Glycine and lysine assays with enzymatic reactions and examination of detection conditions

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Abstract

Glycine is the amino acid which constitutes collagen and it has a role which maintains elasticity with glowing skin. It also exists in the backbone or the brain stem mostly, and works as neurotransmitter of a control system of the central nerves. In contrast, lysine tends to occur at the lowest levels in the human body; further decreases in lysine concentration cause failures in liver function that lead to increased serum saturated fat and cholesterol levels [1]. Analyses of free amino acids in biological fluids may therefore be useful in determining disease status in clinical diagnoses [2-3].

For the analysis of each amino acid concentration, such as high-performance liquid chromatography (HPLC) has been used generally; however, the conventional analytical methods are burdensome in terms of cost, time, and space requirements. HPLC methods for amino acids, for example, take several hours to complete. The development of simple and rapid analytical tools for measuring amino acid concentrations is desirable for the clinical diagnostics and food industries.

We have studied a novel approach for the detection of each amino acid that involved the use of aminoacyl-tRNA synthetase (aaRS) as a molecular recognition element [4-7], because aaRS mediates biosynthesis of proteins and peptides in the body, thus it is expected to a selective binding ability for corresponding amino acid.

In this study, in order to obtain a simple amino acid detection system, for the specific detection of glycine and lysine, glycyl-tRNA synthetase and lysyl-tRNA synthetase were used as the each amino acid recognition element respectively, and these were coupled to the measurement of hydrogen peroxide via several enzymatic reactions, and the results were analyzed by the Trinder's reagent spectrophotometric method and absorbance was measured at 556 nm.

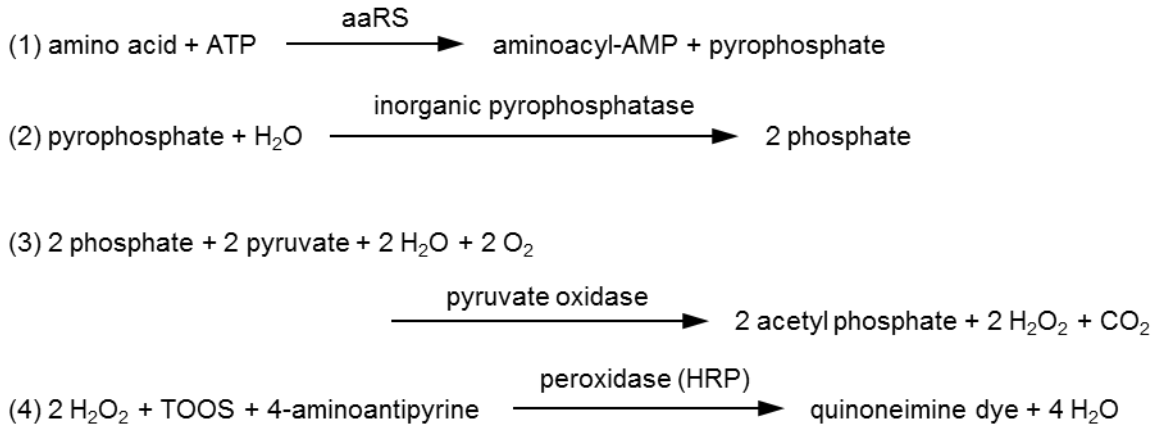
The consecutive enzymatic reactions used in this study are as follows: SerRS binds to its cognate amino acid, serine, and releases inorganic pyrophosphate (**Scheme**, Equation [1]). Hydrogen peroxide (H_2O_2) was produced by the reactions shown in Equations [2] and [3] and Trinder's reagent (TOOS) was injected into the reaction mixture (Equation [4]). The absorbance change at 556 nm was measured using a microplate reader, and the selective and quantitative responses of the biosensor were evaluated. This approach provided selective quantitation of up to 25 μM glycine and also showed selective response to glycine in 100 mM Tris-HCl buffer (pH 8.0) selectively (**Figure 1**).

This work was partly supported by JSPS KAKENHI Grant Number 25330344.

