Temperature-induced reversible control of the catalytic reaction of glucose oxidase using phenothiazine-labeled poly(ethoxyethyl glycidyl ether) as a mediator

Naotaka Nakadan, Shin-ichiro Imabayashi, and Masayoshi Watanabe Department of Chemistry and Biotechnology, Yokohama National University, Yokohama 240-8501, Japan

## Introduction

To shuttle electrons between the FAD group and the electrode, freely diffusing redox mediators with more positive redox potential than that of FAD have been frequently used.<sup>1</sup> Polymer redox mediators, instead of low-molecular-weight mediators, were employed to avoid the leakage of mediators from sensor systems.<sup>2</sup> But the use of polymer mediators resulted in the reduction of catalytic current due to their slow diffusion or loss of the electrochemical activity induced by conformational One advantage of the polymer mediator changes. systems, however, is that it affords polymer-based functions to mediators. We tried to elaborate thermally switchable mediators composed of a redox active phenothiazine(PT) and the thermo-sensitive polymer reported previously.<sup>3</sup> The aqueous solutions of thermosensitive polymers exhibit thermally-induced phase separation and turns from transparent into turbid above the cloud point  $(T_c)$ .

We report here the synthesis and basic characterization of PT-PEEGE with several molecular weights. The redox activity of PT-PEEGE drastically decreased at above  $T_c$ , leading to the thermal switching of the electrocatalytic reaction of GOx with PT-PEEGE mediators.

## Experimental

$$\sum_{S=NH}^{NaH, \bigtriangledown} \underbrace{NaH, \bigtriangledown}_{THF, 0\,°C} \xrightarrow{S}_{S=N}^{N} \xrightarrow{OH} \underbrace{KOH \ 0.5eq, \bigtriangledown}_{diglyme, \ 110\,°C} \xrightarrow{S}_{S=N}^{N} \underbrace{N \xrightarrow{O-(CH_2CHO)_{Th}}_{QH_2}}_{(CH_2)_2}$$



PT-PEEGE was synthesized by base-catalyzed anionic ring-opening polymerization (Scheme 1). Phenothiazine introduced at one end of the polymer was proved to work as a redox mediator for GOx. The number average molecular weight and molecular weight distribution of PT-PEEGE were determined to be 3150 and 1.52 by the <sup>1</sup>H-NMR spectroscopy and gel permeation chromatography, respectively.

The  $T_c$  of 1wt% (3.3mmol dm<sup>-3</sup>) PT-PEEGE aqueous solution was determined to be 28 °C from the temperature dependence of the transmittance of the solution at 500nm.

Cyclic voltammograms (CVs) were recorded at the scan rate of 10 mV s<sup>-1</sup> in 0.05 mol dm<sup>-3</sup> sodium acetate buffer (pH 5.1) using a conventional three-electrode cell equipped with a glassy carbon working electrode (0.071cm<sup>2</sup>), a Ag[AgCl] saturated NaCl reference electrode, and a Pt wire auxiliary electrode after the N<sub>2</sub> purge for 20 min.

## **Results & Discussion**

Figure 1 shows CVs of 5.0 mmol dm<sup>-3</sup> PT-PEEGE measured at 25 (solid line) and 40 °C (dashed line) that are below and above  $T_c$  (28 °C), respectively. A pair of CV peaks with the peak separation of 68 mV appeared at 580 mV for both temperatures, corresponding to a quasi-reversible redox response of the phenothiazine groups.

The peak current, however, decreased by ten-fold from  $1.1 \times 10^{-5}$  A to  $1.2 \times 10^{-6}$  A, while temperature increased from 25 to 40 °C. The diffusion coefficient (*D*) of PT-PEEGE was determined to be  $2.6 \times 10^{-6}$  cm<sup>2</sup> s<sup>-1</sup> at 25 °C

from the sweep rate dependence of the anodic peak current. The hydrodynamic radius of PT-PEEGE molecule is calculated to be 0.84 nm from the Dvalue using the Stokes-



measured at a scan rate of 10 mV s<sup>-1</sup> at 25 (solid line) and 40 °C (dashed line).

Einstein equation. At temperatures higher than  $T_c$ , the polymer solution is phase-separated into the dilute and concentrated phases. In the concentrated phase, PT-PEEGE chains transformed from a coil to a globule state and simultaneously aggregated through hydrophobic interaction. The average *D* and diameter of PT-PEEGE aggregates at 40 °C were estimated by dynamic light scattering to be ca.  $1.0 \times 10^{-7}$  cm<sup>2</sup> s<sup>-1</sup> and 60 nm, respectively. As the diameter of the aggregates is ca. 70 times greater than the hydrodynamic radius of the PT-PEEGE molecule, it seems that most of the hydrophobic PT groups are incorporated into the aggregates and are difficult to electrically communicate with the electrode.

Figure 2 shows CVs of GOx (9  $\mu$ mol dm<sup>-3</sup>) mixed with PT-PEEGE (0.5 mmol dm<sup>-3</sup>) in the presence of 0.05 mol dm<sup>-3</sup> glucose (substrate-saturated condition) at 25 and 50 °C. A typical catalytic wave with a sigmoidal shape appeared at each temperature, suggesting that PT-PEEGE can work as a mediator for GOx. The catalytic current (i<sub>cat</sub>) at 50 °C is significantly smaller than that at 25 °C.

The ratio of the  $i_{cat}$  values at 650mV(1.0 : 0.58 : 0.22) at 25, 40, and 50 °C agrees well with the ratio of the CV peak currents for 0.5 mmol dm<sup>-3</sup> of PT-PEEGE at the three temperatures, (1.0 : 0.55 : 0.22). This supports that

the decrease in the  $i_{cat}$  originates from the phase separation of PT-PEEGE and that only PT-PEEGE molecules remained to be electroactive participated in the electrocatalytic reactions of GOx as mediators.





## References

- [1]Cass, A. E. G.; Davis, G.; Francis, G. D.; Hill, H. A.
  O.; Aston, W. J.;Higgins, I. J.; Plotkin, E. V.; Scott, L.
  D. L.; Turner, A. P. F. Anal. Chem. 1984, 56, 667-671.
- [2]Schuhmann, W.; Wohlschläger, H.; Lammert, R.;Schmidt, H.-L.; Löffler, V.; Wiemhöfer, H.-D.; Göpel, W. Sens. Actuators B, 1990, 1, 571-574.
- [3]Aoki, S.; Koide, A.; Imabayashi, S.; Watanabe, M. *Chem. Lett.*, **2002**, *11*, 1128-1129.
- [4]Schild, H. G. Prog. Polym. Sci., 1992, 17, 163.