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— *Reviews* —

Analysis and Separation of Amyloid β-Peptides Using Aqueous Two-Phase Systems Under Stress Conditions ~ From Aqueous Two-Phase System to Liposome Membrane System ~

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The evaluation of the surface properties of biomolecules and their separation are reviewed especially focusing on the amyloid β-peptides (Aβ) related to Alzheimer's disease by using an aqueous polymer two-phase system (ATPS), a reverse micellar system (RVMS) and a liposome membrane system (LMS) as aqueous two phase systems, which commonly contain either water pools or water phases commonly. The hydrophobicity difference between the phases in each ATPS, RVMS and LMS was quantitatively characterized and compared with each other. The surface properties of peptides/proteins were then evaluated based on the characterized hydrophobicities of the aqueous two-phase systems. The ATPS analysis of Aβ showed that the Aβ(1-40) was more hydrophobic than Aβ(1-42) and that the hydrophobic interaction could play an important role in amyloid fibril formation. The above results also show that the Aβ could interact with the liposome membrane in a LMS similarly to that of lipase although the mutual separation of Aβ(1-40) and Aβ(1-42) could not be achieved effectively in an ATPS or a LMS. Analysis using a new-type of LMS, metal affinity immobilized liposome chromatography (MA-ILC), furthermore showed that not only the hydrophobicity but also the metal affinity of Aβ could play an important role in amyloid fibril formation and its interaction with the liposome membrane. The separation of Aβ(1-40) and Aβ(1-42) was successfully achieved based on the characteristics of the MA-ILC. The above findings imply that the use of the above aqueous two-phase systems could provide valuable information for the understanding of the behavior of the biomolecules in the biological environment and that these techniques could effectively be applied to the separation of the biomolecules.

1. Introduction

In biological systems, a variety of complicated phenomena (such as peptide synthesis, folding of the polypeptide, secretion of proteins etc.) occur in a well-organized manner in order to maintain the cellular

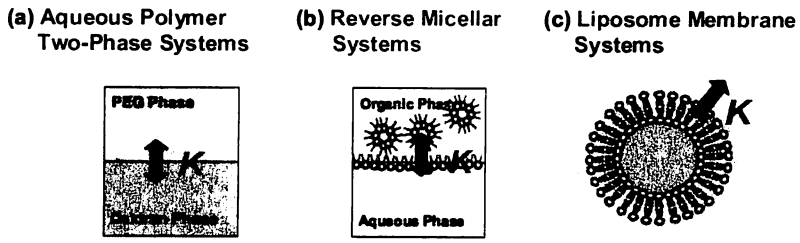

Fig. 1 Schematic diagrams of aqueous two-phase systems

homeostasis. A biological cell can be regarded as self-assembled structure which is composed of a "cytoplasmic aqueous pool" and a "membrane". Among a variety of key elements in the cytoplasm, such as proteins (enzymes), nucleic acids and intracellular organelles, various membranes can be key elements for the induction of biological functions of cells since they wrap a variety of cell contents within each physical boundary, and regulate functions at the interface against the environment by sensing the environmental condition and by recognizing other molecules (nucleic acids, proteins, etc) or other self-assemblies (virus, cells, etc) [1]. Recently, conformational diseases such as Prion disease, hemodialysis amyloidosis, and Alzheimer's disease have become serious problems, because an effective method to cure them has not yet been established [2]. A fundamental understanding on the behavior of biomolecules in the biological environment such as in cytoplasm and on the membrane could provide us with a possible effective treatment of such diseases. Study of the biological membrane, especially of the interaction between the biological membrane and other intracellular components inside a cell, is therefore important and necessary in order to clarify biological roles during the induction of cellular functions.

Aqueous two-phase systems (**Fig.1**) including aqueous phases or water pools in the system are effective systems to be utilized for the reaction and/or separation of biomolecules because they are compatible with the biological environment in which water is abundant [3]. Aqueous polymer two-phase systems (ATPS), which are also known as "coacervates", have previously been utilized in the separation of biomolecules and also for the characterization of their surface properties because the ATPS has a 60~80% water content and is therefore biocompatible [4]. A systematic approach to the evaluation of the physicochemical properties of the two phases in the ATPS and its application for the characterization of the surface properties of biomolecules such as amino acids, peptides, proteins (enzymes), liposomes and bacterial cells has previously been reported in a series of papers [5-10]. It has recently been reported that stimuli-responsive aqueous two-phase systems can also be utilized in polymer recycling and the enhancement of the protein refolding process [11-13].

