Catal Lett (2012) 142:282–286
DOI 10.1007/s10562-011-0743-7

Effects of Polyhydroxy Compounds on Enzymatic Synthesis of L-Tryptophan Catalyzed by Tryptophan Synthase

Lisheng Xu · Zhiyuan Wang · Junzhong Liu · Pingting Mao · Hongjuan Zhang · Ji Gao · Qian Liu · Qingcai Jiao

Received: 1 October 2011 / Accepted: 11 November 2011 / Published online: 29 November 2011

Abstract The effects of polyethylene glycol (PEG) of different molecular (1000, 2000), glycerol, ethylene glycol on the catalytic activity of tryptophan synthase were studied. The results indicated that the addition of PEG 2000 increased the enzymatic activity of tryptophan synthase. The enzymatic activity of tryptophan synthase was enhanced 25.2% by 10 g L^{-1} PEG 2000. Reaction conditions were optimized by using 10 g L^{-1} PEG 2000 at pH 9 and 40 °C. L-Serine conversion rate reach 89.9% under the optimal conditions. The kinetic parameters indicated the specificity of TSase to substrate was improved.

Keywords Tryptophan synthase · Polyethylene glycol · Additives · Kinetic parameters

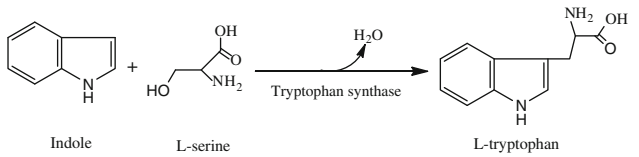
1 Introduction

L-Tryptophan is an essential amino acid, required for metabolism in mammals, which depend on plants and microorganisms for its supply [1]. L-Tryptophan has a potential market as an additive for animal feed, with estimates indicating a potential annual consumption of 11,000 t [2]. There are three methods for microbial production of L-tryptophan, i.e., the precursor-conversion fermentation method, direct fermentation method, and enzymatic method. The enzymatic method using tryptophan synthase (TSase; EC 4.2.1.20) or tryptophanase (Tpase; EC 4.1.99.1) has merits in terms of high purity of the product. Tryptophan synthase, which is mainly found in bacteria and plants, catalyzes L-tryptophan synthesis from indole and L-serine [3–5]. Enzymatic synthesis of L-halotryptophans catalyzed by tryptophan synthase [6]. Structures of tryptophan synthase catalysis were studied [7–9]. Enzymatic conversion method for production of L-tryptophan from indole and L-serine was developed by tryptophan synthase in our laboratory. The mechanism of L-tryptophan from L-serine and indole catalyzed by tryptophan synthase is suggested in Scheme 1. We found that the activity of tryptophan synthase was not high in our previous study. We hope to improve the enzymatic activity of tryptophan synthase by adding polyhydroxy compounds. The effects of polyhydroxy compounds on enzymatic synthesis of L-tryptophan catalyzed by tryptophan synthase were poorly studied.

The native conformation of many proteins and enzymes can be stabilized by surfactants and polyhydroxy molecules such as sugar, polyethylene glycols (PEGs), and glycerol, but there are also experimental results that indicate the direct binding of PEGs to proteins [10–12]. PEGs present attractive physical, chemical, and biological properties such as very low toxicity, good solubility in water and many organic solvents, and low melting point. Furthermore PEGs are economical and easily available in a variety of molecular weights [13]. In the present study, PEG of different molecular (1000, 2000), glycerol, ethylene glycol on the catalytic activity of tryptophan synthase were investigated. The kinetic parameters, fluorescence spectra and other factors such as pH, temperature, substrate mole concentration and reaction time were studied.

