Copper-Binding Peptide-Fragment-Containing Membrane as a Biocatalyst Prepared by Radiation-Induced Graft Polymerization

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The GlyGlyHis (GGH) peptide was introduced to glycidyl methacrylate (GMA)-grafted porous hollow fiber membrane made of polyethylene by radiation-induced graft polymerization. The GGH density in the membrane was 0.352 mmol/g-membrane. The copper sulfate solution was permeated outward through the GGH peptide-containing membrane, and Cu(II) was adsorbed on the membrane. Chemiluminescence between cypridina luciferin analog (CLA) and superoxide from the catalytic reaction with H2O2 and tyramine on the membrane was measured, and the Cu-binding GGH peptide-containing membrane exhibited a very strong chemiluminescence response. Furthermore, we evaluated the molecular structure of the repeating unit model of the Cu-binding GGH peptide-containing polymer brush without trunk polyethylene by computational chemistry.

1. Introduction

The prion protein (PrP) is known as a causative agent of bovine spongiform encephalopathy (BSE) and the human Creutzfeldt-Jacob disease (hCJD), and the PHGGGWGQ peptide at the amino terminus forms a complex with Cu(II) ion. Recently, Kawano has reported that Cu-binding PrP fragments catalyze the generation of superoxide in the presence of certain cofactors such as H2O2, O2, and aromatic monoamines (Fig. 1(a)). It was confirmed that a specific peptide sequence exists for the superoxide generation, and tyrosine-rich peptides enhance the catalytic reaction. Interestingly, this catalytic activity depends on the position of the hydroxyl group of the aromatic monoamine; it acted like the smallest artificial enzyme. The purpose of this...
study is to prepare a functional membrane that shows catalytic activity by introducing Cu-binding PrP fragments into the grafted porous hollow fiber membrane (Fig. 1(b)).

A grafted porous hollow fiber membrane is prepared by radiation-induced graft polymerization (RIGP). RIGP is very useful as a method of introducing various molecules and/or functional groups into a base material, and ion-exchange membranes, chelating resins, and lipase-immobilized membranes were developed using this method. The sample solution, driven by a pressure difference across the membrane, can be transported by convection through the pores of the grafted porous hollow fiber membrane. Therefore, the diffusional mass transfer resistance of the substances to the functional groups can be neglected. Furthermore, a wide variety of functional groups can be introduced at high density on the polymer brush. The low diffusional mass transfer resistance and high functional group density in grafted porous hollow fiber membranes enable the high reaction efficiency and high reaction rate (Fig. 1(b)).

The GlyGlyHis (GGH) peptide was used as a PrP fragment. The GGH peptide has been widely investigated in recent years: its physical properties, the application of the peptide to a sensor for detecting Cu(II) in the ppt range and the characteristics of nanofiber formed using peptides containing the GGH terminus. The structure of Cu(II)-binding GGH was clarified by X-ray structural analysis, and the complex takes the planar-tetracoordinate structure (Fig. 2).

Among aromatic monoamines, tyramine was used as a reactive substrate for superoxide generation by Cu-binding GGH in this study. It is well known that aromatic monoamines such as tyramine are involved in fermentation and corruption processes by microorganisms. HPLC is usually used to detect the aromatic monoamines generated by meat rotting and/or fermentation by microorganisms. However, the real-time detection of aromatic monoamines is difficult. The Cu(II)-binding GGH peptide membrane can be used as a real-time sensor material for aromatic monoamines owing to its high reaction efficiency and high reaction rate.

Fig. 1. PrP fragment-grafted porous hollow fiber membrane as a biocatalyst.
2. Experimental

2.1 Materials

A commercially available porous hollow fiber membrane made of polyethylene, supplied by Asahi Kasei Corporation (Japan), had inner and outer diameters of 1.31 and 3.23 mm, respectively, with a pore diameter of 0.36 mm and a porosity of 70%. It was used as the trunk polymer for grafting. Glycidyl methacrylate, hydrogen peroxide, tyramine, and 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (Cypridina luciferin analog, CLA) were purchased from Tokyo Kasei Co. (Tokyo, Japan). CLA is a chemiluminescence reagent specific to superoxide. The GGH peptide was obtained from the custom peptide service department of Sigma-Genosys Japan, K.K. (Ishikari, Hokkaido, Japan). Other chemicals used in this study were of reagent grade purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2 Preparation of the Cu-binding peptide-containing porous hollow fiber membrane

The preparation scheme for the peptide-containing porous hollow fiber membrane is shown in Fig. 3. An epoxy-group-containing monomer, glycidyl methacrylate (GMA) was grafted onto a porous hollow fiber membrane made of polyethylene by radiation-induced graft polymerization. The reaction conditions are shown in Table 1. The obtained membrane was referred to as the GMA membrane. The degree of grafting was defined as follows.