Reverse micellar systems (RVMS) [14-16] and lipid bilayer membrane vesicles (liposome membrane systems (LMS)) [17-19], analogous to microbial cell membranes, are highly structured supramolecules and can also be regarded as aqueous two-phase systems. A RVMS spontaneously formed in an organic solvent is known to have a nanometer sized "water pool" and an amphiphilic boundary and has previously been utilized for bioseparation and in bioreactors [14-16]. It can also be utilized as a minimal cell because of its structural similarity to micelles [14]. In addition to the similarity to biological membranes, liposome membranes act as a functional boundary with highly selective permeability for small molecules such as ions and amino acids [17]. It is, therefore, possible to concentrate particular materials inside liposomes, as

observed in biological cell systems as well as minimal cell models [18]. Compared with the static properties of liposomes, the dynamic aspects of liposomes are relatively unknown. In general, macromolecules such as proteins cannot pass through the lipid membranes [19]. Liposomes have recently been used to elucidate the potential functions under stress conditions. In a series of studies by Kuboi and his coworkers, it has been reported that a variety of potential functions of liposome were induced, especially when the liposomes were exposed to stress conditions [20-26]. In an adequate stress condition, it has been reported that the liposome can interact with partly-denatured protein and, furthermore, can enhance the refolding of proteins [23, 27-28]. Under heat stress conditions, the water-soluble cytoplasmic protein has been reported to be translocated across the phospholipid membrane [24-25, 29]. In studies of the mechanisms of the interaction between the membrane and protein, membrane-membrane interaction is, therefore, important in order to control cellular functions.

In this article, we review the features of several types of aqueous two-phase systems such as ATPS, RVMS and LMS, focusing on the characterization of the difference in hydrophobicity between two phases in these systems, and, also, on the characterization and the separation of target peptides, amyloid β-peptides related to Alzheimer's disease.

2. Comparison of the Hydrophobicity of Aqueous Two-Phase Systems and its Application to the Evaluation of the Surface Hydrophobicity of Biomolecules

A systematic approach to the characterization and quantitative determination of surface properties of biomolecules has previously been reported by using an aqueous polymer two-phase system (ATPS) [5,7]. Previous researchers mainly focused on the use of functional polymers in the ATPS, such as charged ligands (tetramethylacetate-PEG or PEG-sulfonate) [30-31], hydrophobic ligands (palmitate-PEG [32-33]), and biospecific ligands (antibodies-PEG) [34]. If such ligands are not attached to the polymer, the partition coefficient of biomolecules in an ATPS has been found empirically to depend upon several factors, which act independently. The partitioning coefficients of biomolecules, such as amino acids, peptides, proteins (enzymes), liposomes and cells, may therefore be expressed as follows [3, 35]:

$$\ln K = K_{\text{electorostatic}} + K_{\text{hydrophobic}} + K_{\text{salt}} + K_{\text{ligand}} + \ldots \quad (1)$$

where $K_{\text{electorostatic}}$, $K_{\text{hydrophobic}}$, K_{Salt} and K_{ligand} represent the contribution to the partitioning of the biomolecules by electrostatic, hydrophobic, salt and ligand effects, respectively. From consideration of these effects, the surface of biomolecules can be characterized systematically [5]. It is then considered that the ATPS method can also be applied to the characterization of the surface properties of bacterial cells, which are highly organized biomolecular assemblies. Albertsson [3] has found that the pH dependence of the partition coefficient of cells in an ATPS containing some types of salt showed a common cross point at pI (cross partition method). At the pI and low ionic strength, the values of ln $K_{\text{electorostatic}}$ and ln K_{salt}, can be ignored, and the following relationship can then be obtained,

$$\ln K = K_{\text{hydrophobic}} \quad (2)$$

Nozaki and Tanford [36] evaluated the hydrophobicities of several amino acids in water/ethanol and water/dioxane systems. Kuboi et al. [5] have elaborated a relationship between the Nozaki-Tanford values

