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Catalytic and Thermodynamic Properties of a Tannase Produced by *Aspergillus niger* GH1 Grown on Polyurethane Foam

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Abstract Tannase is an inducible enzyme with important applications in the food and pharmaceutical industries. This enzyme was produced by the fungus *Aspergillus niger* GH1 under solid-state fermentation using polyurethane foam as solid support and tannic acid as sole carbon source and tannase inducer. Physicochemical properties of *A. niger* tannase were characterized, and the kinetic and thermodynamics parameters on methyl gallate hydrolysis were evaluated. The enzyme was stable in a pH range of 2–8 and a functional temperature range of 25–65 °C. The highest k_{cat} value was 2,611.10 s^{-1} at 65 °C. Tannase had more affinity for methyl gallate at 45 °C with a K_M value of 1.82 mM and an efficiency of hydrolysis (k_{cat}/K_M) of 330.01 s^{-1} mM^{-1}. The lowest E_a value was found to be 21.38 kJ/mol at 4.4 mM of methyl gallate. The lowest free energy of Gibbs (ΔG) and enthalpy (ΔH) were found to be 64.86 and 18.56 kJ/mol, respectively. Entropy (ΔS) was −0.22 kJ/mol K. Results suggest that the *A. niger* GH1 tannase is an attractive enzyme for industrial applications due its catalytic and thermodynamical properties.

Keywords *Aspergillus niger* GH1 · Tannase · Thermodynamic parameters · Catalytical properties

Introduction

Tannase or tannin acyl hydrolase (EC, 3.1.1.20) is an enzyme that catalyzes the hydrolysis of ester and depside bonds present in hydrolysable tannins, e.g., tannic acid, releasing glucose, and gallic acid [1]. It can be extracted from vegetal and animal sources, but for its industrial production, microbial sources are preferred [2].

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Tannase has several important applications mainly in food and pharmaceutical industries. The principal uses of tannase are in the elaboration of instantaneous tea and the production of gallic acid by hydrolysis of tannin-rich materials [3]. But due to its hydrolytic and synthetic properties, tannase have several potential uses, such as the clarification of tannin-rich beverages [4], the synthesis of antioxidants used in food industry [5, 6], the elucidation of the structure of polyphenolic compounds [7], and the additive in laundry detergents [8]. Tannase could also be utilized for improvement of nutritional properties of legume flours and animal feed [9, 10], bioremediation of tannin-contaminated wastewaters [11], and in the pre-treatment of lignocellulosic materials used for ethanol production [12].

Filamentous fungi, mainly of the genus *Aspergillus*, have been widely used for tannase production [13]. Although tannase production by *Aspergillus* can occur in the absence of tannic acid, this fungus tolerates tannic acid concentrations as high as 20% without having a deleterious effect on both growth and enzyme production [14].

Tannase production has been studied in submerged fermentation (SmF) and under solid-state fermentation (SSF) conditions [15]; however, two major differences are found when submerged and solid-state conditions are compared: (a) Tannase production and productivity are higher in SSF than in SmF and (b) tannase location under SSF conditions is mostly extracellular, while it is bounded to the mycelium under SmF conditions. In SSF, inert supports such as sugar cane pith or polyurethane foam, impregnated with a defined culture medium, have been also used [16, 17].

Catalytic and thermodynamic properties of fungal enzymes depend on the production system [3]. Knowledge of these properties is necessary for the design of efficient bioprocess based on the use of enzymes. Since very few reports exist concerning thermodynamic parameters of fungal tannases [18–21], the aim of this work is to provide thermodynamic parameters of an extracellular tannase produced by *Aspergillus niger* GH 1. The knowledge of these parameters will allow establishing the optimal conditions of enzymatic process for an efficient treatment of tannin removal and for production of gallic acid.

Materials and Methods

Microorganism and Inocula

The *A. niger* GH1 strain from the DIA-UAdeC collection (Saltillo, Coahuila, México) was used in this study. Two hundred fifty-milliliter Erlenmeyer flasks containing 30 mL of mycological agar were inoculated with *A. niger* spores. Erlenmeyer flasks were incubated at 30 °C for 6 days. After this, spores were harvested with Tween 80 sterile solution (0.01%, *v*/*v*), and these were then counted on a Neubauer chamber.

