Colloids and Surfaces B: Biointerfaces 116 (2014) 343–350

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Relationship between the mobility of phosphocholine headgroup and the protein–liposome interaction: A dielectric spectroscopic study

Toshinori Shimanouchi, Noriko Yoshimoto, Azusa Hiroiwa, Keiichi Nishiyama, Keita Hayashi, Hiroshi Umakoshi*

Division of Chemical Engineering, Grad. Sch. of Engineering Science, Osaka University, 1-3 Machikaneyama-cho, Toyonaka, Osaka 560-8531, Japan

ARTICLE INFO

Article history:
Received 6 February 2013
Received in revised form 27 June 2013
Accepted 15 July 2013

Keywords:
Protein
Hydrogen bond
Liposome
Lipid composition

ABSTRACT

Proteins could affect the headgroup mobility of phospholipid within liposome membranes through the protein–liposome interaction. The variation of headgroup mobility of phospholipid was then investigated by using the dielectric dispersion analysis. The eight proteins (Mw = 4.2–28.7 kDa) were used to investigate the protein–liposome interaction. It has been revealed that the strength of the protein–liposome interaction at 25 °C was linearly correlated with the stability of intramolecular hydrogen bondings of proteins, better than with their hydrophobicity and the surface charge density. Overall, liposomes composed of binary lipid system, appeared to strongly interact with proteins, in contrast to liposomes composed of single, ternary, and quaternary lipid systems. This is probably because liposomes composed of binary lipid system favored to form the microscopic environment where proteins could interact. The present result suggested the heterogeneous phase state of lipid membranes was one of dominant factors for the interaction between proteins and lipid membranes.

1. Introduction

The dynamic clustering of specific lipid molecules within a fluid lipid matrix of cell membranes provides the scaffold for the accumulation of functional membrane proteins, which is the lipid raft hypothesis [1]. Various biological processes such as endocytosis [2], membrane fusion [3], lipid sorting [4], and signal transduction [5] are mediated by the formation of lipid raft. In addition, the lipid raft is thought to be related to the pathological events including the formation of the senile plaque associated with Alzheimer's disease [6]. The phase separation or domain formation behavior in a lipid membrane has already been observed in various systems before the proposal of lipid raft hypothesis [1].

Previous studies on the phase separation or domain formation has been mainly performed by using the thermodynamic or the spectroscopic approach, such as the differential scanning calorimetry (DSC) [7–9] the fluorescence resonance energy transfer (FRET) [8], the depolarization of fluorescence probes [10], the eximer formation [11], and the direct observation of giant liposome stained by the fluorescence probes [12–14]. Those approaches have the difficulty in the elucidation of the mechanism how the specific lipid molecules forms the dynamic clustering as lipid raft. Meanwhile, the direct observation of phase separation or lipid raft is undoubtedly important. The research with respect to the lipid raft has been developed experimentally and theoretically. For examples, it has been reported that some proteins have been reported to interact with the *boundary* of lipid raft at the Monte-Carlo calculations [15]. If so, the protein–lipid raft interaction should be related to the formation of boundary of lipid rafts.

Such boundaries of lipid raft might be generated by the headgroup mobility of phospholipids. The dielectric dispersion analysis, which is a useful tool for the assessment of headgroup mobility [16–22], has revealed that the interaction between phospholipid headgroups contributed to the domain formation of the binary phase system [22].