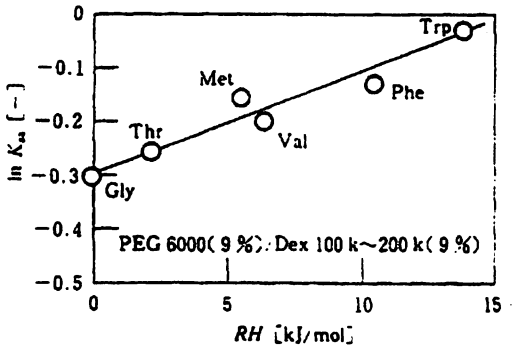

Fig.2 Relationship between the partition coefficients of amino acids and their hydrophobicities determined by Nozaki and Tanford in ATPS [36]

and the partition coefficients of amino acids and the following equation on the definition of *HF* values can then be obtained.

$$\ln K = HF \times (RH + B) \quad (3)$$

where *RH* is the relative hydrophobicity based on the Nozaki-Tanford value and *B* is the normalization constant defined as the ratio of the partition coefficient and the hydrophobicity of glycine, $\ln K_{Gly}/\Delta G_{Gly}$. They further determined the hydrophobicity differences between the two phases in an ATPS (hydrophobicity factor, *HF*) as for example shown in **Fig.2** and the surface net hydrophobicity of protein (*HFS*) from the slope of Eq.(4) using the ATPS,

$$\ln K = HFS \times HF \quad (4)$$

The above equation can provide quantitative data based on the surface properties of a variety of biomolecules such as amino acids, peptides, proteins (enzymes), liposomes and bacterial cells. As an analogy, the hydrophobicity of other types of aqueous two-phase systems such as reverse micellar systems (RVMS) [16] and liposome membrane systems (LMS) [23] can also be evaluated based on the partition behavior of amino acids between two phases. **Figure 3** shows the *HF* values of some aqueous two-phase systems such as RVMS and LMS, which are evaluated based on the relationship between the partitioning behaviors of amino acids and their hydrophobicity (**Fig.3(a)**). As shown in **Fig.3(b)**, the ATPS was in general found to provide the wide spectra of *HF* values from 0.005 ~ 0.35mol/kJ, depending on the type

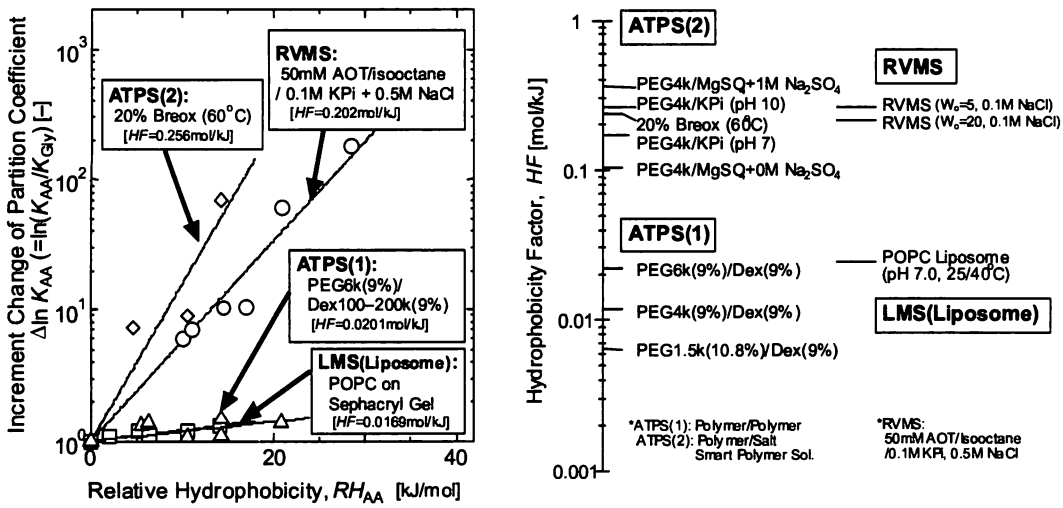

Fig.3 *HF* values of various aqueous two-phase systems. (a) Relationship between the partition coefficients of amino acids and their hydrophobicities determined by Nozaki and Tanford in various aqueous two-phase systems such as ATPS, RVMS and LMS. (b) The *HF* Ladder for the systems.