L. Xu · Z. Wang · J. Liu · P. Mao · H. Zhang · J. Gao · Q. Liu · Q. Jiao (✉)
State Key Laboratory of Pharmaceutical Biotechnology, School of Life Science, Nanjing University, Nanjing 210093, People's Republic of China
e-mail: jiaoqc@yahoo.com.cn

L. Xu
Department of Chemistry and Life Science, Suzhou University, Suzhou 234000, People's Republic of China



Scheme 1 The mechanism of reaction catalyzed by tryptophan synthase

2 Experimental

2.1 Materials and Reagents

Tryptophan synthase and L-serine were purchased from Sigma (St. Louis, Mo, USA). Glycerol, ethylene glycol, PEG 1000, PEG 2000 and indole were purchased from Shanghai Chemical and Medicine Co. Ltd. All chemicals are of analytical grade.

2.2 TSase Activity Assay

By measuring the tryptophan synthesized from L-serine and indole, TSase activity was determined with amino acid analyzer (Hitachi L-8900, Japan). The reaction was carried out in 50 mL containing 5 mmol L-serine, 5 mmol indole, additives of polyhydroxy compounds and 0.5 g TSase at different temperature and pH. One enzyme unit (U) was defined as the amount of TSase that synthesize 1 μmol of L-tryptophan per min from L-serine and indole. The specific activity is defined as unit/g of TSase.

2.3 Determination of Kinetic Parameters

The kinetic parameters, K_m and V_{max} values of TSase were determined by measuring initial rates of the reactions with L-serine as substrate. K_m and V_{max} values were calculated from Lineweaver–Burk plots by linear regression analysis.

$$V^{--1} = \{K_m/V_{max}\}[S]^{-1} + V_{max}^{-1}$$

Where [S] was the concentration of substrate, v and V_{max} represented the initial and maximum rate of reaction, respectively.

2.4 Fluorescence Spectra Measurement

The fluorescence spectra of TSase at various concentrations of PEG 2000 were recorded on Shimadzu RF-5300PC spectrofluorometer (Kyoto). The fluorescence spectra of TSase were measured in the presence of PEG 2000 at 25 °C in a 1 cm path length cell. TSase was excited at 295 nm, and the emission spectra were recorded from 300 to 400 nm.

3 Results

3.1 Effect of Polyhydroxy Compounds on TSase Activity

The effect of additives including Glycerol, ethylene glycol, PEG 1000, PEG 2000 were examined at 30 °C. Among the above additives, PEG 2000 has positive effect. As show in Fig. 1, TSase activity was enhanced to a maximum 25.2% compared to that in pure buffer at L-serine concentration of 100 mmol L^{-1}. TSase activity increased with the increasing PEG 2000 concentration supply, peaking at PEG 2000 concentration of 10 g L^{-1}, and decreased as the PEG concentration increased from 15 to 20 g L^{-1}. We can classify the four polyols in terms of their protective effect as follows: ethylene glycol < glycerol < PEG 1000 < PEG 2000.

3.2 Effect of pH and Temperature on TSase Activity

Enzyme activity can be affected by environmental factors such as pH and temperature. In this study, using L-serine and indole as substrate, a pH range from 4 to 10 was used to determine the optimal initial pH. As shown in Fig. 2, with the initial pH increasing from 4 to 9, TSase activity increased significantly and reached its maximum at pH 9 when PEG 2000 concentration was 10 g L^{-1}. TSase activity reached its maximum at pH 8 without PEG 2000. The optimal temperature was about 40 °C at pH 8.0 when PEG 2000 concentration was 10 g L^{-1} (Fig. 3). The optimal temperature was about 35 °C at pH 8.0 without PEG 2000 (Fig. 3).