Abbreviations: DDA, dielectric dispersion anaylysis; ε', relative permittivity [–]; ε'', dielectric loss [–]; $\Delta\varepsilon_i$, amplitude of relaxation at the ith relaxation [–]; f_{ci}, characteristic frequency at the second relaxation [MHz]; $f_{c2,0}$, characteristic frequency at the second relaxation in the absence of proteins [MHz]; Δf_{c2}, $\Delta f_{c2} = f_{c2} - f_{c2,0}$ [MHz]; ρ_{pr}, the stability of hydrogen bonding of the backbone of the main chain of the protein molecule [–]; LH_{pr}, local hydrophobicity of proteins [–]; Z, surface charge of protein [C]; ASA, accessible surface area of protein [nm^2]; Z/ASA, surface charge density [C/nm^2]; ΔG_{el}, total free energy on electrostatic interaction between protein and liposome [J/mol]; $\Delta G_{non\text{-}el}$, total free energy on non-electrostatic interaction between protein and liposome [J/mol]; $\Delta G_{ele}/\Delta G$, electrostatic contribution to total free energy [%]; l_d, liquid-disordered phase; l_o, liquid-ordered phase; s_o, solid-ordered phase; G-F, gel-fluid phase; F, fluid phase; CAB, carbonic anhydrase from bovine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; DLPC, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimirystoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; POPA, 1-palmitoyl-2-oleoyl-phosphatidic acid; POPG, 1-palmitoyl-2-oleoyl-phosphatidylglycerol; SM, sphingomyeline; Ch, cholesterol; DG, diacylglycerol.

* Corresponding author. Tel.: +81 06 6850 6287; fax: +81 06 6850 6286.
E-mail addresses: umakoshi@cheng.es.osaka-u.ac.jp, hiroshi.umakoshi@gmail.com (H. Umakoshi).

0927-7765/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.07.028

Besides, the headgroup mobility of lipid sometimes appeared to generate the pothole/crevice on the membrane of liposomes even composed of single phospholipid component [21]. Therefore, the investigation on the relationship between the headgroup mobility and the protein–liposome interaction would give the better understanding of the lipid raft.

In this study, the dielectric dispersion analysis (the microwave dielectric measurement) was adopted to evaluate the strength of the protein–liposome interaction, because this technique have succeeded in the evaluation of protein–liposome interaction in terms of headgroup mobility [18,20]. The eight proteins with different physicochemical properties (charge density, hydrophobicity, and intramolecular hydrogen bonding stability) were herewith used to compare the reduction of headgroup mobility with the protein–liposome interaction. Finally, we discussed the relationship between the protein–liposome interaction and the phase state of twenty three kinds of liposomes.

2. Materials and methods

2.1. Lipid membrane

Various zwitterionic phospholipids DOPC, DPPC, DMPC, DLPC, DSPC, and POPC were purchased from Avanti Polar lipids (Alabaster, AL, USA). SM, diacyl grycerol (DG), stearic acid (SA), and linoreic acid (LA) were purchased from Sigma. The abbreviations of lipids were listed in the last of the manuscript. Insulin from human, lysozyme from egg, myoglobin, β-lactoglobulin, and ubiqutin were purchased from Wako Pure Chemical Ltd. (Osaka, Japan). Carbonic anhydrase from bovine (CAB), β_2-microglobulin, apolipoprotein A1, were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1-Pyrenedecanoic acid was obtained from Dojindo (Kumamoto, Japan). Other reagent were of analytical grade.

2.2. Liposome preparation

Large unilamellar vesicles were prepared by the previous method [21]. In brief, a desired quantity of phospholipids was dissolved in chloroform in a round bottom flask. The solvent was removed under vacuum using a rotary evaporator. This process was performed twice using chloroform and was pursued once more using diethyl ether in order to form a homogeneous thin layer of phospholipids on the wall of the flask. For the preparation of the liposome solution, an appropriate quantity of distilled water was added into the round bottom flask containing the lipid film and, thereafter, five freezing–thawing cycles were applied before forcing the solution 15 times through a polycarbonate filter (50, 100, 200, 400 nm) using an extruder device (Avestin, LiposoFast).

Giant unilamellar vesicles (GUVs) of POPC were prepared by the high-temperature hydration method [21,23]. For the preparation using method (i), the dried POPC was hydrated with distilled water at 65 °C without shaking for 3 h. The floating lipid assembly was picked up by a micropipette for transfer to another tube. More than 100 GUVs were monitored under the photomicroscope (IX51-11-FL/PH-S, Olympus) to determine the diameter distribution. The mean diameter of GUVs obtained here was 5.2 μm.