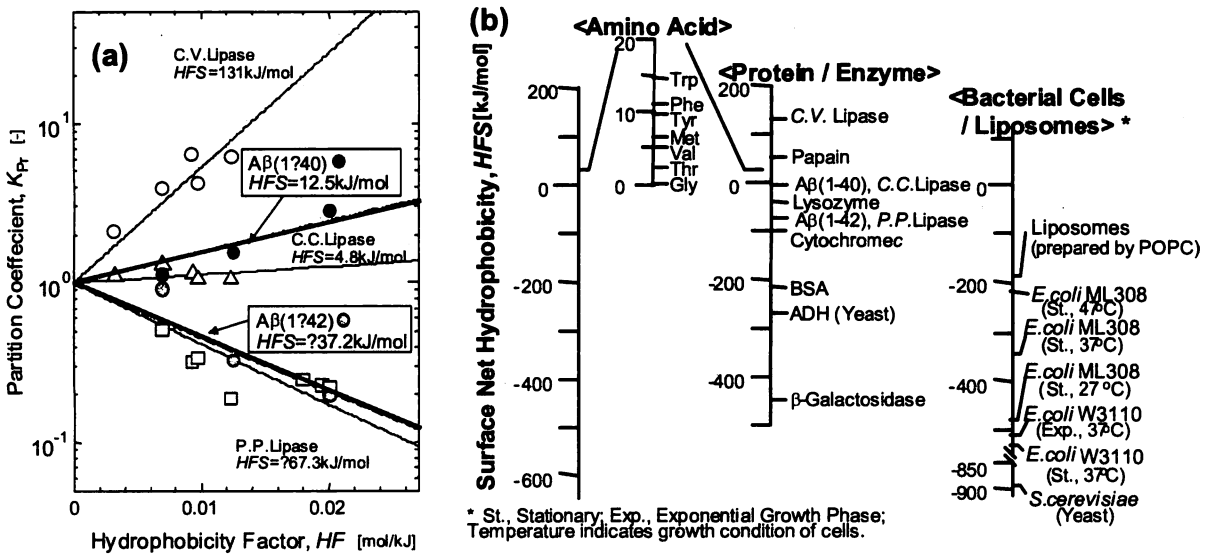

Fig.4 *HFS* values of various target molecules. (a) Relationship between the partition coefficient of proteins and the hydrophobicity of the aqueous two-phase systems (*HF*). (b) The *HFS* ladder of various target molecules.

and concentration of phase forming components such as poly(ethylene glycol), dextran, phosphate salts and the random copolymer of PO and EO (BREOX) [13]. The *HF* values for the RVMS were higher than that usually found for ATPSs and they varied with the water contents and types of detergents. The LMS indicated a lower *HF* value and, especially in the case of the POPC liposome, the *HF* value was nearly equal to that of PEG6k(9%)/dextran(9%) two phase systems, which can be classified as an ATPS with a higher *HF* value in PEG/dextran aqueous polymer two-phase systems. It was thus found that the *HF* values for various types of aqueous two-phase systems could be characterized on the same scale based on the Nozaki-Tanford value and the partitioning behaviors of target biomolecules in the systems could be controlled by the *HF* values.

As described in the above equation (4), the evaluated *HF* values of the systems can be utilized for the evaluation of the surface hydrophobicity of the biomolecules based on their partitioning behaviors [5]. **Figure 4** shows the evaluated *HFS* values by using an ATPS. The partition coefficient of hydrophobic molecules were increased with increasing *HF* values while that of hydrophilic molecules decreased as shown in **Fig.4(a)**. The slope of the relationship between the partition coefficient and the *HF* values can herewith be determined as the surface net hydrophobicity (*HFS*) of the biomolecules based on Eq.(4). The *HFS* values of various target materials are also shown in **Fig.4(b)**. Although the target molecules were quite varied, i.e. amino acids, peptides, proteins (enzymes), liposomes, and bacterial cells, their surface hydrophobicity was found to be characterized by using the same hydrophobicity scale (*HFS* value). Especially in the case of the proteins (enzymes), the effect of their conformation should be considered in a design for analysis and separation of the proteins [37]. The *HFS* values of α-lactalbumin (α-LA) have previously been characterized, where its hydrophobicity was varied in the presence and absence of metal ion (Ca ion) because of the conformational change of α-LA between the native and apo state [37]. Successful separation has already been achieved in an ATPS by utilizing the above-characterized properties as design parameters [37]. More drastic conformational change of proteins could also be induced during the