\[
\text{Degree of grafting (\%)} = \frac{100 \times \text{weight of polyGMA grafted}}{\text{weight of trunk polymer}}
\]

Subsequently, the epoxy group of polyGMA was converted to GlyGlyHis peptide (GGH) under the conditions shown in Table 2. The molar conversion of the GGH peptide from the epoxy group was defined as follows.
Molar conversion (%) = 100 (moles of GGH) / (moles of epoxy group before introducing GGH) (2)

To immobilize Cu(II) onto the obtained GGH peptide-containing membrane, a CuSO₄ solution was permeated outwards through the membrane. The Cu(II) concentration of the feed solution was set at 1.0 ppm. The initial pH of the feed solution was adjusted to 3 with HCl and NaOH. The amount of Cu(II) adsorbed onto the GGH peptide-containing membrane in equilibrium with $C_0$, i.e., the equilibrium adsorption capacity, $q_e$, was calculated from the following integration:

$$q_e = \int_0^{V_e} (C_0 - C) \, dV/W,$$
where $C_0$ and $C$ are the Cu(II) concentrations of the feed and effluent, respectively. The terms $V$, $V_e$, and $W$ are the effluent volume, the effluent when $C$ reached $C_0$, and the mass of the membrane, respectively.

2.3 Measurement of superoxide by chemiluminescence detection

The Cu-binding GGH peptide-containing membrane and other chemicals were dissolved in phosphate-buffered saline and the generation of superoxide was monitored from the chemiluminescence of CLA with a Luminescencer PSN (ATTO Co., Ltd., Japan) under the conditions shown in Table 3. The CLA chemiluminescence specifically indicates the generation of superoxide.\(^{(22)}\)

2.4 Geometry optimization of the Cu(II)-binding GGH peptide-GMA

The crystal structure model of the Cu(II)-binding GGH peptide was created on the basis of the X-ray structural analysis data of Carerman et al.\(^{(18)}\). The calculated crystal structure model of the Cu(II)-binding GGH peptide was calculated using the Density Functional Theory (DFT) at the B3LYP/6-311g+(d, p) level with Gaussian03 series program\(^{(23)}\) according to the calculation procedure of Kim et al.\(^{(24)}\). GMA moiety was added to the amino terminal of the calculated crystal structure model of the Cu(II)-binding GGH peptide, and the resultant structure model was geometry optimized using the DFT at the B3LYP/6-311g+(d, p) level (the calculated Cu(II)-binding GGH peptide-GMA). The valence angles and bond distances of all the models were measured and the Root Mean Square Deviation (RMSD) was calculated using the Scigress Explorer Ultra version 7.6.0.52.\(^{(25)}\)

3. Results and Discussion

3.1 Properties of peptide-containing membrane

The GGH peptide was introduced to the epoxy group of the GMA polymer brush by immersing the GMA membrane in the GGH aqueous solution at 353 K for 24 h. In this study, the degree of grafting of the GMA membrane was set to 85%. The molar conversion of the GGH peptide from the epoxy group, defined using eq. (2), was 12%, and the GGH density in the membrane was 0.352 mmol/g-membrane. The GGH peptide-containing membrane had inner and outer diameters of 1.38 and 3.31 mm,

Table 3
Materials and conditions for detecting superoxide.

| Material                                      | Value       |
|-----------------------------------------------|-------------|
| Cu(II) binding GGH peptide membrane (mg)      | 2           |
| pH 7.0 phosphate-buffered solution (mmol/L)   | 50          |
| $H_2O_2$ (mmol/L)                             | 0.15        |
| Tyramine (mmol/L)                             | 0.25        |
| CLA: chemiluminescence reagent (mmol/L)       | $3 \times 10^{-3}$ |
| Reaction temp. (K)                            | 303         |
respectively. The breakthrough curve of Cu(II) is shown in Fig. 4. Cu(II) was efficiently adsorbed on the GGH peptide-containing membrane. The molar ratio of GGH peptides in the membrane to the total amount of Cu(II) adsorbed on the membrane was 1.26. Approximately one GGH peptide captured one Cu(II) ion in the membrane.

