Effect of Modification Site on the Electron Transfer Reaction of Glucose Oxidase Hybrids Modified with Phenothiazine *via* Poly(ethylene oxide) Spacer

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[Introduction] The covalent immobilization of mediators to the glucose oxidase (GOx) surface is an effective method to establish the direct electron transfer (ET) between the buried FAD center and an electrode. We found that a rapid ET from $FADH_2$/FADH to PT^+ was achieved by attaching phenothiazine labeled-poly(ethylene oxide) (PT-PEO) spacers to the lysine residues on the GOx surface. Particularly, the GOx hybrids prepared by attaching PT-PEO with the molecular weight of 3000 to surface lysine residues possess about 2000 times higher ET rate than the GOx hybrids with directly attached PT groups[(1), (2)]. The above studies suggest that the length and structure of a spacer chain and the number of mediators significantly affect the ET properties according to the so-called "wipe-mechanism". In contrast, the effect of the location of modified mediators has not been systematically elucidated using a common mediator and spacer chain. The aim of the present study is to elucidate the effectiveness of the PEO spacer in generating a fast ET between the FAD center and the electrode for acidic amino acid residue-modified GOx hybrids, and to evaluate the effect of the location of mediator modification (acidic amino acid residues *vs.* lysine residues) on the ET reaction of GOx hybrids. We newly synthesized two kinds of GOx-hybrids, GOx-(PT-PEO_{Am}) and GOx-(PT-PAm) hybrids, in which the PT groups are covalently modified *via* a PEO spacer and directly to acidic amino acid residues on the GOx surface, respectively. The electrocatalytic properties of these systems were investigated, and the obtained results were compared with those for the GOx hybrids where the PT groups were attached to lysine residues, in terms of the effect of the location of PT modification and the spacer length.

[Experimental] PT-PEO_{Am} with different molecular weights, which have an amino end group for the covalent immobilization to acidic amino acid residues, were synthesized and covalently bonded to surface aspartic or glutamic acid residues[(3)]. PT-PAm was prepared according to the reported procedure[(4)]. Sulfo-NHS was used for carbodiimides-promoted amide bond formation between amine group of mediators and carboxylic acid group of GOx surface to achieve the immobilization of PT-PEO_{Am} or PT-PAm to surface aspartic or glutamic acid residues. Cyclic voltammograms (CVs) were recorded from 0.3 to 0.7 V at the scan rate of 10 mVs^{-1} in the absence and presence of 0.05 mol dm^{-3} glucose using a BAS CV-50W electrochemical analyzer.

[Results and Discussion] The catalytic current (i_{cat}) measured under a diffusion-limited and substrate saturation condition as a function of the number of PT groups per GOx molecule for GOx-(PT-PEO_{Am}) and GOx-(PT-PAm) hybrids. To analyze the effect of the modification site and the PEO spacer on the catalytic reaction of GOx hybrids, the average first-order rate constant for the oxidation of $FADH_2$/FADH by PT^+, k_{obs}, was calculated from the i_{cat} value. Under a glucose-saturated and diffusion-limited condition, i_{cat} is a function of the $D_{GOx\text{-}hybrid}$ and k_{obs} values. Fig.1 represents the dependence of the k_{obs} for GOx hybrids on the modification site and the number and molecular weight of attached mediators. Without a PEO spacer, as shown in Fig.1(a), the GOx-(PT-PAm) hybrid clearly possesses a larger k_{obs} than the GOx-(PT-PA) hybrid at the same number of PT groups, although the pH condition of each is different. It is highly probable that the ET distance from $FADH_2$/FADH to PT^+ is shorter for PT groups of the GOx-(PT-PAm) hybrid than for those of the GOx-(PT-PA) hybrid, because the distance between the modification site and the FAD center corresponds to the ET distance. In contrast, similar k_{obs} values were obtained for both GOx-(PT-PEO_{Am}) and GOx-(PT-PEO) hybrids in which PT groups are bonded to the GOx surface *via* PEO of the optimum length (molecular weight of 3000), as shown in Fig.1(c). This suggests that the disadvantage in attaching PT groups to the sites far from the FAD center for the lysine modification can be compensated by using sufficiently long PEO spacers, which enable PT groups to access the broad area on the GOx surface. Therefore, the ET reaction depended only on the length of the PEO spacer, irrespective of the modification sites. In the intermediate case between the two extreme cases described above, the GOx-(PT-PEO_{Am} 1000) hybrid shows a slightly higher k_{obs} value than that of GOx-(PT-PEO) hybrid. The short PEO spacer cannot completely compensate the disadvantage in modifying amino acid residues far from the FAD center[(5)].

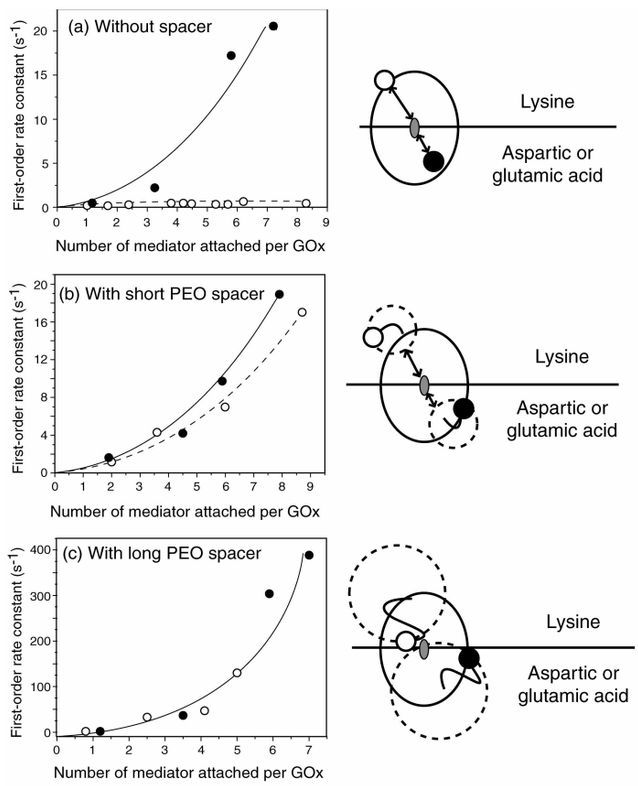

Fig. 1 Relationship between the number of mediators attached per GOx molecule and the rate constants for the ET from $FADH_2$/FADH to PT^+ in GOx hybrids modified (a) without spacer, (b) with molecular weight 1000, and (c) with molecular weight 3000.

[References]

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