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Characterization of Hollow Fiber Immobilized Liposome Membrane by Using Aqueous Two-Phase Partitioning Systems

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The modification of the surface of a polysulfone (PSf) membrane in a hollow fiber module was performed based on the evaluated hydrophobicity of the polymers used in its modification to develop a new type of membrane module immobilizing liposome. The surface net hydrophobicity of the polyvinylpyrrolidone (PVP), to be modified, was first characterized by using the aqueous two-phase partitioning method. The hollow fiber module using the PVP-modified PSf membrane was characterized based on the adsorption behaviors of standard amino acids, showing that the membrane surface was found to have a hydrophilic surface nearly equal to the difference in aqueous two-phase systems. The immobilized liposome membrane (ILM) in the above module was found to have a hydrophobic potential, thus promoting the adsorption of the hydrophobic molecules on its surface.

1. Introduction

Hollow fiber membrane modules are widely applied for bioremediation, water purification, biomedical uses and could be a powerful tool in solving the above problems [1]. Membrane modules, consisted of hollow fiber membranes, have been produced by some membrane companies and utilized in many research areas [2]. A polysulfone (PSf)-based hollow fiber membrane is a porous membrane and the pore size is varied in a gradual manner (wide on the outside, narrow on the inside of a fiber) and is also used for medical uses such as artificial kidneys [3]. However, the modification of such a membrane surface is needed for such a use because some hydrophobic domains of the membrane surface could often adsorb biomolecules and cells and could negatively affect the biological system [4]. It is important to establish a rational design of the membrane surface.

Liposome, a closed phospholipid bilayer membrane, is a type of biocompatible membrane which can recognize molecules through combined interactions such as electrostatic and hydrophobic interaction and hydrogen bond stabilities [5-7]. Some new aspects of the liposome membrane itself, which could be induced under stress conditions, have recently been reported [8-14]. Recently, an immobilized liposome membrane module has reportedly been designed [15]. However, it is necessary to establish a basic strategy to characterize the PSf surface, to minimize the liposome-membrane interaction, and the immobilized liposome PSf membrane, so as to determine the design parameters of the immobilized liposome membrane module.

The purpose of this study is the design of the immobilized liposome membrane module. The aqueous two-phase partitioning method [5-7] was used to characterize the surface of the polymer-composite membrane to give a hydrophilic surface. The modified PSf module obtained with and without the immobilization of liposome was further characterized based on the adsorption behavior of amino acids that were similarly used to evaluate the hydrophobicity of the aqueous two-phase systems [7].

2 Experimental

Poly (ethylene glycol) (PEG 1540, 4000, 6000, and 50k; Mw 1.5 kDa, 3kDa, 7 kDa and 50kDa, respectively) and dextran (Dex) (100~200k; Mw 100-200 kDa) were purchased from Wako Pure Chemicals Ltd. (Osaka, Japan). Polyvinylpyrrolidone (PVP) K50 (Mw: 50 kDa) and PVP-co-vinylcaprolactam (PVP/VC) (Mw: 50 kDa) were purchased from BASF (Ludwigshafen, Germany). A siliconized-PEG (PEG-11 Methyl Ether Dimethicone: Si-PEG) (Mw: 50 kDa) was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from NOF Co. Ltd. (Nagoya, Japan). All other chemicals of analytical grade were purchased from Wako. The PSf and PVP-modified PSf membrane module, prepared by gthe amma-ray irradiation method [16], were gifted by Toray.

The basic composition of the aqueous two-phase systems (ATPSs) for the partitioning of the polymer were 7~13 wt% PEG 1540, 4000, 6000 and 7~13 wt% Dex 100-200k. The ATPSs were prepared by mixing the stock solutions of 30 wt% PEG and 30 wt% Dex with the suspension described above. The pH values of these systems were adjusted by the addition of a high concentration HCl solution. The surface properties such as surface net hydrophobicity (*HFS*) of the proteins were analyzed by using the aqueous two-phase partitioning method as was applied for the characterization of protein surfaces [5-7]. When the pH value is then selected at the pI at lower ionic strength, the partitioning coefficient of biomolecules was mainly dependent on the hydrophobic effect and the following relationship can be obtained.

$$\ln K = HFS \times HF \quad (1)$$

where the hydrophobic factor (*HF*), which has been defined from the partition coefficient of amino acids [12], is an indicator of the hydrophobicity difference in the two-phase system and

the *HFS* value is defined as the surface net hydrophobicity of the sample molecule. After the sample polymer to be analyzed was added to the ATPS, the top and bottom phases were separated and a small portion of the phases was dried at 80°C overnight. The polymer concentration was determined from the mass balance of dry weight of the sample polymer and ATPS-forming polymers.