denaturation or inactivation process of proteins (enzymes). The surface hydrophobicity of the proteins has been systematically investigated under several kinds of stress conditions such as acidic pH, denaturant, and heating [10,38,39], where the *HFS* values were shown to be significantly increased during their conformational change from the native to the unfold state. The stress condition for variation of the *HFS* values was also shown to be dependent on the type of proteins [10,38]. The previous findings show that the difference in the *HFS* values of the proteins under stress conditions could be used for the design of stress-mediated separation in aqueous two-phase systems [10,12,38]. It has recently been reported that the polymer assemblies could act as molecular chaperones, which can assist in protein refolding, and that they could enhance the refolding of the target proteins [12, 13, 40]. By combined use of the ATPS and the above artificial chaperone, the extractive refolding process can also be designed, where the unit operations of stress-mediated separation and refolding of proteins are included in the process [40]. Another type of hydrophobicity, local hydrophobicity (*LH*), can also be evaluated by using the ATPS method, where the binding of a PEG bound-hydrophobic ligand, Triton X-405, with the target molecules was used for its evaluation [7,10] as described above. The local hydrophobicity of the target molecules of lower molecular weight has already been shown to be correlated with their *HFS* values although the larger molecules could not be correlated in such a manner owing to the contribution of their conformational state [7]. Especially, in the case of the peptides of lower molecular weight, the *HFS* values could effectively be available for the measurement of the surface characterization of the molecules.

It was thus shown that the ATPS can effectively be utilized for the analysis of the surface properties of the biomolecules and can also be utilized for the separation of proteins by selecting the analyzed properties such as *HFS*, *LH*, and the *HF* of ATPS together with the stress conditions including pH, temperature, and salt/metal ion concentration as operational parameters. In the following, the above methodology to evaluate the surface properties of proteins was extensively applied to the characterization and the separation of specific peptides, amyloid β-peptides (Aβ), which are known to be related to Alzheimer's disease.

3. Analysis and Separation of Amyloid β-Peptide in ATPS

It is well known that amyloidgenic proteins form fibrous aggregates, which are resistant to proteolysis and to detergent solubilization. Conformational diseases, such as prion disease, Alzheimer's disease, and dialysis amyloidosis, may be caused by amyloidgenic proteins, and have become a social issue in recent years. The establishment of a method or technology to solve such problems is a newly emerged issue. For example, especially in the case of Alzheimer's disease, many researchers have studied the mechanism of the amyloid formation of amyloid β-peptides, Aβ(1-40) and Aβ(1-40) (**Fig.5**), *in vivo* and *in vitro*. The causes of Alzheimer's disease are considered as (i) excess stabilization of membrane surface caused by the amyloid-fibril of Aβ on the membrane [42], (ii) the channel-like pore formation

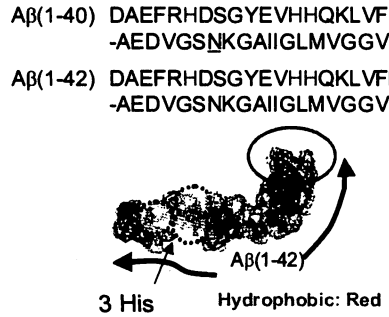

Fig.5 Amino acids sequence of amyloid β-peptides

Table 1 Surface properties of Aβ peptides characterized by using ATPS

	pH=pI			pH7.5		
	pI [-]	HFS [kJ/mol]	LH[-]	Z[C/nm^2]	HFS [kJ/mol]	LH [-]
Aβ(1-40)	5.2	12.5	2.62	-0.114	1.84	1.34
Aβ(1-42)	5.2	-37.2	0.89	-0.126	-142.7	0.14
C.C.Lipase	4.3	4.8	0.51	-	-	-
P.P.Lipase	4.4	-67.3	0	-	-	-
β-LG	5.2	22.33	0.47	-0.112	-	1.25
Melittin	12.5	12.06	0.135	0.224	-	0.619

of Aβ on the membrane [43], (iii) excess oxidative conditions near the membrane owing to the metal-Aβ complex [44], (iv) cross-linking of the Aβ and membrane protein by specific enzymes (i.e. tissue transglutaminase) on the biological membrane [45] and, also (v) modulation of the above functions by varying the membrane composition, such as cholesterol and fatty acid content [46]. It is postulated that the variation of protein conformation or its bioconversion should be closely related to the cause of the physiological condition of Alzheimer's disease. It is important to establish a method to monitor the conformational change of Aβ, especially Aβ(1-40) and Aβ(1-42).