Culture Media

Composition of culture medium (in grams per liter) was $NaNO_3$ (7.65), KH_2PO_4 (3.04), $MgSO_4$ (1.52), KCl (1.52), and tannic acid (25). The mineral medium was sterilized at 121 °C for 15 min and cooled. Then, tannic acid was added and the pH adjusted to 5.5–6 with 1 N KOH. The liquid medium was filter-sterilized through 0.45 μm nylon membranes (Millipore, Billerica, MA, USA) and inoculated with the spore suspension at 2×10^7 spores per gram support. Polyurethane foam (PUF), gifted by Poliuretanos de México S. A. de C. V., was used as solid support. It was cut into 0.5 cm cubes and washed twice with water at 60 °C followed by a wash with cold water. The PUF was then dried at 60 °C in an

incubator. Reactors containing 150 g of PUF were impregnated with 350 mL of inoculated medium, prepared as described above in a ratio of 70:30, and were incubated at 30 °C for 30 h.

Tannase Recovery

The PUF was washed with 50 mM citrates buffer (pH 5.0). Enzymatic crude extract containing extracellular tannase was obtained by compressing PUF. Extract was filtered through a nylon membrane of 0.45 μ (Millipore, Billerica, MA, USA) using a vacuum microfiltration system. The enzyme was recovered to apparent homogeneity from solid-state culture using a protocol of solvent precipitation, dialysis, and gel chromatography. Briefly, enzyme was precipitated with acetone in a 1:1 ratio. Further, the precipitated protein was re-dissolved in 50 mM acetate buffer (pH 5.0) and dialyzed against water. Dialyzed extract was filtered through a cellulose membrane 0.45 μm and then was applied into a HiTrap™ G25 column (Amersham, Piscataway, NJ, USA). Enzyme was eluted with 5 mL of the same buffer and a flow of 1 mL/min.

Tannase and Protein Assays

Tannase activity was assayed using HPLC methodology, essentially as described by Beverini and Metche [22] with slight modifications. In brief, enzyme (50 μL) was added to 1 mL of 3 mM methyl gallate. The reaction mixture was incubated at 30 °C for 30 min. The reaction was stopped with 2 M HCl. Each sample was filtered through a 45-μm membrane prior to HPLC analysis. One unit of enzyme (international units) was defined as the amount of enzyme able to release 1 μmol gallic acid per minute of culture filtered under the standard assay conditions. Protein estimation was done as described by Bradford [23].

Effect of Temperature and pH on Tannase Activity

Effect of temperature was studied in a range of 20–65 °C at different concentrations such as 0, 1.4, 3, 4.4, and 5.8 mM of substrate (methyl gallate) in citrate buffer 50 mM at pH 5. Effect of pH on tannase activity was studied using acetate buffer at which covered a pH range of 3.5–5.5, 0.2 M phosphate buffer covering a range of 6–8, and finally buffer 0.2 M Tris–HCl to cover a pH 8.5–9. Effect of pH on tannase activity was evaluated using methyl gallate as substrate at different concentrations (1.4, 3, 4.4, and 5.8 mM) for each buffer. For both studies, effect of temperature and pH on tannase activity was evaluated as described above. Initial velocity (V_o) was expressed as micromoles per liter per minute.

Estimation of Kinetic Constants K_M and k_{cat}

K_M (affinity constant) values were calculated in a temperature range of 25–65 °C using methyl gallate as substrate at initial concentration in the range of 1.4–5.8 mM. Enzyme kinetic studies were carried out for 30 min, and initial enzyme activity rates were calculated. Kinetic constants were then determined. Values of kinetic parameters K_M obtained from Lineweaver–Burk plots were re-evaluated using the Michaelis and Menten equation under the simulator and algorithm Excel Solver. This procedure was done minimizing the errors of the estimated kinetic parameters. The k_{cat} (turnover number) was calculated from the following equation: k_{cat}=(S.A.) M_r, where (S.A.) is the specific activity of the enzyme and M_r (225,000 Da) is the molecular mass previously estimated by Mata-

Gómez and co-workers [24]. The catalytic efficiency (k_{cat}/K_M ratio) was calculated from the K_M values to each temperature.