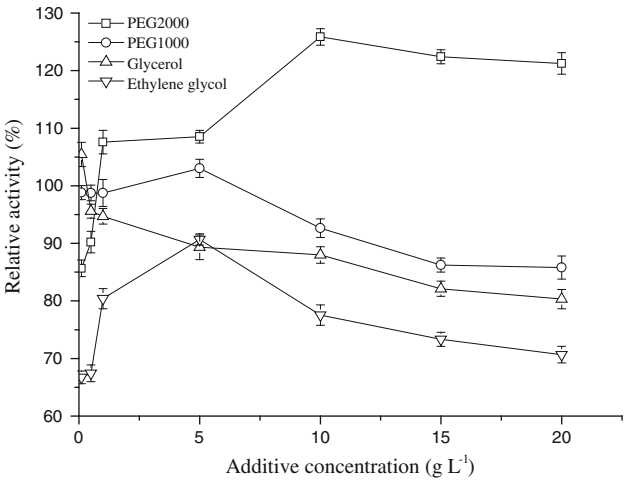


Fig. 1 Effect of polyhydroxy compounds concentration on the TSase activity. The reaction was carried out in 50 mL containing 5 mmol L-serine, 5 mmol indole, additives and TSase at 30 °C, pH = 7. The concentrations of L-tryptophan were measured after 2 hours

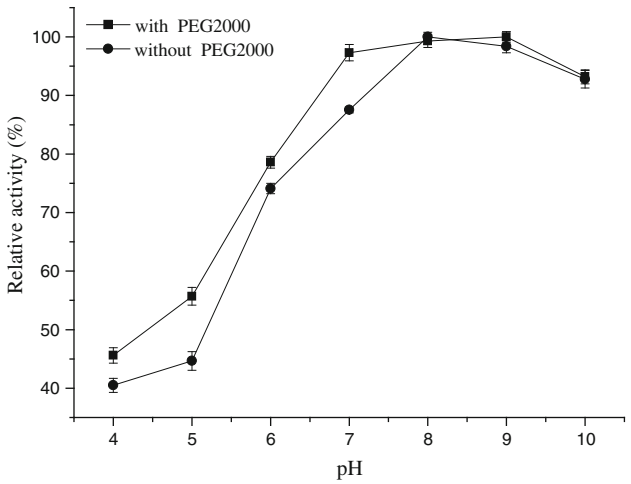


Fig. 2 Effect of pH on the TSase activity. The reaction was carried out in 50 mL containing 5 mmol L-serine, 5 mmol indole, 0.5 g PEG and TSase at 30 °C (*filled square*). The reaction was carried out in 50 mL containing 5 mmol L-serine, 5 mmol indole, 0 g PEG and TSase at 30 °C (*filled circle*). The concentrations of L-tryptophan were measured after 2 hours

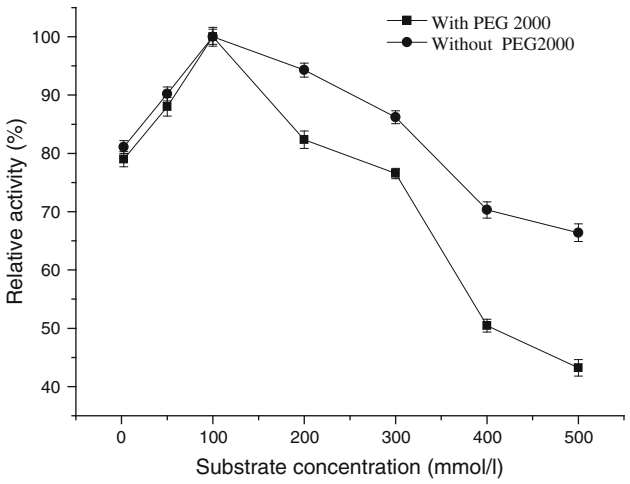


Fig. 4 Effect of substrate concentration on the TSase activity. The reaction was carried out in 0.5 g PEG and TSase at pH = 9 (*filled square*). The reaction was carried out in 0 g PEG and TSase at pH = 8 (*filled circle*). The concentrations of L-tryptophan were measured after 2 hours

3.3 Effect of Substrate Concentration and Reaction Time

The relationship between synthesis of TSase activity and substrate (L-serine) is shown in Fig. 4. The highest TSase activity occurred at L-serine concentration 100 mmol L^{-1}. The results indicated that TSase activity increased with L-serine concentration increased. TSase activity inhibition was observed at a high substrate concentration. The optimal substrate concentration would be 100 mmol L^{-1} when PEG 2000 concentration was 10 g L^{-1}. The reaction time was 2 hours when PEG 2000 concentration was 10 g L^{-1}. L-Serine conversion rate reach 89.9% under the optimal conditions. The optimal reaction time was 3.5 hours with PEG 2000 (Fig. 5).

