Supplementary Material (ESI) for Chemical Communications

Immobilization of enzymes on microchannel surface through cross-linking polymerization

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1) Reproducibility of the procedure developed in this study

In the amount (μg) of protein immobilized under optimized condition, the average and standard deviation values were 380 and 22, respectively. The coefficient of variations in the protease activity (yield of the hydrolysate / %) of this microreactor at each reaction time were within 3%. It was important to strictly control the reaction conditions such as pH, concentration, temperature, etc for high reproducibility of this procedure.

2) The protease activity per enzyme in the microreactor

In the PA/GA-prepared microreactor (amount of the captured protein = 380 μg, 15.2 nmol), the reaction yield reached 100% at residence time of 3 min (flow rate = 3.925 μl min^{-1}). The amount of the collected product solution (corresponding to 1 mM) was 3.925 μl for 1 min, corresponding to 3.925 nmol. If it is simply assumed that all proteins in this microreactor are active chymotrypsin, 1 nmol of the enzyme in this microreactor has an ability of hydrolyzing 258 pmol of substrate per min. In contrast, the hydrolysis abilities of the microreactors prepared using GA alone (0.25% and 2%) were lower than that of the PA/GA-prepared microreactor (Fig. S1). The procedure using GA alone might lead to deactivation of enzymes by over-reactivity of GA against the protein. It was found that the protease activity per enzyme in the microreactor prepared using PA alone was higher than those of GA alone. However, the procedures using 4% PA alone and 0.25% GA alone showed very low reproducibility due to the unsuccessful immobilization of enzyme.

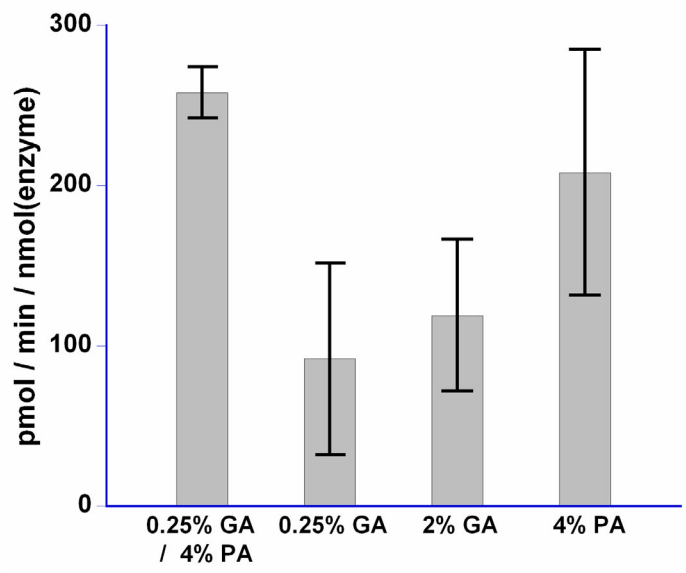

Figure S1. The protease activity per enzyme in the microreactor prepared using 0.25% GA and 4% PA, 0.25% GA only, 4% GA only, and 4% PA only. The amounts of GPNA hydrolyzed per min by the enzyme (1 nmol) in each microreactor are shown. Error bars indicate standard deviations.

3) Influences of the GA/PA ratio and the concentration of total aldehyde for the microreactor preparation

In Figure S2-A, the protease activity decreased with decreasing the amount of GA due to the unsuccessful membrane formation. In contrast, higher amount of GA promoted evenness of the membrane and tube obstruction. It was found that the GA/PA ratio (0.25% : 4%PA) is most suitable for microreactor preparation. Figure S2-B shows that the optimal concentration of aldehyde with a constant GA/PA ratio was 0.25% GA/4% PA – 0.5% GA/8% PA (●). However, preparations at such GA concentration without PA (▲) led to the failure of membrane formation, indicating that the combination of GA and PA is essential for stable preparation of the microreactor.

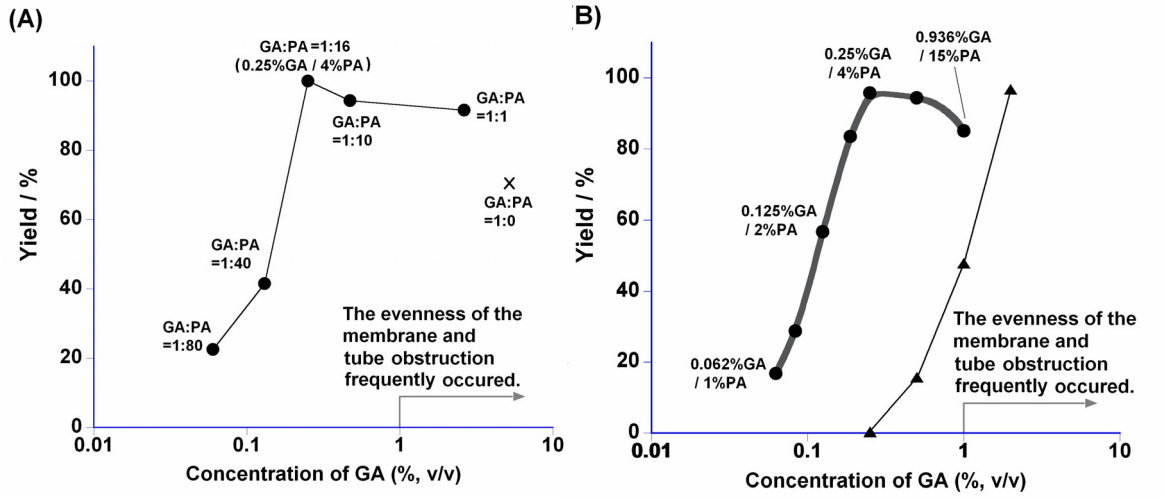

Figure S2. The dependency of protease activity on the GA/PA ratio and the concentration of total aldehyde. (A) The graph shows comparison of reaction yields of microreactors prepared using GA/PA with various combination ratios. The volume rate of aldehyde reagents (GA and PA) was kept a constant against total volume of reaction solution. There was no success in microreactor preparation at 1:0 of GA/PA ratio (5.25% GA) due to rapid tube obstruction (×). (B) Protease activities of microreactors prepared using GA/PA with various concentrations at a constant GA/PA ratio (●) and using GA only with various concentrations (▲). Horizontal axes in Figure S2-A and B indicate logarithmic plots of concentration of GA included in the reaction mixture.