Determination of Activation Energy (E_a) and Thermodynamic Parameters (ΔG, ΔH, and ΔS)

Activation energy (E_a) and thermodynamic activation parameters (ΔG, ΔH, and ΔS) were calculated as described by Kasieczka-Burnecka and co-workers [25]. E_a was determined from the slope ($-E_a/R$) of Arrhenius plots (log V as a function of $1/T$). Thermodynamic activation parameters were estimated by using the following equations: $\Delta G=\Delta H-T\Delta S$, $\Delta H=E_a-RT$ and $\Delta S=2.303R$ (log $k_{cat}-10.753-\log T+E_a/2.303RT$), where ΔG corresponds to Gibb's free energy (kilojoules per mole), ΔH is the enthalpy (kilojoules per mole), and ΔS is the change in entropy (kilojoules per mole kelvin), R is the universal gas constant (8.3145 J/mol K), and T is the absolute temperature (in kelvin).

Results

Tannase Production and Recovery

A. niger GH1 produced extracellular tannase in SSF. After washing with buffer, it was obtained as a crude extract with a volumetric activity of 81.9 IU/L and a specific activity of 4.3. Precipitation with acetone leaded to 6.5-fold purification. After the precipitation, the enzyme was fractioned using a HiTrap™ G25 Sephadex column (Amersham, Piscataway, NJ, USA). Fractions containing tannase activity were pooled and utilized for tannase characterization.

Effect of pH and Temperature

A. niger tannase remained active at pH 2.0–8.0. It showed an optimum activity at pH 6 (Fig. 1) at 30 °C as described previously by Mata-Gómez and co-workers [24]. Figure 2 shows the effect of temperature at different concentrations of substrate. Tannase activity was calculated as V_o (micromoles per liter per minute). Optimum temperature for enzyme

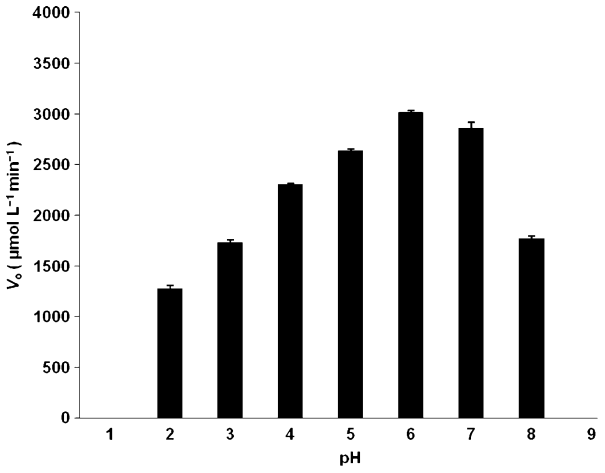

Fig. 1 Effect of pH on tannase activity from *A. niger* GH1

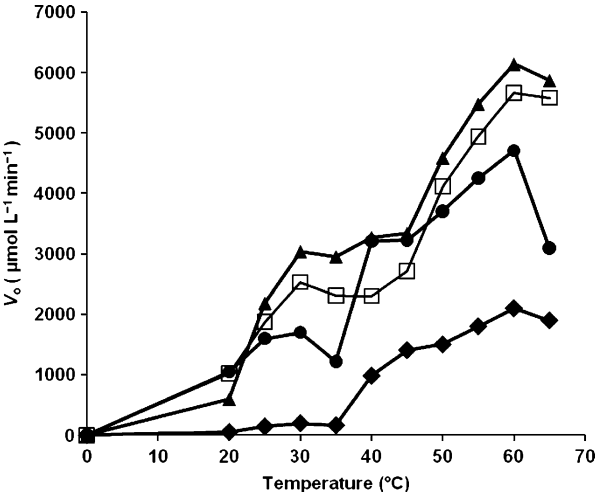

Fig. 2 Temperature–activity profiles of *A. niger* GH1 tannase at pH 5 using methyl gallate as substrate 1.4 mM (*closed diamonds*), 3 mM (*closed circles*), 4.4 mM (*closed triangles*), and 5.8 mM (*open squares*)

activity was found to be 60 °C at a methyl gallate concentration of 4.4 mM. However, tannase activity remained in a range of temperature from 20 °C to 65 °C. On the other hand, the effect of reaction time under optimal conditions (60 °C and pH 5) on tannase activity also was evaluated (Fig. 3). A value of 105.6 IU/L of tannase activity was obtained at 20 min of incubation, and then no significant increase was found (Fig. 3).