2.3. Dielectric dispersion analysis

Various compounds were dissolved with liposome in distilled water. The dielectric permittivity (ε') and dielectric loss (ε'') was measured by an RF impedance analyzer (Agilent, 4219B, 1 MHz to 1 GHz), according to the previous report [21]. Prior to analysis of dielectric spectra using Debye-typed equations, the electrode polarization effect ($G_{dc}/2\pi f C_0$) was excluded to maintain accuracy in the low frequency range.

2.4. Surface properties of protein

2.4.1. Surface charge density.

Charge of protein (Z) was calculated from the pK_a value of the constituted amino acid residues. The accessible surface area (ASA) was applied to the surface are of protein molecule. The Z/ASA value was utilized as the surface charge density of protein molecule.

2.4.2. Local hydrophobicity.

In short, the aqueous two-phase partitioning method was used to estimate the local hydrophobicity of proteins [24]. The mixture of poly(ethylene glycol) 4000 (9 wt%) and dextran 100–200 k (9 wt%) was used as two phase system. The protein (final concentration 1 mg/ml) and the hydrophobic probe Triton X-405 (final concentration 1 mM) were added to the two phase system. The mixture was incubated for 1 h to separate the top and bottom phases. The concentrations of proteins in the top (PEG-rich) and bottom (Dex-rich) phase were measured to estimate the partition coefficient K_{pr} ($=C_{pr,t}/C_{pr,b}$). The LH_{pr} value were estimated according to the following equation: $LH_{pr} = \Delta \ln K_{pr} = \ln K_{pr,T} - \ln K_{pr,0}$. Note that $K_{pr,T}$ and $K_{pr,0}$ represent the partition coefficient of proteins with and without 1 mM of Triton X-405.

2.4.3. Intramolecular hydrogen bonding stability.

The data base of index on hydrogen bonding stability of proteins, ρ_{pr}, was referred to the previous literature [25]. The ρ_{pr} value is defined below:

$$\rho_{pr} = \frac{C_3}{Q} \tag{1}$$

where C_3 is the total number of third-body correlations and Q is the total number of backbone hydrogen bond. The algorithm to calculate the C_3 value is introduced at the previous paper [25].

2.5. Thermodynamic analysis

Protein–liposome interaction was analyzed by the binding model as previously reported [26]. In short, the $\Delta f_{c2}/f_{c2,0}$ value was utilized to monitor the binding onto the liposome at equilibrium. The protein–liposome interaction (ΔG) value can be divided into the electrostatic (ΔG_{el}) and non-electrostatic contribution ($\Delta G_{non\text{-}el}$), that is to say, $\Delta G = \Delta G_{el} + \Delta G_{non\text{-}el}$. The ΔG and $\Delta G_{non\text{-}el}$ values can be estimated based on the complex formation model and distribution model.

For the complex formation model, the apparent constant of binding (K_{app1}) was calculated from the following complex formation model.

$$P + L_n \Leftrightarrow P \times L_n \tag{2}$$

where P stands for protein, L for the lipid, and n for the number of lipids bound to one protein. Therefore, the binding site to which one protein binds consists of n lipid molecules. $P \times L_n$ is the complex formed between one protein and n lipid molecules. The equilibrium constant K_{app1} (M^{-1}) is given by the following equation.

$$K_{app1} = \frac{[P \times L_n]}{[P][L_n]} \tag{3}$$

Then, $\Delta G = -RT \ln K_{app1}$ means the total free energy on the interaction between protein and liposome. It is assumed that the observed decrease in frequency ($\Delta f_{c2}/f_{c2,0}$) divided by the maximally observed frequency change, $(\Delta f_{c2}/f_{c2,0})_{max}$, is directly proportional to the concentration of the binding sites occupied divided by the concentration of all available binding sites. This leads to the following relationship:

$$\frac{(\Delta f_{c2}/f_{c2,0})}{(\Delta f_{c2}/f_{c2,0})_{max}} = \frac{[P \times L_n]}{[L_n]_{tot}} \tag{4}$$