The modified hydrophobicity factor (*HF*') [5,7] for the bulk aqueous phase and the polymer membrane or liposome immobilized on the membrane was also estimated from the adsorption behavior of the amino acids such as glycine and phenylalanine. The concentration of amino acid was determined by using the fluorescamine method which can be applied to the detection of primary amines [17].

3. Results and Discussion

3.1 Characterization of PVP Using ATPS and Its Use for Membrane Modification

In the medical use of a hollow fiber module, the surface modification of the hollow fiber membrane is important to avoid excess and unfavorable adsorption of biomaterials such as proteins and cells [4]. The polysulfone (PSf) membrane is often used as an artificial kidney after hydrophilic modification of its surface [16]. The surface modification using appropriate polymers is needed for surface reforming. As described in Eq.(1), the partition coefficient of the solute is dependent on the hydrophobicity (*HF*) of the aqueous two-phase systems [12]. The partition coefficients of several polymers were first measured in PEG/Dextran two-phase systems with higher *HF* values as shown in **Fig.1**. Among the possible candidates for the polymer for surface modification, PVP was found to be hydrophilic and suitable for the surface modification of the PSf membrane surface. After the PVP was partitioned in the ATPSs with

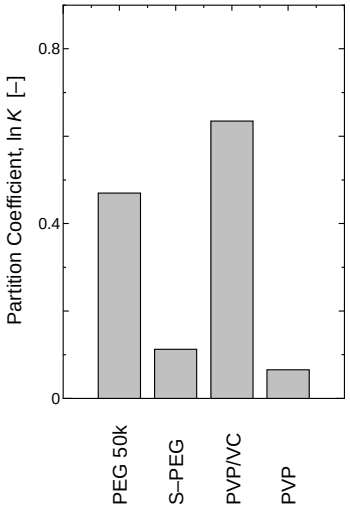

Fig.1 Partitioning of various polymers in PEG6000 (9%) / Dextran 60-90k (9%) aqueous two-phase system (*HF* = 0.192mol/kJ).

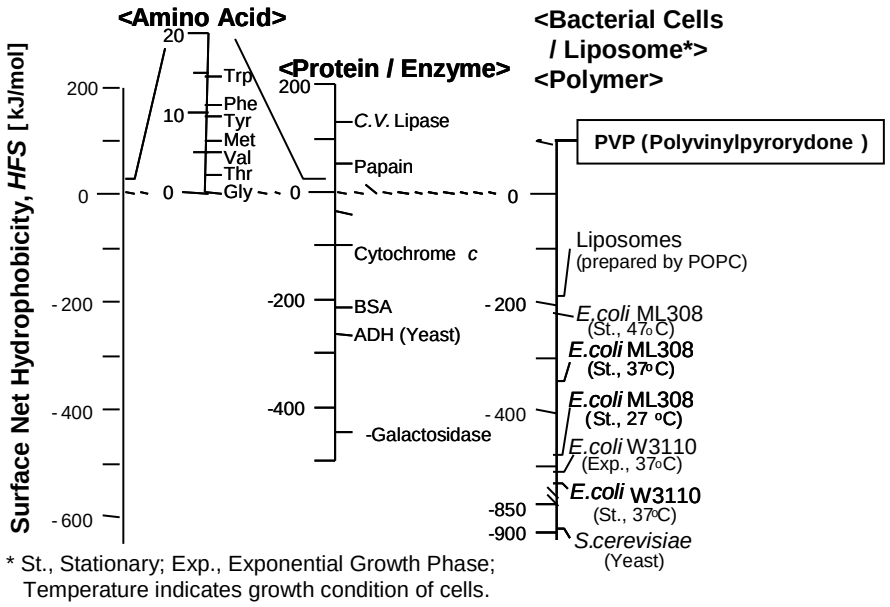

Fig.2 Ladder of surface net hydrophobicity (*HFS*) of PVP by the aqueous two-phase partitioning method.

