Application of a microfluidic gas-diffusion unit to flow-injection analysis of L-lysine with an L-lysine α-oxidase column

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A fluorometric flow-injection analysis (FIA) system with an immobilized L-lysine α-oxidase column as a recognition element was developed and applied to determination of L-lysine by using a microfluidic gas-diffusion device.

L-Lysine α-oxidase (from *Trichoderma viride*) was purchased from Yamasa Shoyu Co., Ltd., Chiba, Japan). Controlled-Pore glass (CPG, mean pore diameter 24.2 nm, particle size 120 – 200 mesh) as the supporting materials for the enzyme immobilization was purchased from Funakoshi Co., Ltd. (Tokyo). A gas-diffusion device and Teflon PTFE filter tubes (inner diameter: about 190 μm, outer diameter: about 250 μm) were kindly obtained from Dainippon Ink and Chemicals, Inc. (Tokyo). The L-lysine α-oxidase was covalently immobilized onto alkylaminated CPG as previously described [1]. The immobilized preparations were packed into a small polymer column and then mounted in a water-jacketed holder. The enzyme column (0.2 ml packed volume) was used as a recognition element for L-lysine. A schematic diagram of the flow system is shown in Fig. 1. This FIA system armed with the microfluidic gas-diffusion unit has advantages over the sensitivity in comparison with a conventional gas-diffusion unit [2, 3] because of the higher efficiency of gas permeability.

A 100 μl of standard L-lysine solutions with various concentrations was separately prepared and injected into the flow line of 100 mM phosphate buffer solution (pH 8.0, 0.2 ml/min) as a carrier solution in the sensing system. The injected L-lysine was catalyzed to form 2-oxo-6-aminohexanoate, H_2O_2, and NH_3 by the L-lysine α-oxidase column, and then the produced ammonia was transferred to the microfluidic gas-diffusion unit consisting of a double tubing structure. The fluorescent intensity of isoindole derivatives formed in the coupling reaction with ammonia molecules released in the enzymatic hydrolysis of urea and orthophthalaldehyde (OPA) reagents (20 mM OPA, 60 mM thioglycolate and 4 mM sodium tetraborate) was varied by gaseous ammonia diffusion across the gas-diffusion tubing, and subsequent increase in fluorescent intensity was successively monitored by a flow type of a fluorometer (λ_{ex} = 415 nm, λ_{em} = 485 nm). A good linear relationship between the concentration of L-lysine and the peak height (changes in fluorescent intensity) was obtained. The coefficient was calculated to be 0.997 and the relative standard deviation for urea determination with each concentration was about 3 %. The determination range was 5.0 ~ 200 μM.

This system should be applicable to various enzymes producing ammonia or carbon dioxide such as urease and other amino acid oxidases.

[1] I. Satoh, I.Sakurai, *Ann. NY Acad. Sci*., **864**, 493-496 (1998).
[2] Y. Iida, A. Koga, N. Hara, K. Matsumoto, I. Satoh, *J. Flow Injection Analysis*, **19**, 133-136 (2002).
[3] Y. Iida, N. Hara, K. Matsumoto, I. Satoh, *IEEJ Trans. SM*, **123**, 306 – 312 (2003).

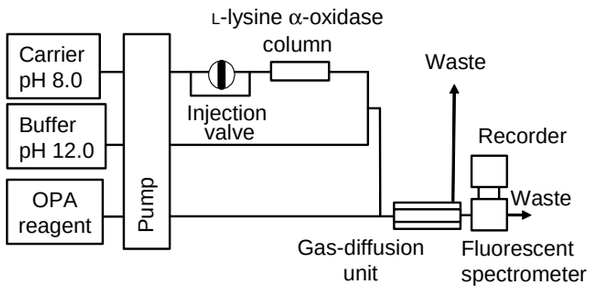

Fig. 1 Schematic diagram of the FIA system

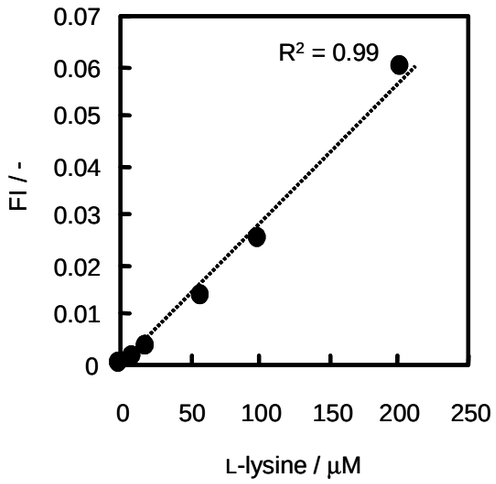

Fig. 2 Calibration graph for L-lysine