The surface properties of Aβ of different types of amino acid sequences were characterized by using the ATPS as described above (black and shaded circles in **Fig.4**). The *HFS* values of the Aβ peptides measured at pI and pH 7.5 are summarized in **Table 2**, together with those of other types of amyloidgenic proteins (melitin and β-Lactoglobulin) and two types of lipases, which bind to the lipid aqueous interface. In general, the *HFS* value for Aβ(1-40) was greater than that for Aβ(1-42). The local hydrophobicity (*LH*) of Aβ peptides was also evaluated by utilizing the effect of the hydrophobic ligands on their partitioning behavior in the ATPS as described in Eq.(1), showing that a similar tendency for the hydrophobicity of Aβ peptides could be obtained; Aβ(1-42) > Aβ(1-40). The increase in the *LH* values of peptides/proteins [39] under stress conditions, which can be determined by using the ATPS with and without the nonionic detergent, Triton X-405, has been previously reported to be well related to the aggregate formation of the peptides/proteins. It has also been reported that Aβ(1-42) is known to easily form an fibrilous aggregation as compared with that of Aβ(1-40) [48]. Although further investigation is needed, the above results on the hydrophobicity difference of Aβ peptides correspond well with the previous findings although the contribution of hydrogen bonds between the main chains of peptides should be investigated further. It was thus found that the Aβ peptides could be effectively characterized by using the ATPS.

In order to compare with other proteins, the *HFS* values of lipases of different origins were also determined as shown in **Fig.4(a)**, indicating that the *HFS* value of C.C. lipase was much higher than that of P.P. lipase. It has been reported that the lipase activity was dependent on its *HFS* value, implying that the lipid-lipase interaction required for its catalytic reaction could be affected by the hydrophobicity of the lipase [51]. Similarly for different lipases, the *HFS* values also varied depending on the type of Aβ, Aβ(1-40) and Aβ(1-42) (**Fig.4(a)**). The above finding on the relationship between the activity of lipase and the *HFS* values implies that Aβ(1-40) can also interact with the liposome membrane. Among the possible Aβ

peptides, the interaction of Aβ(1-42) and Aβ(1-40) with the phospholipid (1-palmitoyl-2-oleoyl-sn-glycero-phosphatidylcholine) membrane was also measured by using the immobilized liposome chromatography (a method in LMS). This showed that a similar tendency to that in the ATPS could be obtained (data not shown). The above results also show that both the ATPS and LMS methods could be applied for the evaluation of the surface properties of the Aβ peptide and that the hydrophobicity as well as hydrogen bonding could be important factors. The above results imply that the condition of the mutual separation of the Aβ(1-42) and Aβ(1-40) could be designed based on the characterized properties. It has also been discussed that such an interaction of Aβ with the liposome membrane could be related to the hydrophobicity and the hydrogen bonds [49-50]. The above findings on the difference of Aβ peptides may imply that the separation of the Aβ peptides could be achieved based on the characterized results also by selecting pH (stress) as an operational parameter. However, there are some difficulties in the separation process of the Aβ peptides using the ATPS and LMS techniques because there is no significant difference in the hydrophobicity and possible aggregate formation of Aβ peptides during the long-time operation. In order to accomplish the mutual separation of the Aβ peptides, a more distinct difference in the interaction should be employed. The difference of the interaction of these Aβ peptides with a lipid membrane under the metal ion (Cu) stress condition is also of interest. The effect of environmental stress including metal ion concentration on their separation was furthermore introduced in the following section.

4. Analysis and Separation of Amyloid β-Peptides in a LMS with Metal Ions

A metal affinity effect could be a possible factor for improving the separation of peptides/proteins including Aβ. Immobilized metal affinity chromatography (IMAC) was first developed by Porath et al for the separation of metalloenzymes [52]. IMAC has widely been known as a tool for the purification of His-tagged recombinant proteins [53]. A metal affinity-ATPS (MA-ATPS) has also been developed as an alternative separation method for IMAC, where the extraction efficiency of a His-tagged membrane protein in an iminodiacetate-bound PEG was enhanced in a Triton-water ATPS [54]. This could be because of the higher *HF* value in comparison with the PEG/dextran two-phase systems [55]. Both IMAC and MA-ATPS have a problem with the higher stability of the metal-ligand complex, implying that all the His-harboring target products would adsorb strongly on the gel matrix in the column and furthermore the peptides/proteins with the same His number could not be separated. The previous findings also show that Aβ(1-42) and Aβ(1-40) with the same His number (His_6, His_{13} and His_{14}) (**Fig.5**) could not be separated by using the IMAC and MA-ATPS method.