3.2 Generation of superoxide in the presence of tyramine

Chemiluminescence between cypridina luciferin analog (CLA) and superoxide from the catalytic reaction was measured for three samples: without membrane, with GMA membrane, and with Cu-binding GGH peptide-containing membrane. The results are shown in Fig. 5. The GMA membrane, without a Cu-binding GGH peptide, did not show...
a chemiluminescence response, similarly to the blank solution without a membrane. On the other hand, the Cu-binding GGH peptide-containing membrane exhibited a very strong chemiluminescence response. Therefore, tyramine was reacted with O$_2$ and H$_2$O$_2$ on the Cu-binding GGH peptide-containing membrane, and the superoxide was generated in the same manner as previously reported, that is, PrP fragments catalyze the generation of superoxide.$^{(3)}$

3.3 Optimized structural model of the Cu-binding GGH peptide-GMA

We created the three models on the basis of the X-ray structural analysis data of the Cu(II)-binding GGH peptide, and using the computational chemistry: the Cu(II)-binding GGH peptide crystal, calculated Cu(II)-binding GGH peptide crystal, and the calculated Cu(II)-binding GGH peptide-GMA. The molecular structure of the calculated Cu(II)-binding GGH peptide-GMA is shown in Fig. 6. The model is the repeating unit of the Cu(II)-binding GGH peptide-containing polymer brush without trunk polyethylene. The calculated geometric parameters of all the models are shown in Table 4. No significant differences were observed for all bond distances and angles between the calculated Cu(II)-binding GGH peptide crystal and X-ray structural analysis data.$^{(18)}$ Figure 7(a) shows the overlay of the Cu(II)-binding GGH peptide crystal and the calculated Cu(II)-binding GGH peptide crystal for the quality of the structural prediction.$^{(18)}$ It was confirmed that the computational method is suitable for the structural prediction of the Cu(II)-binding GGH peptide derivatives. The valence angles and bond distances of the calculated Cu(II)-binding GGH peptide-GMA model were compared with that of the Cu(II)-binding GGH peptide crystal model. The valence angle of N2-Cu(II)-N4 was moved about 20°. The GGH moiety of the calculated Cu(II)-binding GGH peptide-GMA was distorted when GMA was added to the Cu(II)-binding GGH peptide crystal (Fig. 7(b)). The GGH peptide moiety in the calculated Cu(II)-binding GGH peptide-GMA...
forms a square-planar coordinate with Cu(II). The space for the superoxide generation reaction is maintained on the Cu(II) in the GGH peptide.

### Table 4
Geometric parameters of Cu(II)-binding GGH peptide moiety.

| Molecular structure model                  | Bond distance (Å) | Valence angle (°) |
|-------------------------------------------|------------------|-------------------|
| Cu(II)-binding GGH peptide crystal        |                  |                   |
| Cu(II)-N1                                 | 2.04             | N1-Cu(II)-N2      |
| Cu(II)-N2                                 | 1.90             | N1-Cu(II)-N4      |
| Cu(II)-N3                                 | 1.95             | N2-Cu(II)-N3      |
| Cu(II)-N4                                 | 1.96             | N2-Cu(II)-N4      |
| Calculated Cu(II)-binding GGH peptide     |                  |                   |
| crystal                                   |                  |                   |
| Cu(II)-N1                                 | 2.00             | N1-Cu(II)-N2      |
| Cu(II)-N2                                 | 1.85             | N1-Cu(II)-N4      |
| Cu(II)-N3                                 | 1.88             | N2-Cu(II)-N3      |
| Cu(II)-N4                                 | 1.88             | N2-Cu(II)-N4      |
| Calculated Cu(II)-binding GGH peptide-GMA |                  |                   |
| crystal                                   |                  |                   |
| Cu(II)-N1                                 | 2.01             | N1-Cu(II)-N2      |
| Cu(II)-N2                                 | 1.87             | N1-Cu(II)-N4      |
| Cu(II)-N3                                 | 1.87             | N2-Cu(II)-N3      |
| Cu(II)-N4                                 | 1.94             | N2-Cu(II)-N4      |

Fig. 7. RMSD overlay of the GGH moiety (a) of the Cu(II)-binding GGH peptide crystal and calculated Cu(II)-binding GGH peptide crystal (highlighted), (b) of the Cu(II)-binding GGH peptide crystal and calculated Cu(II)-binding GGH peptide-GMA (highlighted). The hydrogen atoms were omitted for clarity.
4. Conclusions

We prepared the porous hollow fiber membrane containing the Cu-binding GGH peptide as a biocatalyst by radiation-induced graft polymerization. The Cu-binding GGH peptide-containing membrane exhibited catalytic activity in the presence of tyramine and H$_2$O$_2$ to produce superoxide, and a strong chemiluminescence reaction between cypridina luciferin analog (CLA) and superoxide was observed. The superoxide generated by the Cu(II)-binding GGH peptide membrane can be used in various fields such as sterilization or deodorization. Furthermore, the Cu-binding GGH peptide-containing membrane in this study can be applied to a tyramine sensor in combination with the chemiluminescence detection system.

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