Enzyme Immobilization in Glass Fiber Filters for Enzymatic Cascade Reactions in a Flow-Through System

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Introduction. Carbonic anhydrase (CA) plays important roles in living organisms. It catalyzes the hydration of CO_2 to yield HCO_3^- (*aq*) and H^+ (*aq*). This natural CA activity is often applied in bioreactors to control the CO_2 concentration in the atmosphere under mild conditions. CA has also esterolytic activity towards *p*-NA (*p*-nitrophenylacetate). In this research, we have investigated a model cascade reaction using bovine CA (BCA) and horseradish peroxidase (HRP) for basic studies in a glass fiber filter-based flow-through reactor. To immobilize BCA and HRP on the filters, dendronized polymer-enzyme conjugates were first prepared which then were adsorbed in the filter and finally examined with a BCA-HRP-catalyzed cascade reaction.

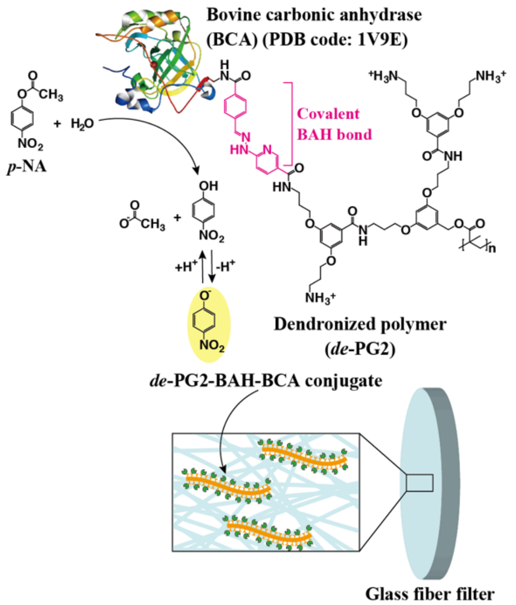

Fig. 1. Schematic illustration of dendronized polymer-BCA conjugates adsorbed in glass fiber filter.

Experiments. A conjugate between a water soluble, second generation dendronized polymer (abbreviated as *de*-PG2) and BCA was prepared through stable bisaryl hydrazone (BAH) bonds.[1] The conjugate was immobilized in glass fiber filters *via* stable non-covalent interactions.[2] The activity of the immobilized BCA was tested with 1 mM *p*-NA as substrate (pH = 7.2) (Fig. 1) in a flow-through-spectrophotometer system. A HRP conjugate was also prepared and immobilized in the same way. Using two filters with individually immobilized enzymes, a BCA-HRP catalyzed cascade model reaction was investigated with 2',7'-dichlorodihydrofluorescein diacetate ($DCFH_2$-DA) (50 µM) and H_2O_2 (10 µM) as substrates. Hydrolysis of $DCFH_2$-DA (colorless) and subsequent oxidation leads to the formation of DCF (2',7'-dichlorofluorescein) with absorption at $\lambda = 503$ nm.

Results and Discussion. The flow-through activity of immobilized BCA conjugate in glass fiber filter with *p*-NA is shown in Fig. 2 (A). Product formation is clearly observed (*p*-nitrophenolate with absorption at 405 nm). Without BCA, the rate of non-enzymatic hydrolysis is much slower, but not negligible. The resulting net product formation for up to 3 h is shown in Fig. 2 (B). Similar test were made with HRP filters. For the BCA-HRP cascade reaction, several combinations were tested: 1) BCA filter-HRP filter, 2) empty filter-HRP filter, and 3) only HRP-filter. In the case of 1), formation of DCF as final product was observed (~0.7 µM). In the case of 2) and 3), DCF formation was less effective (~0.3 µM and ~0.2 µM). This confirms the role of the BCA filter for running the cascade reaction efficiently.

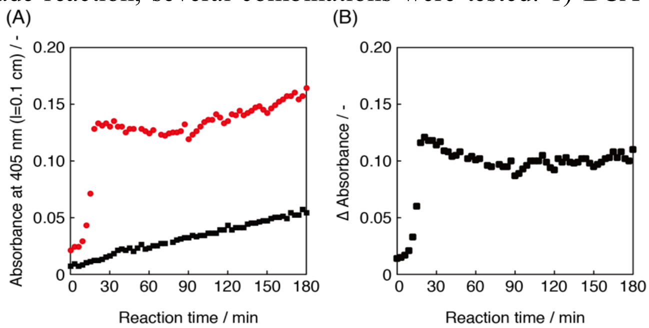

Fig. 2. (A) Flow-through activity measurements of BCA filter (red circles) and control filter (no BCA, black square): flow rate 3 µL/min. (B) Actual flow-through activity (red minus black).

References. [1] Küchler, A.; Messmer, D.; Schlüter, A. D.; Walde, P. *Methods Enzymol.* 590, 445-474 (2017). [2] Yoshimoto, M.; Schweizer, T.; Rathlef, M.; Pleij, T.; Walde, P. *ACS Omega* 3, 10391-10405 (2018).