3.4 Effect of Kinetic Parameters and Fluorescence Spectra of TSase

The kinetic parameters of TSase activity was presented in Table 1. The K_m, V_{max}, K_{cat} and K_{cat}/K_m of the enzyme in the absence of PEG 2000 were 4.309 mM,

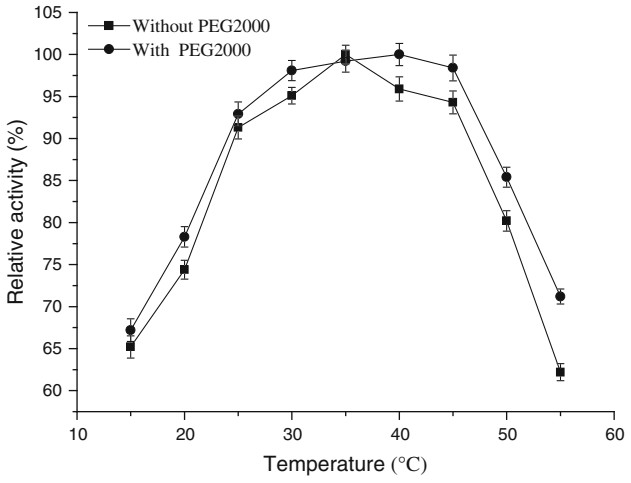


Fig. 3 Effect of temperature on the TSase activity. The reaction was carried out in 50 mL containing 5 mmol L-serine, 5 mmol indole, 0.5 g PEG and TSase at pH = 9 (*filled square*). The reaction was carried out in 50 mL containing 5 mmol L-serine, 5 mmol indole, 0 g PEG and TSase at pH = 8 (*filled circle*). The concentrations of L-tryptophan were measured after 2 hours

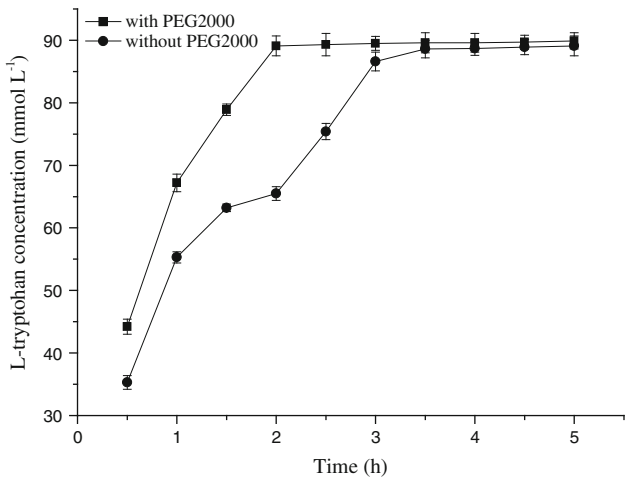


Fig. 5 Effect of reaction time on the TSase activity. The reaction was carried out in 50 mL containing 5 mmol L-serine, 5 mmol indole, 0.5 g PEG and TSase at 40 °C, pH = 9 (*filled square*). The reaction was carried out in 50 mL containing 5 mmol L-serine, 5 mmol indole, 0 g PEG and TSase at 35 °C, pH = 8 (*filled circle*)

Table 1 Kinetic parameters of TSase in the presence of PEG 2000 at 40 °C, pH = 9

PEG 2000 (g L^{-1})	V_{max} (mmol min^{-1} per^{-1} g^{-1})	K_m (mM)	K_{cat} (s^{-1})	K_{cat}/K_m (mM^{-1} s^{-1})
0	0.163	4.309	12.3	2.85
5	0.169	3.369	12.7	3.77
10	0.314	1.386	23.6	17.0
15	0.236	2.317	17.7	7.64
20	0.209	3.316	15.7	4.73