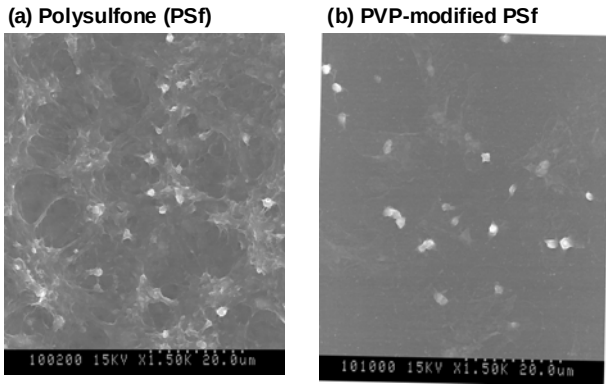

Fig.3 SEM image of (a) PSf surface and (b) PVP-modified surface. Both membranes were treated with blood to observe its adsorption on the membranes.

different *HF* values, the partition coefficient of the PVP was measured. The relationship between the PVP partition coefficient and the hydrophobicity factor of the ATPS was first investigated. The partition coefficient increased with increasing *HF* value. The slope of the line, which can be defined as surface net hydrophobicity (*HFS*), was found to be 110 kJ/mol. The obtained *HFS* value of PVP is also shown in **Fig.2.** The PVP surface was found to be hydrophobic in comparison with the other molecules, implying that PVP could cover the hydrophobic parts of the PSf membrane. It is considered that, among the polymers, the PVP polymers have a moderate hydrophilic surface which can be used for the hydrophilic modification of the PSf surface.

The PSf membrane surface was modified with the PVP polymer. **Figure 3** shows the SEM image of the membrane surface. The PSf membrane was found to have a rough surface before modification with PVP as shown in **Fig.3(a)**. **Figure 3(b)** shows the SEM image of the PSf membrane after PVP modification, showing that the surface had changed to a smooth surface. These surfaces were treated with a blood platelet solution. The bright dots in **Fig.3** show the blood platelets on the membrane, showing that there is no significant change between both membranes. It was thus found that the hydrophilic modification of the PSf membrane could be obtained by using PVP because the hydrophobic parts of the PSf membrane could be covered by the hydrophobic PVP polymers.

3.2 Hydrophobicity of the PVP-modified PSf Membrane.

The PVP-modified PSf membrane [16] so obtained was used for the preparation of a hollow fiber module (**Fig.4**) in order to investigate its possible use as an artificial kidney. An immobilized liposome membrane for use as an artificial kidney has recently been proposed [15]. The hollow fiber module, which is often used as an artificial kidney, can be used for the basic support of the immobilized liposome membrane as shown in **Fig.4** because it has a hydrophilic surface which minimizes its interaction with the solid

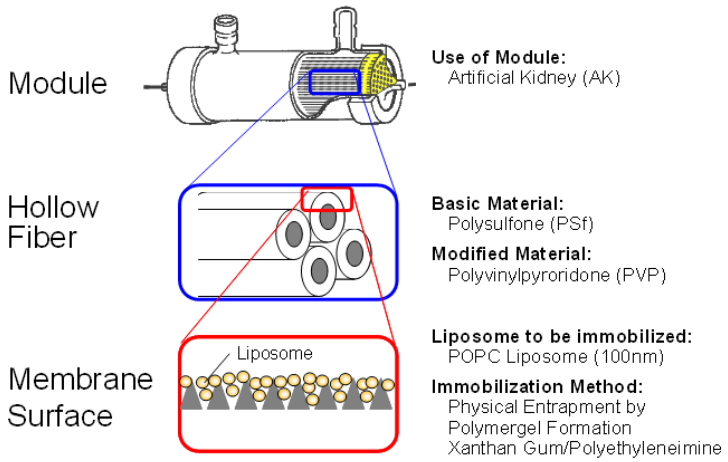

Fig.4 Schematic illustration of an immobilized liposome membrane for use as an artificial kidney and its optimized condition