References

[1] A. Sánchez, D.A. Rubano, G.W. Shavlik, R. Hubbard, M.C. Horning, *Arch Latinoam Nutr.*, 38, (1988) 229-238.
[2] Y. Noguchi, Q.W. Zhang, T. Sugimoto, Y. Furuhata, R. Sakai, M. Mori, M. Takahashi, T. Kimura, T., *Am. J. Clin. Nutr.*, 83, (2006) 513S-519S.
[3] Y. Miyagi, *et al*., *PLoS ONE*, 6, (2011) 1-12.
[4] A. Kugimiya, M. Morii, T. Ohtsuki, *Anal. Biochem.*, 378, (2008) 90-92.
[5] A. Kugimiya, E. Takamitsu, *Mater. Sci. Eng. C*, 33, (2013) 4867-4870.
[6] A. Kugimiya, R. Fukada, D. Funamoto, *Anal. Biochem.*, 443, (2013) 22-26.
[7] A. Kugimiya, E. Matsuzaki, *Appl. Biochem. Biotech.*, (2014) accepted.

Figures



Scheme 1 Enzymatic reaction equations

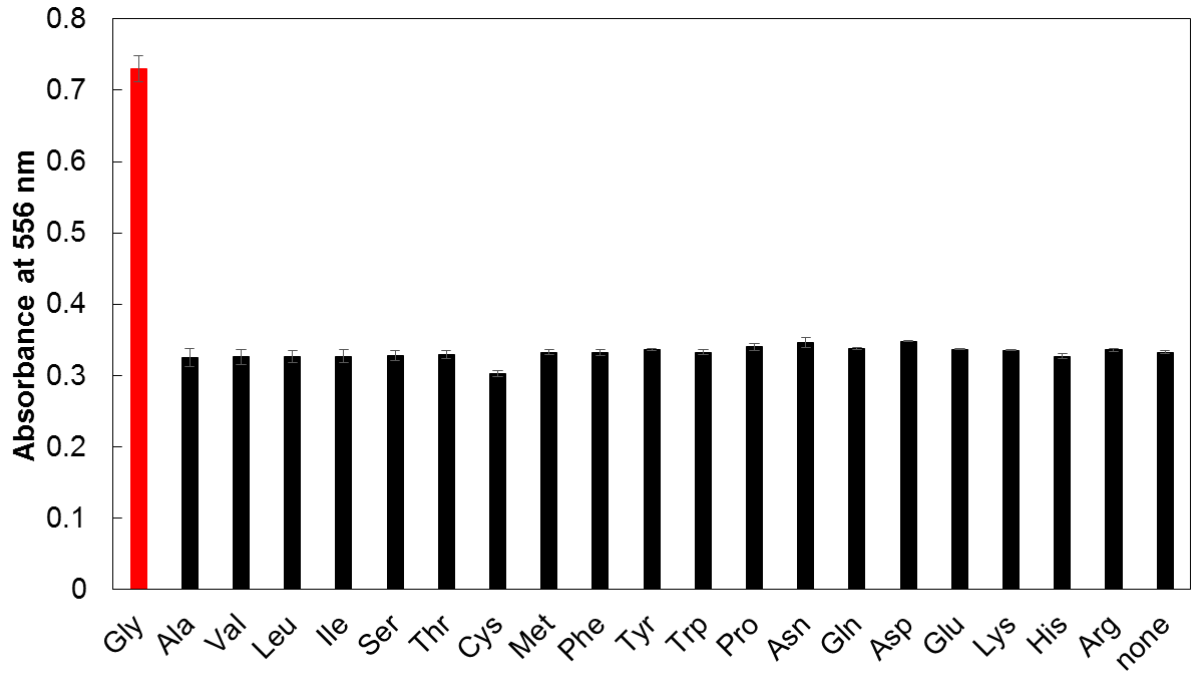


Figure 1 Selectivity test for 20 natural amino acids during Glycine sensing. Twenty natural amino acids, each at a concentration of 50 μM, were added to the reaction mixture, and the absorbance at 556 nm was measured using a microplate reader. Data represent the average of 3 measurements, and the standard deviation is indicated by error bars.