity at specified conditions. Activity is expressed in terms of nkat/mL.

Product(s) standard curve: The absorption standard curve for the product was obtained by carrying the enzymatic reaction for 5 h at 40°C and pH 6.0, using the catechin solution from 0.125 to 1.0 mM. At the end of the reaction, the absorbance was measured at 395 nm, and the following relationship between the catechin conversion and absorbance was obtained: $C = 0.274 \times A - 0.224 \times A^2$, where C and A represent the concentration of the product (mM) and absorbance at 395 nm, respectively. To determine the extent of this transformation at other pHs, the correction factors 0.812, 0.936, 0.899, 1.000, and 0.679, for the absorbencies at 395 nm for the pH values of 3.0, 4.0, 5.0, 6.0, and 7.0 were used, respectively. This method for the determination of the product(s) concentration assumed that the reaction was complete, and that the same product(s) is/are generated at each pH examined.

Results and Discussion

The transformation of tannic acid and catechin by the enzyme preparation secreted by *Trametes versicolor* is shown in Fig.1. The tannic acid was transformed with the formation of product(s) with a weak absorbance maximum at 375 nm. Khan and Overend [1990] showed that the degradation of catechol by this enzyme resulted in a formation of a product with a maximum absorbance at 375 nm, that was further identified as muconic semialdehyde. The transformation of catechin resulted in the formation of product(s) which had a maximum absorbtion peak near 395 nm. Since the change in the adsorbance was stronger with catechin, this compound was further used for the characterization of the enzyme. The wavelength of 395 nm was chosen to follow the course of this enzymatic reaction, since catechin contributed only a fraction of the absorbance of products measured at this wavelength.

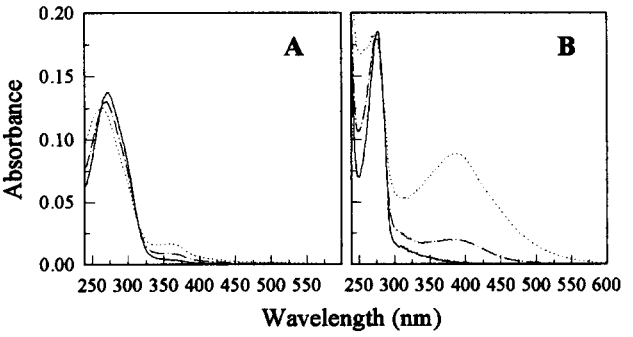

Figure 1. UV-visible spectra of tannic acid (**A**) and catechin (**B**) after the addition of enzyme preparation: (——) initial, (– · –) 5 min., (·····) 30 min.. Conditions: pH 4.5, 25°C; substrate at 1 mM; enzyme: 4.0 nkat/mL.

Examining the effects of pH and temperature on the enzyme activity, it was found that the optimum pH was 6.0 for all the tested temperatures with the exception of the two extreme cases

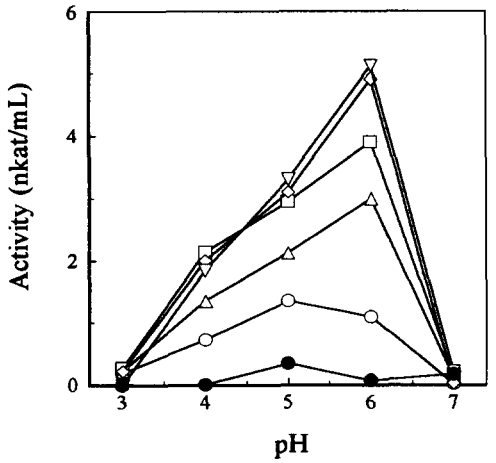

Figure 2. Effect of pH on enzymatic activity at various temperatures of reaction: (O) - 15°C; (Δ) - 30°C; (□) - 40°C; (◊) - 50°C; (▽) - 60°C; (●) - 75° C. Catechin concentration: 0.05 mM; enzyme: 20 nkat/mL.

i.e., for 15°C and for 75°C (Fig. 2). For these two temperatures, the optimum pH value was 5. These results showed that the enzyme activity increased with increasing temperature up to 60°C but almost no activity was detected at 75°C. This could be an indication of considerable thermal denaturation and inactivation of the enzyme at temperatures exceeding 60°C. The thermal deactivation was, however, more pronounced in acidic conditions, pH 3.0 and 4.0, suggesting a lower degree of structural stability in these conditions. Indeed, at these two pH levels, the maximum activity was observed at only 40 °C. These results were confirmed during the investigation of the effect of the enzyme preincubation at various temperatures and pH levels on its stability. The tests showed that the enzyme lost 90% of its activity at 75°C after one hour of incubation at pH 6.0. At the same pH, the enzyme preincubated for 20 hours at 50°C still retained 35% of its initial activity. The enzyme was most stable at pH 5.0 and 6.0 when preincubated at 25°C, showing almost no decrease in activity. On the other hand, a 3 hour preincubation at this temperature at pH 4.0 resulted in a 50% loss of activity. The reduced thermostability of this enzyme at lower pH levels was noticed previously by Lacki and Duvnjak [1995] in studying the conversion of sinapic acid.