0.163 mmol min^{-1} per^{-1} g^{-1}, 12.3 $s^{-1}$and 2.85 mM^{-1} s^{-1}, respectively. When the concentration of PEG 2000 was 10 g L^{-1}, the value of K_m, V_{max}, K_{cat} and K_{cat}/K_m of the enzyme were 1.386 mM, 0.314 mmol min^{-1} per^{-1} g^{-1}, 23.6 $s^{-1}$and 17.0 mM^{-1} s^{-1}, respectively. Fluorescence spectra of TSase was measured in the presence of PEG 2000. The maximal intensity was observed at 330 nm. No change in the spectra was observed at the concentration 10 g L^{-1} of PEG 2000, suggesting that 10 g L^{-1} of PEG 2000 did not affect the structure of TSase (Fig. 6). The emission peak at 330 nm was not shifted, but the shape of the spectra between 310 and 350 nm changed with the increase in PEG 2000 concentration. This indicated that the microenvironment of TSase amino acid residues was changed with an increase of the PEG 2000 from 20 to 30 g L^{-1}.

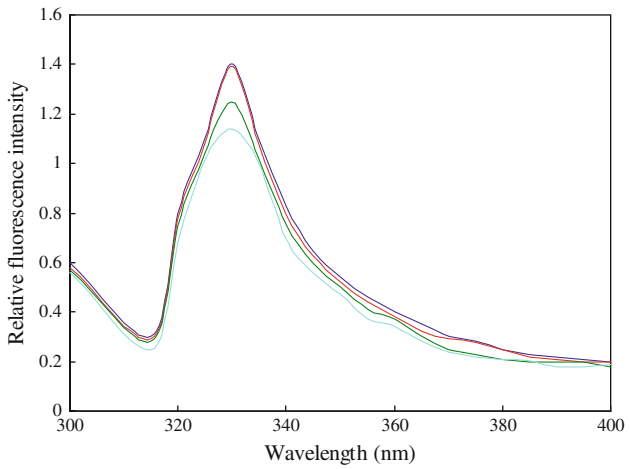


Fig. 6 Fluorescence spectra of TSase in the presence of PEG 2000. (*Red line*: 10 g L^{-1} PEG 2000, *Black line*: 0 g L^{-1} PEG 2000, *Green line*: 20 g L^{-1} PEG 2000, *Blue line*: 30 g L^{-1} PEG 2000). The fluorescence spectra of TSase were measured in the presence of PEG 2000 at 25 °C in a 1 cm path length cell. TSase was excited at 295 nm, and the emission spectra were recorded from 300 to 400 nm

4 Discussion

From these experiments, we can classify the four polyols in terms of their protective effect as follows: ethylene glycol < glycerol < PEG 1000 < PEG 2000. The hydroxyl groups of polyhydroxy compounds improved enzymatic stabilization. Enzymatic stabilization is related with the number of hydroxyl of polyhydroxy compounds. It is noticeable that this classification follows the increase in the polyol molecular masses. These results are in accordance with previous studies on the effect of polyhydric alcohols on enzyme stability [14–16]. Some studies have shown that polyhydric alcohol behavior is much different. Bull and Breese reported that *n*-alcohols bound strongly to protein molecules and dehydrate them which lead to their denaturation [17]. However, Nurul Alam et al. [18] found that *n*-alcohols act as activators of thermolysin-catalyzed peptide synthesis. The mechanism by which polyhydric alcohols stabilize biological macromolecules is generally well documented. Polyhydric alcohols do not denature proteins and contribute to enzymatic stabilization [19]. In the present study, the effect of additives including Glycerol, ethylene glycol, PEG 1000, PEG 2000 were examined. The result showed that PEG 2000 has positive effect, but ethylene glycol has negative effect. The reason may be PEG 2000 has been used to improve the TSase stabilization. The speed of reaction, catalyzed by TSase is significantly affected by pH and temperature. Generally, a high pH and temperature inhibit reaction. The TSase activity reached its maximum at pH 9.0 and 40 °C when PEG 2000 concentration was 10 g L^{-1}, which shifted 1 pH Units by PEG 2000 and was beneficial to the stability of TSase. The speed of reaction was improved by PEG 2000. The entails that the enzymatic efficiency expressed as K_{cat}/K_m is raised to about six times that obtained in buffer. The higher ratio of K_{cat}/K_m increase indicated TSase has a better specificity to the substrate due to the changes of environment around L-serine. The kinetic parameters indicated the specificity of TSase to substrate was improved. PEG could dehydrate availability and reduce hydrolyze effect of protein so as to stabilize the protein structure [13]. Fluorescence spectra of TSase indicated that the structure of TSase was not changed with adding 10 g L^{-1} of PEG 2000. The microenvironment of TSase amino acid residues was changed with an increase of the PEG 2000. PEG 2000 can form hydrogen bonds and be helpful to form a solvent layer of the protein surface. PEG 2000 can draw away water from their environment for their own hydration, generating a solvent layer of TSase that increases the surface tension and solution viscosity. Furthermore, PEG 2000 is a nonpolar polyol and can bind to the hydrophobic sites of proteins. PEG