Kinetic and Activation Thermodynamic Parameters

The kinetic properties of tannase from *A. niger* GH1 were investigated, k_{cat} and K_M parameters, as well as the efficiency hydrolysis, k_{cat}/K_M, were determined using methyl gallate as substrate at a range of temperatures from 20 °C to 65 °C (Table 1). The highest turnover number, k_{cat}, was 2,611.10 s^{-1} at 65 °C. The comparison of K_M values for different temperatures exhibits that tannase has more affinity for methyl gallate at 45 °C with a K_M value of 1.82 mM and an efficiency of hydrolysis (k_{cat}/K_M) of 330.01 s^{-1} mM^{-1}. Whereas the highest efficiency for hydrolysis (k_{cat}/K_M) of tannase using methyl gallate was 350.18 s^{-1} mM^{-1} at 60 °C with a value K_M of 4.47 mM. Michaelis–Menten plot of

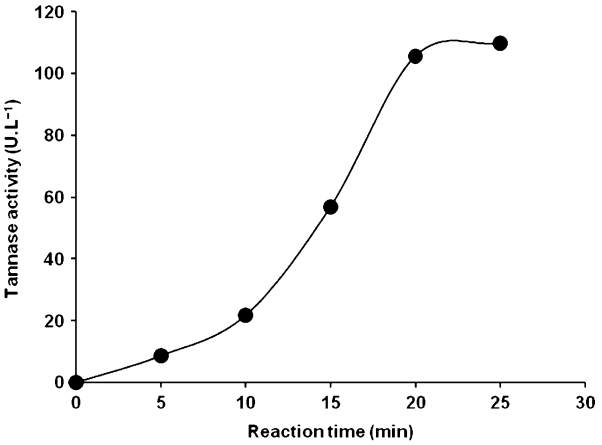

Fig. 3 Time course of incubation on tannase reaction from *A. niger* GH1 at optimal conditions (60 °C and pH 5) using methyl gallate (4.4 mM) as substrate

Table 1 Temperature dependence of kinetic parameters on methyl gallate hydrolysis by *A. niger* GH1 tannase

Temperature (°C)	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M (s^{-1} mM^{-1})
20	4.94	17.02	3.45
25	5.11	52.48	10.26
30	5.17	78.01	15.08
35	5.06	60.99	12.06
40	2.06	580.09	281.96
45	1.82	601.36	330.01
50	4.06	1,109.11	273.45
55	4.35	1,364.41	313.67
60	4.47	1,564.39	350.18
65	12.05	2,611.10	216.68

extracellular tannase at 60 °C and pH 5 is shown in Fig. 4. The values of kinetic constant K_M and V_{max} under these conditions were previously reported by Mata-Gómez and co-workers [24].

Activation energy (E_a) was calculated at different concentrations of substrate based on the slope from Arrhenius plot (Fig. 5). Table 2 shows the temperature dependence of activation energy (E_a) and thermodynamic activation parameters (ΔG, ΔH, ΔS) in methyl gallate hydrolysis catalyzed by tannase. The highest E_a value on methyl gallate hydrolysis was 84.94 kJ/mol at 1.4 mM, and the lowest was 21.38 kJ/mol at 4.4 mM. The lowest free energy of Gibbs (ΔG) was 64.86 kJ/mol observed at 20 °C in all concentrations of methyl gallate. The values of free energy of Gibbs for 3, 4.4, and 5.8 mM of methyl gallate were found to be 88.79, 84.70, and 91.62 kJ/mol, respectively, at 30 °C. By contrast, at 65 °C and 5.8 mM concentration of methyl gallate, the enthalpy (23.89 kJ/mol) was lower than the other methyl gallate concentration.

Discussion

Tannase purification fold 6.5 was lower as compared to 24–46-folds reported previously by several other authors [24, 26–28]. Some protocols for tannase purification report

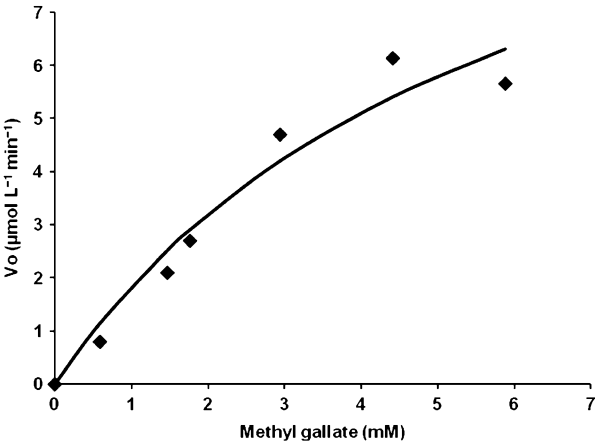

Fig. 4 Michaelis–Menten plot of *A. niger* GH1 tannase using methyl gallate as substrate (1.4–5.8 mM). Reaction was carried out at 60 °C and pH 5; experimental (*closed diamonds*) and adjusted (*line*) data