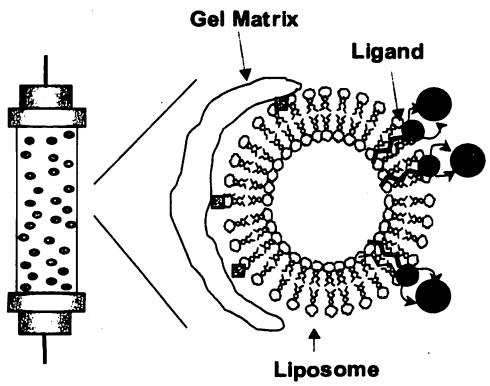

Fig.6 Schematic illustration of MA-ILC. HIDA-modified Liposome was immobilized on gel matrix.

The toxicity (stress) of metal ions in the Alzheimer's disease has also become an important issue. Recently, the toxicity of the

metal-induced reactive oxygen species (ROS) and the positive role of Aβ has been reported by some researchers [47,56]. The following model of the role of Aβ, which can act together with other antioxidant systems, has recently been reported [56]. Cu(II) was found to be toxic to the biomembrane in the biological system via the variation of the membrane property by binding to the membrane and oxidation by the metal-induced reactive oxygen species (ROS). It has been reported that Aβ(1-40) could interact with the membrane-immobilized Cu(II) to inhibit the ROS generation and the peroxidation of the liposome by forming a fibrilous aggregation on the membrane [47]. As a consequence, it can protect the membrane from Cu(II) toxicity [47]. It has also been shown that the Aβ-Cu(II) complex could act as a metal enzyme on the liposome surface [57]. A new model of the control system for Cu(II)-induced ROS stress in the membrane, mimicking the biological system, was proposed on the basis of the Aβ abilities of metal capture and ROS modulation [47,49,50]. It is thought that such biological functions of Aβ could be extended for the design of the adsorption sites of Aβ and a metal ion on the ligand-modified membrane (liposome) could be a candidate for the bio-inspired adsorption site.

Metal affinity immobilized liposome chromatography using a gel matrix immobilizing the ligand(hexadecyl-iminodiacetate(HIDA))-modified liposome has been designed and developed to solve this problem in IMAC and MA-ATPS (**Fig.6**) [47]. MA-ILC has been used to analyze the adsorption behavior of peptide on a metal-immobilized membrane surface [52]. The MA-ILC column could adsorb Cu(II) ion through the immobilized HIDA although the amount of the Cu(II) adsorption was lower than that for a general immobilized metal affinity (IMAC) column. MA-ILC, IMAC and ILC (immobilized liposome chromatography) were evaluated on the basis of their retention properties from the adsorption behavior of 25 peptides that differ in the number of histidine residues. MA-ILC displayed interesting selectivities, combining with those of IMAC and ILC, for the peptides. The retention properties of peptides on MA-ILC

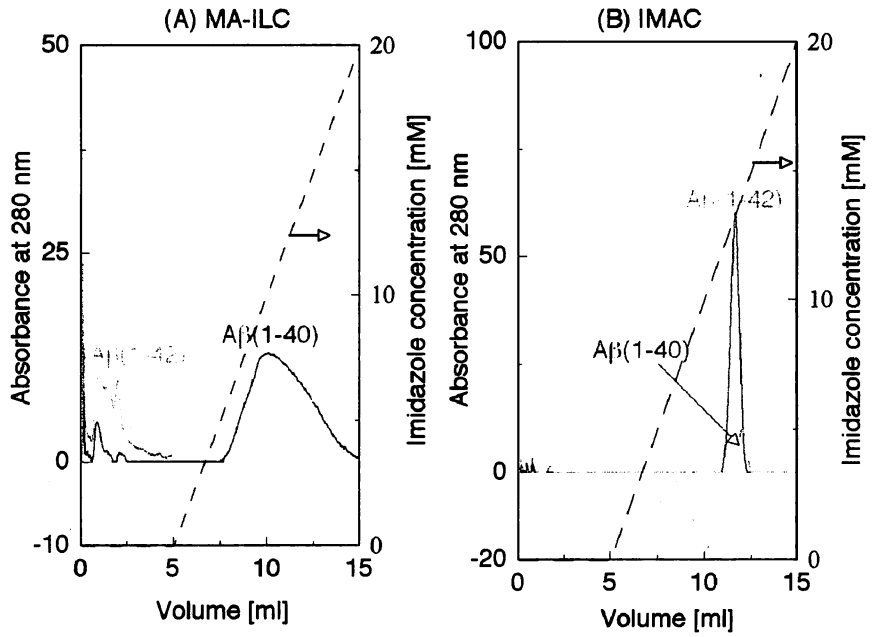

Fig.7 Chromatographic profiles for amyloid β-peptides on (a) MA-ILC and (b) IMAC. The retained peptides were eluted by using an imidazole concentration gradient. A 30 mg sample was applied to the column equilibrated in 20 mM sodium phosphate buffer containing 0.5 M NaCl, pH 7.0.