2000 could dehydrate availability and reduce hydrolyze effect of TSase so as to stabilize the TSase structure at higher temperature. Hence, from the view of dynamics, it could draw a conclusion that PEG 2000 is able to improve catalytic activity of TSase.

5 Conclusion

In summary, PEG 2000 was found to be efficient additive in the TSase-catalyzed reactions. The enzymatic activity of tryptophan synthase was enhanced 25.2% by 10 g L^{-1} PEG 2000 at pH 9 and 40 °C. L-Serine conversion rate reach 89.9% under the optimal conditions. No change in the fluorescence spectra was observed at the concentration 10 g L^{-1} of PEG 2000, suggesting that 10 g L^{-1} of PEG 2000 did not affect the structure of TSase. The entails that the enzymatic efficiency expressed as K_{cat}/K_m is raised to about six times that obtained in buffer. PEG 2000 is able to improve catalytic activity of TSase.

Acknowledgment This work was supported by the National Technology-Innovation Fund (02CJ-13-01-16) and the Open Fund of State Key Laboratory of Pharmaceutical Biotechnology of Nanjing University, P.R. China.

References

1. Mateus DMR, Alves SS, Fonseca MMR (2004) J Biosci Bioeng 5:289
2. Toromanoff E (1986) Biofutur 49:39
3. Bang WG, Lang S, Sahm H, Wagner F (1983) Biotechnol Bioeng 25:999
4. Phillips RS (2004) Tetrahedron Asymmetr 15:2787
5. Dallmann K, Orosz LS, Szajani B (1997) Biotechnol Lett. 19:123
6. Goss RJ, Newill PL (2006) Chem Commun 47:4924–4925
7. Dierkers AT, Niks D, Schlichting I, Dunn MF (2009) Biochemistry 48:10997
8. Ehrmann A, Richter K, Busch F, Reimann J, Albers SV, Sterner R (2010) Biochemistry 49:10842
9. Nishio K, Ogasahara K, Morimoto Y, Tsukihara T, Lee SJ, Yutani K (2010) FEBS J 9:2157
10. Sheth SR, Leckband D (1997) Proc Natl Acad Sci USA 94:8399
11. Kita Z, Arakawa T, Lin TZ, Timasheff SN (1994) Biochemistry 33:15178
12. Timasheff SN (1998) Adv Protein Chem 54:355
13. Zhi LF, Jiang YC, Wang YS, Hu MC, Li S, Ma YJ (2007) Biotechnol Prog 23:729
14. Ye WN, Combes D, Monsan P (1988) Enzym Microb Technol 10:498
15. Athès V, Guerra P, Combes D (1999) J Mol Catal B: Enzym 7:1
16. Combes D, Graber M, Ye WN (1990) Ann NY Acad Sci 613:559
17. Bull HB, Breese K (1978) Biopolymers 17:2121
18. Nurul Alam M, Tadasa K, Kayahara H (1996) Biotechnol Lett. 18:45
19. Mondher M, Emmanuel P, Veronique L, Mohamed M (1998) Enzym Microb Technol 23:392