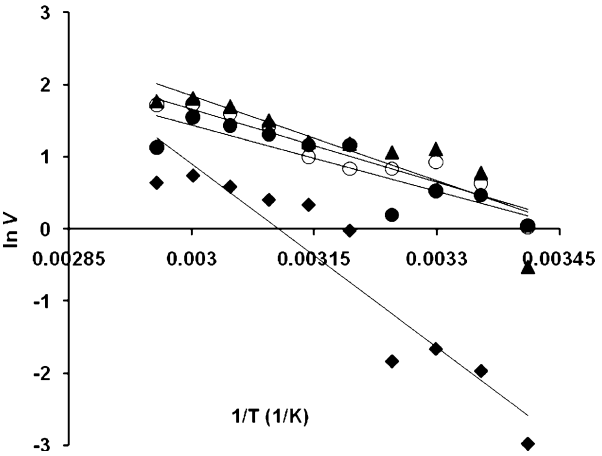

Fig. 5 Arrhenius plot for activation energy (E_a) estimation of tannase from *A. niger* GH1 at different concentrations of methyl gallate 1.4 mM (*closed diamonds*), 3 mM (*closed circles*), and 4.4 mM (*closed triangles*) in a temperature range of 25–65 °C and pH 5

ammonium sulfate precipitation as the first step followed by some techniques (generally chromatographic, ultrafiltration, etc.) for enzymes separation which are more sophisticated [26, 27, 29]; because of this, the purification folds are higher than those obtained using precipitation.

We have previously reported the effect of pH on *A. niger* GH1 tannase activity [24]. The enzyme showed high stability at extreme pH (2.0–8.0) and optimum activity at pH 6.0. These results are similar to other reported for tannases from several organisms including different strains of *A. niger* [29–31]. Most of the characterized tannases are optimally active in a pH range of 4.5–6.0, e.g., Kasieczka-Burnecka and co-workers [25] reported two *Verticillium* sp. tannases with an optimum activity at a pH of 5.5. Tannases from *Aspergillus awamori* and *Aspergillus heteromorphus* also are optimally active at pH 5.5 [32, 33]. *A. awamori nakazawa* exhibited optimum activity at pH 5.0 [34], and tannase from *Penicillium variable* was found to be stable at pH range of 3.0–8.0 [35]. The effect of pH on enzyme activity is given by the nature of the amino acids present at the tannase active site. Amino acid undergoes protonation and deprotonation, given place to induced conformational changes by the ionization of other amino acids [29]. Enzymes are very sensitive to changes in pH, and they function best over a very limited range, with a definite pH optimum [29].

Tannase from *A. niger* GH1 showed to be stable at a broad range of temperature (20–65 °C) and an optimum activity at 60 °C. These results indicate that the enzyme studied in this work is a thermostable enzyme because it shows activity at a wide range of temperatures. Most of tannases have been reported to have optimal temperature of activity between 30 °C and 40 °C [2]. However, it have been found a few thermoactive and thermostable tannases, e.g., Ramírez-Coronel et al. [30] reported the production of thermostable tannases by *A. niger* in SSF, and this enzyme have its optimal activity at 70 °C; more recently, Gonçalves and co-workers characterized an extracellular tannase from *Emericella nidulans* showing hypertolerance to temperature, and this enzyme showed temperature optima of 45 °C with a half-life of about 72 h at 90 °C [36]. Enzymes with high temperature optimum and thermostability are preferred for industrial applications [37, 38].

