Note

Substrate Preference of γ -Glutamyltransferase from Caps of Fruiting Bodies of *Lentinus edodes*

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γ -Glutamyltransferase (γ -GT) is found particularly at high levels in the peridermal region of caps of fruiting bodies of a commonly eaten mushroom, shiitake, *Lentinus edodes* (Berk) Sing. When the caps are macerated, the enzyme comes into contact with the endogenous substrate lentinic acid (LA)¹⁾ and forms desglutamyl-lentinic acid (DGLA),²⁾ which gives rise to pyruvate, ammonia and a thiosulfinate with the concomitant action of C-S lyase.³⁾ From the thiosulfinate arises a cyclic pentathiepane bearing the characteristic flavor of the shiitake mushroom.

In the previous studies we have shown that the mushroom γ -GT shares many properties with the animal γ -GT.^{2,3)} In spite of this, the two enzymes seem to be distinct entities; in contrast to the animal γ -GT, the mushroom γ -GT is highly resistant to treatment with proteolytic enzymes²⁾ and is significantly activated in the presence of chaotropic anions.⁴⁾ The present study was undertaken to

ascertain whether or not the mushroom γ -GT has distinct substrate specificity as well.

The enzyme preparations were obtained in microsomal fractions from shiitake fruiting bodies and hog kidneys by centrifugation of their homogenates at $105,000 \times g$ for 90 min. The enzyme reaction was followed by measuring the release of free *p*-nitroaniline and DGLA from γ -glutamyl-*p*-nitroanilide (γ -GPNA) and LA, respectively. The amount of free *p*-nitroaniline was determined by measuring absorbance at 410 nm, and the amount of DGLA in terms of pyruvate formed in the assay system coupled with C-S lyase from the shiitake mushroom.⁵⁾

Kinetic analysis revealed that the substrate specificity of the two enzyme is relatively stringent (Fig. 1). With γ -GPNA as substrate, the two enzymes gave similar values for the apparent Michaelis constant (K_m). With LA, the mushroom enzyme gave a significantly lower K_m value than that the kidney enzyme gave. A difference was also found in the maximal rate (V_{max}). The mushroom γ -GT gave similar V_{max} values for the two substrates, but the kidney γ -GT gave a significantly lower value for LA than for γ -GPNA. A further significant difference between the two substrates was detected in the kinetic parameter ratio, V_{max}/K_m , which has the property of a first-order rate constant at low unsaturating substrate levels; the mushroom γ -GT gave a 4.6 times higher ratio for LA than for γ -GPNA, whereas the kidney γ -GT gave a ratio for LA corresponding to 1/80 of the ratio observed for γ -GPNA. The net results of these findings are that LA serves as an active and specific substrate for the mushroom γ -GT, but is virtually inert for the kidney γ -GT.

The finding that LA is a practically inert substrate for the kidney γ -GT implied that LA may not be so readily metabolized in animal bodies as other γ -glutamyl peptides

FIG. 1. Lineweaver-Burk Plots for the Reactions of γ -Glutamyltransferases from Shiitake Fruiting Bodies (left) and Hog Kidneys (right).

The enzyme reaction was carried out with LA (●) and γ -GPNA (○) at 37°C in 0.1 M Tris-HCl buffer (pH 8.0). The amounts of *p*-nitroaniline and DGLA (in terms of pyruvate) were measured as previously described.^{2,3,5)} Nonlinear regressions of the kinetic parameters ($\pm$ standard errors) from these data, K_m (mM), V_{max} (nmol/ml per min) and V_{max}/K_m (per min), were as follows: with γ -GPNA, 3.3 ± 0.1 , 45 ± 0.1 and 13.7 ± 0.4 for the kidney enzyme, and 5.5 ± 1.7 , 39 ± 3 and 7.1 ± 1.0 for the mushroom enzyme, respectively; and with LA, 41 ± 9 , 7.1 ± 1.0 and 0.17 ± 0.04 for the kidney enzyme, and 1.28 ± 0.25 , 42 ± 4 and 33 ± 7 for the mushroom enzyme, respectively.

FIG. 2. Transfer of the γ -Glutamyl Moiety of Glutathione to Desglutamyl-lenticic Acid by γ -Glutamyl-transferase from Shiitake Fruiting Bodies.

In a series of test tubes was placed a reaction mixture consisting of 2 mM DGLA, 10 mM glutathione, 0.18 M Tris-HCl (pH 8.0) and the mushroom enzyme in an amount capable of releasing *p*-nitroaniline at a rate of 0.03 μ mol/ml per min from 3.5 mM γ -GPNA (○). At the indicated intervals during incubation at 37°C, the tubes were removed from the incubation bath and heated at 100°C for 2 min to terminate the reaction. The amount of LA formed was calculated from the decrease in DGLA (●).⁵⁾

are. γ -GT occurring in different tissues of a given animal species is known to exhibit similar relative activities with various substrates. It is therefore not unreasonable that LA will be little acted on by those γ -GT as well. No significant hazard was hitherto identified in association with shiitake consumption. This may also reflect the possible metabolic inertness of LA in the human body.

It can be envisaged from the available data²⁾ that the mushroom γ -GT functions in dual modes. The first mode of function is to catalyze hydrolysis of the γ -glutamyl linkage in LA to yield DGLA. The thiosulfinate arising from the subsequent action of C-S lyase on DGLA has been shown to have a potent effect against a number of bacterial species.^{6,7)} Therefore, the mushroom γ -GT is capable of initiating a self-protection mechanism against microbial infection of a wounded region of the mushroom cap.

The second mode of γ -GT function is to catalyze the transpeptidation reaction. In this reaction, *S*-alkylcysteine sulfoxides were proved to be much more active acceptors than glycylglycine,²⁾ which has been known to be among the best acceptors for the kidney γ -GT.⁸⁾ Therefore, it is highly probable that γ -GT is involved in the synthesis of

LA during the developmental stages of shiitake fruiting bodies.

To verify this hypothesis we followed conversion of DGLA into LA in the presence of the mushroom γ -GT and an excess amount of glutathione (Fig. 2). Formation of LA reached a plateau at 90% of the theoretical maximum value. No such ready formation of lenticic acid was observed with the kidney γ -GT. The hypothesis strengthened by this experiment resembles the idea presented by Hasegawa and Matsubara⁹⁾ that γ -GT is involved in the synthesis of γ -glutamyl peptides during glutamic acid fermentation with *Corynebacterium glutamicum*. The hypothesis presented here would also substantiate the interpretation of the finding of Aoyagi *et al.*¹⁰⁾ that *N,N'*-bis- γ -glutamylcystine as well as LA are among the most predominant peptides recovered in the ethanol extracts of dried shiitake mushrooms. However, since a ubiquitous γ -glutamyl donor, glutathione, has not been identified in the mushroom, the possibility cannot be excluded that the synthesis of these γ -glutamyl peptides is brought about by the action of γ -glutamylcysteine synthetase coupled to cleavage of ATP. Further work is required to determine which of the two possibilities is the case.

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