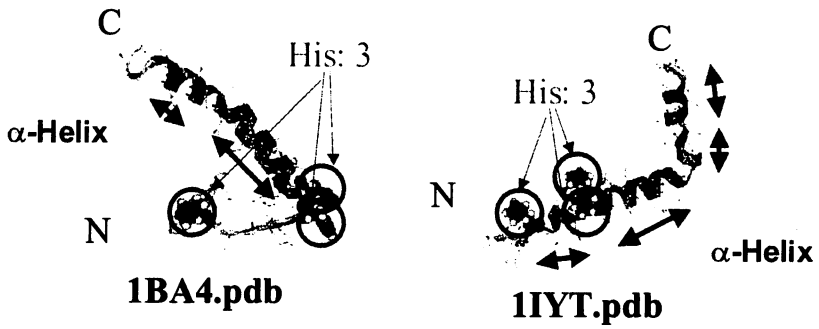

Fig.8 Difference in the secondary structure of Aβ(1-40) (left) and Aβ(1-42) (right)

depend on both the number of histidine residues in the peptides and the hydrophobicity of the peptides. By using multi linear regression analysis, it was found that complex formation of histidine with Cu(II) and the membrane interaction of hydrophobic aromatic residues, such as tryptophan and phenylalanine, with liposome could be key factors in regulating the retention of the peptides on the column. MA-ILC was thus found to be a new effective technique for separating proteins or peptides without an additional reagent in the elution process.

Based on the above MA-ILC findings, the interaction of Aβ on the metal-bound-liposome was investigated. Although a similar retention of Aβs was obtained in the case of IMAC, the elution profile of the Aβ(1-40) and Aβ(1-42) was found to be quite different in the MA-ILC (**Fig.7**). This results show that the Aβ peptides can be successfully separated in the MA-ILC although they both have same number of His residues and their amino acid sequences are almost identical except for two residues. The retention of the Aβs was furthermore found to be dependent on the variation of the contents of their secondary structure (**Fig.8**). It has been reported that the binding of several metal ions with ligands on the liposome membrane is different from that for the organic/water systems with the same ligand [58]. The above results on the variation of the retention of two types of Aβs such as Aβ(1-40) and Aβ(1-42) could also be related to the stability of the Cu-His complex on the membrane and the hydrophobicity of the Aβ surface. It was thus found that Aβ(1-40) and Aβ(1-42) could share their roles on the metal-bound liposome surface by changing their conformational state and a new type of LMS could provide information on the some coordination bond and hydrogen bond on the liposome membrane.

5. Conclusive Prospectus

The possible analytical application of aqueous two-phase systems such as ATPS, RVMS, and LMS have been reviewed, especially focusing on the evaluation of the surface properties of amyloid β-peptides (Aβs) and their separation. The characterization of the surface properties of biomolecules could effectively be achieved based on multi-analysis using both ATPS and LMS in order to elucidate the beneficial characteristics of both systems. The surface properties of Aβ such as hydrophobicity and membrane metal affinity were characterized by using ATPS and also LMS (MA-ILC). This shows that Aβ(1-40) had a hydrophobic surface and a more stable metal-His complex on the liposome membrane compared with

Aβ(1-42). An effective separation of Aβ(1-40) and Aβ(1-42), which differ in only two amino acids in their sequences, could be successfully performed particularly by using MA-ILC, based on the basic findings. In addition, a LMS can act as a micro- or nano-reactor because it has a nano-scaled water pool surrounded by a phospholipid bilayer membrane. Recent research shows that the ligand-modified liposome can act as an antioxidant enzyme [57,59] and that the enzyme-entrapping liposome can act as a micro-reactor for effective bioconversion [60]. The use of the membrane itself could provide new possible applications. By using liposome-immobilization techniques and micro-array technology, such reactor characteristics of liposomes can be applied to the design of micro factories on a chip, including (i) stimuli-inducible reactors, (ii) stimuli-responsive separators, and (iii) sensor functions to identify the type and/or state of the materials.

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