The value of kinetic constants (K_M and k_{cat}) depends on the particular substrate used and the enzyme source. K_M is a parameter, which is considered as a measure of the affinity of the enzyme by the substrate, to a lesser value of K_M, greater affinity. The turnover number (k_{cat}) is considered as a measure, which represents the moles of substrate transformed by

Table 2 Temperature dependence of activation energy (E_a) and thermodynamic parameters (ΔG, ΔH, and ΔS) on methyl gallate hydrolysis catalyzed by *A. niger* GH1 tannase

Substrate (mM)	Temperature (°C)	ΔG (kJ/mol)	E_a (kJ/mol)	ΔH (kJ/mol)	ΔS (kJ/mol K)
1.4	20	64.86	84.94	82.50	0.06
	25	148.15		82.46	−0.22
	30	148.26		82.42	−0.22
	35	149.97		82.37	−0.22
	40	145.20		82.33	−0.20
	45	146.11		82.29	−0.20
	50	145.47		82.25	−0.20
	55	145.89		82.21	−0.19
	60	146.48		82.17	−0.19
	65	146.00		82.12	−0.19
3	20	64.86	25.47	23.04	−0.14
	25	88.69		23.00	−0.22
	30	88.79		22.95	−0.22
	35	90.51		22.91	−0.22
	40	85.74		22.87	−0.20
	45	86.65		22.83	−0.20
	50	86.01		22.79	−0.20
	55	86.42		22.75	−0.19
	60	87.02		22.70	−0.19
	65	86.54		22.66	−0.19
4.4	20	64.86	21.38	18.94	−0.16
	25	84.59		18.90	−0.22
	30	84.70		18.85	−0.22
	35	86.41		18.81	−0.22
	40	81.64		18.77	−0.20
	45	82.55		18.73	−0.20
	50	81.91		18.69	−0.20
	55	82.32		18.65	−0.19
	60	82.92		18.61	−0.19
	65	82.44		18.56	−0.19
5.8	20	64.86	28.30	25.86	−0.13
	25	91.52		25.82	−0.22
	30	91.62		25.78	−0.22
	35	93.34		25.74	−0.22
	40	88.57		25.70	−0.20
	45	89.48		25.65	−0.20
	50	88.84		25.61	−0.20
	55	89.25		25.57	−0.19
	60	89.84		25.53	−0.19
	65	89.37		25.49	−0.19

1 mol of enzyme per unit of time, generally per second. Tannase from *A. niger* GH1 showed different values of K_M and k_{cat} for methyl gallate at different temperatures (Table 1). In this work, K_M value of 4.1 mM was obtained at 65 °C (optimal temperature). However, the

higher affinity for methyl gallate was attained at 45 °C with a K_M value of 1.82 mM. Kasieczka-Burnecka and co-workers [25] reported a two cold-adapted extracellular tannases from *Verticillium* sp. p9. Both tannases had K_M values of 3.65 and 2.43 mM using methyl gallate as substrate. A K_M value of 0.2 mM was obtained for tannase from *A. niger* van Tieghem by Sharma and co-workers [28], a value lower than the value reported in this work. These results show that tannase from *A. niger* GH1 exhibits kinetic constants similar to other fungal tannases reported in literature. Turnover number (k_{cat}) was found to be directly proportional to temperature. The major value of k_{cat} was attained at 65 °C (2,611.10 s^{-1}). Kasieczka-Burnecka and co-workers [25] reported a k_{cat} value of 228.3 and 170.9 s^{-1} at 25 °C and 20 °C, respectively, for a two cold-adapted extracellular tannases from *Verticillium* sp. p9. In this work, the highest k_{cat}/K_M values, 330.01 and 350.18 s^{-1} mM^{-1}, were found at 45 °C and 60 °C, respectively. Values of hydrolysis efficiency, k_{cat}/K_M, of 217.5 and 237.4 s^{-1} mM^{-1} were lower than those reported in this work [25].

The activation energy (E_a) in hydrolysis of methyl gallate by *A. niger* GH1 tannase was evaluated. The lowest value of E_a was found to be 21.38 kJ/mol (Table 2) at 4.4 mM of methyl gallate lower than 28.04 and 33.86 kJ/mol for *Verticillium* sp. p9 tannases [25]. Similarly, Yu and co-workers [21] reported an E_a value of 23.297 kJ/mol for a mycelium-bound tannase from *A. niger*. The lowest value of free energy of Gibbs (64.86 kJ/mol) was observed at 20 °C (Table 2). ΔG values (range to 64.86–149.97 kJ/mol) obtained at this work were higher compared to values close to 60 kJ/mol for tannase from *Verticillium* sp. p9 [25]. Also, mycelium-bound tannase from *A. niger* exhibited a value of ΔG of 113.77–118.31 kJ/mol [21]. Values of ΔH (range of 18.56–25.86 kJ/mol) at 3×10^{-3}, 4.4×10^{-3}, and 5.8×10^{-3} M of methyl gallate at 20–65 °C were similar than to 20.6 kJ mol^{-1} reported for tannase from *Verticillium* sp. p9 [25]. ΔS and ΔH for mycelium-bound tannase also were determinate showing values of −0.1511 kJ/mol K and 66.48 kJ/mol, respectively [3]. The lowest values of entropy (ΔS) of activation for methyl gallate hydrolysis were found in a range of −0.13 to −0.16 kJ/mol K at 3, 4.4, and 5.8 mM at 20 °C lower than those values of 130 kJ/mol K reported by Kasieczka-Burnecka and co-workers [25]. In addition, the activation energy (E_a) was quite small to 4.4 mM of methyl gallate which suggests that the catalysis of the enzyme is given at low levels of energy as described by Yu and co-workers [21]. Since industrial point view, the use of enzymes with low values of activation energy reduces production costs in terms of energy. Entropy values of tannase were all negative which is a unique typical behavior in biocatalytic systems as reported by Yu and co-workers [21].