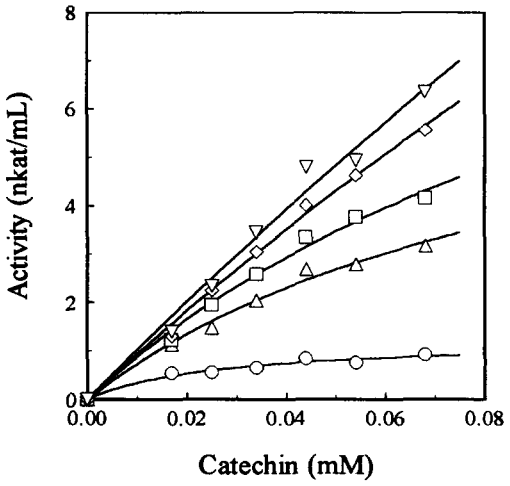

Figure 3. Effect of catechin concentration on enzymatic activity at pH 6.0 and various temperatures: (O) - 15°C; (Δ) - 30°C; (□) - 40°C; (◊) - 50°C; (▽) - 60°C. Enzyme: 20 nkat/mL.

The effect of substrate concentration on the enzyme activity at five temperatures is illustrated in Fig.3. The results showed a typical Michaelis-Menten type of enzyme kinetics. The data at each temperature were fitted to the Michaelis-Menten relationship using a nonlinear regression analysis. The obtained kinetics parameter values are presented in Table 1. According to these results an increase in temperature caused a decrease in the affinity of the enzyme for the substrate. Since the maximum activity increased with temperature, it can be assumed that the deactivation of the enzyme was negligible in the temperature range between 15°C and 60°C. Hence, the data

presented in Table 1 pertaining to the maximum activity and the Michaelis-Menten constant values can be used to calculate the energy of activation for this reaction, E_a, and the change of enthalpy, ΔH, for the enzyme-substrate complex formation. The calculated values are 81.676 kJ/mol and -66.124 kJ/mol, for E_a and ΔH, respectively.

Table 1. Effect of temperature on the enzyme kinetics parameters at pH 6.0.

T [°C]	Maximum Activity [nkat/mL]	Michaelis-Menten constant K_M [mM]
15	1.23	0.026
30	7.87	0.097
40	11.96	0.123
50	48.12	0.516
60	111.32	1.121

According to Michaelis-Menten kinetics, the enzymatic activity is directly proportional to the enzyme concentration. This prediction is, however, only valid if the concentration of the enzyme is small compared to the concentration of the substrate. For higher enzyme concentrations the deviations from linearity are expected. Similar results were obtained in this study (Fig.4), although the saturation of the system with the enzyme is not well defined.

Conclusions

The enzyme preparation secreted by *Trametes versicolor* transformed tannic acid and catechin. The optimum pH and temperature for the transformation of catechin by this enzyme are 6.0 and 60°C, respectively. However, these conditions are not suitable for reactions intended to last long periods of time, such as for continuous processes, since the enzyme showed considerable deactivation at 50°C. Non-optimal conditions at pH 6.0 and 30°C is a more plausible choice, ensuring greater stability of the enzyme while offering 60-80% of the optimal activity.

Since the catechin and tannic acid are considered the model compounds representing condensed and hydrolysable tannins, respectively, this enzyme could be used for the degradation of tannins in animal feeds, and foods destined for human consumption. One such application would be an enzymatic upgrading of canola meal.

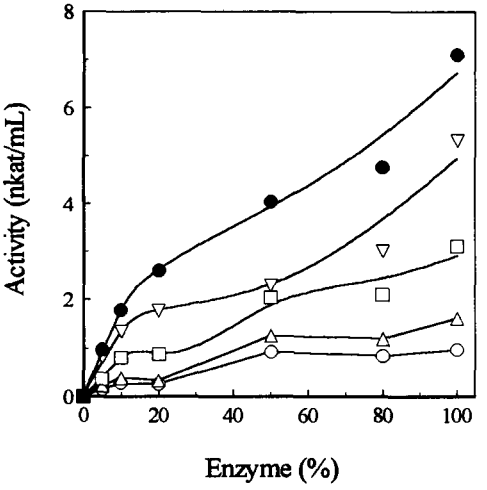

Figure 4. Effect of enzyme concentration on enzymatic activity at various catechin concentrations at pH 6.0 and 30°C: (●) - 0.068 mM; (▽) - 0.054 mM; (□) - 0.044 mM; (△) - 0.025 mM; (○) - 0.017 mM. Concentration of 1% represents 0.4 nkat/mL.

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