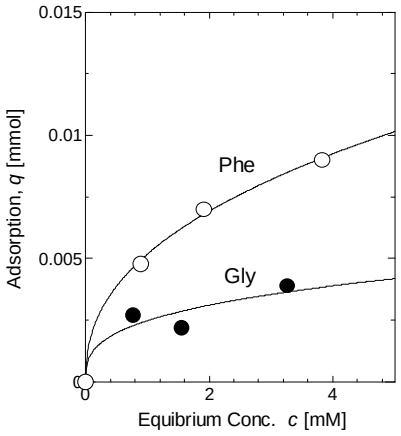

Fig.5 A typical example of amino acid adsorption on ILM-AK

surface, described above, and has some empty space for liposome entrapment [3]. The PVP-PSf membrane module immobilizing the liposome by using the hydrophilic polymer gel matrix [15] can herewith be defined as the immobilized liposome membrane for the artificial kidney (ILM-AK). The hydrophobicity of the PVP-PSf membrane with and without the immobilized liposome was assessed based on the adsorption behavior of the standard amino acids. **Figure 5** shows a typical example of the adsorption behaviors of the standard amino acids on ILM-AK. It was found that there was a difference in the adsorption behavior of glycine (Gly) and phenylalanine (Phe), showing that Phe adsorption was higher than Gly adsorption at any concentration, depending on their hydrophobicity. It has been reported that the hydrophobicity factor of the aqueous two-phase system (ATPS) can be defined by measuring their partitioning coefficient [5] based on the hydrophobicity defined by Nozaki-Tanford values [18-19]. The above methodology was extended to the reversed micellar system (RVMS) and also liposome membrane systems (LMS) [7]. The determined *HF* values based on partitioning behavior have been reported to reflect the hydrophobic nature of the systems [5,7]. This concept was extended to the characterization of PVP-PSf and ILM-PVP-PSf in an attempt to characterize the membrane surface, where the equilibrium constant was plotted against the hydrophobicity (Nozaki-Tanford values [18,19]) and the modified *HF* values (*HF'*) were obtained. The *HF'* values are shown in **Fig.6**, together with the *HF'* values of other systems such as ATPS, RVMS, and LMS. The *HF'* value for AK, obtained using the PVP-modified PSf membrane, was found to be hydrophilic and the value was equivalent to that of ATPS (PEG/Dextran systems) and LMS (POPC liposomes), both of which are know to be hydrophilic. A similar *HF'* value can also be obtained in the case of ILM-AK, showing that its surface is also hydrophilic. However, a slight increase in the *HF'* value was obtained in the case of ILM-AK. These phenomena could imply that the POPC liposome immobilized inside the PVP-PSf membrane could also provide a hydrophobic potential.

It has been reported that the liposome could induce a variety of functions, such as a molecular chaperone-like function [8] and LIPOzyme functions [12-14], because of its "recognition function" of the biomolecules. The above-prepared ILM-AK could be used as an effective tool to separate the "amyloidgenic proteins", which are related to Alzheimer's disease or hemodialysis amyloidosis because the hydrophilic basic material (PVP-modified PSf) has a less adsorptive nature and the immobilized liposome could recognize such proteins. The adsorption of the lysozyme and insulin on the AK and ILM-AK was also investigated. Although there is no adsorption on the AK, the ILM-AK was found to show a higher recognition ability for amyloidgenic protein, where it adsorbs insulin (approximately 70% adsorption) but less lysozyme (approximately 40% adsorption) [21]. It has been reported that the amyloidgenisity of the proteins is related to the stability of the hydrogen bonds of the main chains of the proteins [20], where insulin has unstable hydrogen-bonds on its surface.

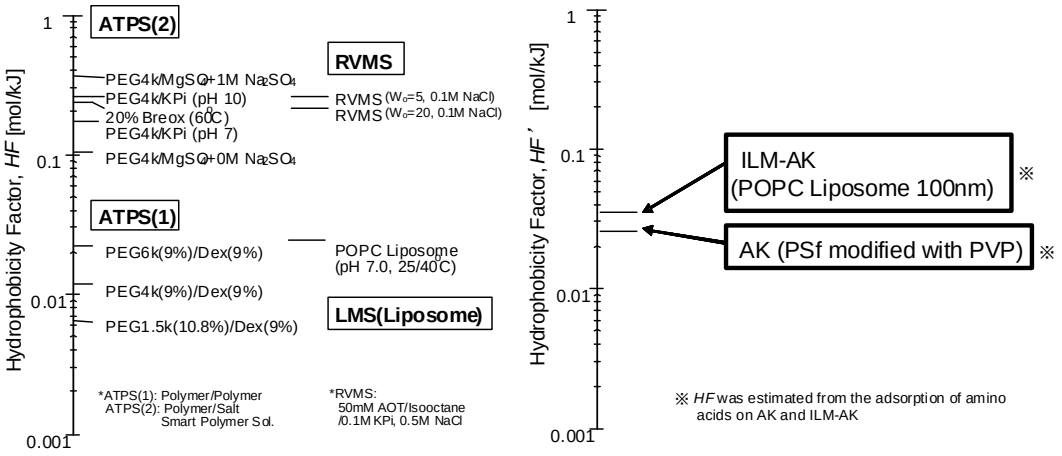

Fig.6 Hydrophobicity factors of various systems
※ *HF'* for ILM-AK and AK was estimated from the adsorption behavior of amino acids

Although further investigation is needed, the prepared ILM-AK could enable the recovery of "potentially-amyloidgenic protein".

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