The first of the works related to this topic was that published by Tewari et al. [18] on the effect of temperature (20–35 °C) on the hydrolysis reaction of 3,4,5-trihydroxybenzoic acid propyl ester (*n*-propyl gallate) by tannase to release 3,4,5-trihydroxybenzoic acid (gallic acid) + propan-1-ol. The change in binding of the hydrogen ion for this biochemical reaction in aqueous solution was calculated both from an equilibrium model for the biochemical reaction and from the dependence of the apparent equilibrium constant on pH. It was found that the equilibrium yield of 3,4,5-trihydroxybenzoic acid propyl ester is significantly enhanced by carrying out the reaction in toluene rather than in the aqueous buffered solutions [19].

A kinetic and thermodynamic study was reported on the esterification of propyl gallate from gallic acid and 1-propanol by mycelium-bound tannase from *A. niger* in organic solvent [21]. The activity of tannase increased with temperature up to a threshold at 47.5 °C, and successive fell beyond this value enlightened the occurrence of reversible biocatalyst inactivation. The experimental results confirmed that the deactivation process of

mycelium-bound tannase follows first-order kinetics pattern, and the mycelium-bound enzyme showed improved stability in organic solvent. In consideration of both the activity and stability of tannase, the optimum reaction temperature for tannase-catalyzed esterification should be 40 °C. A kinetic model of esterification by mycelium-bound tannase was developed based on the Ping–Pong Bi–Bi kinetic mechanism, considering not only the effects by substrates and products but also tannase denaturation. A reasonable quality of fit was observed by fitting experimental rate data to the kinetic mode with an average correlation coefficient of 0.977. Additionally, Yu and Li [21] demonstrated that if neglecting the inactivation of tannase, the kinetic model fitted the corresponding experimental data poorly. These results indicated that it is important to study the enzyme stability to help simulate the enzymatic reaction process.

In contrast, Raab et al. [20] reported the galloylation (esterification with gallic acid) of catechins using a tannase from *A. niger* in room temperature ionic liquids. Immobilization of the tannase on Eupergit C substantially increased the esterification activity. Six out of seven tested ionic liquids proved adequate media for the esterification of (−)-epicatechin, with the highest yield (3.5%) in 1-butyl-3-methylimidazolium 2-(2-methoxyethoxy)-ethylsulfate. Synthesis of esters was favored with increasing concentrations of gallic acid (6.0% yield, 2 M gallic acid) and decreasing water content. However, water concentrations lower than 20% (*v*/*v*) resulted in a decrease of conversion due to inactivation of the tannase. Significant differences in the reaction yields were observed for the galloylation of epicatechin (5.4%), epigallocatechin (3.1%), and catechin (1.3%), but not for the individual (−)- and (+)-enantiomers. Raab et al. [20] demonstrated that tannase shows a broad specificity for the alcohol moiety and an absolute specificity for the acid portion of the ester.

Finally, a kinetic and thermodynamic study of *A. niger* GH1 tannase was performed. It is evident that temperature affects directly kinetic and enzyme thermodynamic parameters. When temperature was increased, more coalitions between substrate and enzyme molecules accelerated the reaction giving place to a better biocatalysis. This effect can be observed when the turnover number (k_{cat}) increased considerably. The entropy value of tannase was negative, which is unique in biocatalytic systems. The results indicate that the new *A. niger* GH1 tannase is an attractive enzyme for industrial applications due its thermostability, catalytic capacity, and thermodynamical properties.

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