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Electrochemical behavior of ferritin at the polarized water|1,2-dichloroethane interface

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ABSTRACT

The electrochemical behavior of ferritin at the polarized water|1,2-dichloroethane (DCE) interface was studied, mainly under acidic conditions. No obvious voltammetric responses were observed under neutral and alkaline conditions where ferritin formed an uncharged nanocage structure. On the other hand, large electrochemical responses were obtained in the positive potential region under acidic conditions where ferritin was disassembled into positively charged subunits. These responses varied slightly with the concentration of the supporting electrolyte in the organic phase and changed drastically with the concentration of ferritin and under repetitive sweeps. In addition, depending on the applied potential and the concentration of ferritin, a white film was formed at the interface. This electrochemical behavior could be attributed to ion transfer and the adsorption/desorption of positively charged ferritin subunits with the interfacial activity accompanied by the facilitated ion transfer of the organic supporting electrolyte anion.

1. Introduction

Ferritin is a well-known iron storage and detoxification protein, which is ubiquitous in animals, plants and bacteria. Ferritin forms a nanocage structure in which up to 4500 iron atoms can be accommodated [1]. The diameter and inner cavity are approximately 12 and 8 nm, respectively [2]. Ferritin is composed of 24 subunits of two types known as heavy (H) and light (L) chains, classified according to the subunit molecular weight, 21 and 19 kDa, respectively [1,2]. The nanocage structure reversibly assembles and disassembles depending on the pH conditions. The pH-dependent structures of ferritin have been investigated by synchrotron small-angle X-ray scattering (SAXS) [3]. It has been found that the intact hollow spherical ferritin was stable over the pH range 3.40–10.0, while stepwise disassembly of the hollow sphere takes place below pH 3.40. Since these unique features could play an important role in developing a molecular capsule or container, much attention has been paid to ferritin in fields including nanocomposite materials, semiconductors, drug delivery system (DDS) and so on [4–7]. In fact ferritin forms a nanocage structure with several channels through which metal ions and/or organic molecules could pass *in vivo*, however, ferritin could function as an attractive molecular capsule if one can control the reversible assembly reaction.

The interface between two immiscible electrolyte solutions (ITIES) is a useful model for biomembrane systems in which charge transfer and adsorption processes are controlled as a function of the Galvani potential difference between two liquid phases [8,9]. The physicochemical study of bioactive species in biomimetic liquid–liquid systems is important since it is difficult to investigate *in vivo* the complicated pharmacokinetics involved in molecular association, acid-base equilibrium and distribution on or across a biomembrane [10–12]. Furthermore, electrochemical ion transfer at the ITIES offers an attractive label-free method of determining non-redox active species [13]. There are a number of studies of the electrochemical characterization and determination of small ionizable drugs [14–16], polyelectrolytes [17], macromolecules [18,19], and proteins [20–23] at the ITIES. We have recently investigated the interfacial mechanism of dendritic polymers and their association behavior with anionic water-soluble porphyrins [24], ionizable drugs [25] and bioactive flavin species [26] through spectroelectrochemical techniques. In these works, it was demonstrated that NH_2- and COOH-terminated polyamidoamine (PAMAM) dendrimers are useful molecular capsules and that hyperbranched polymers are pharmacokinetic modifiers of bioactive species.

In this study, the voltammetric behavior of ferritin was studied at the water|1,2-dichloroethane (DCE) interface prior to an application of ferritin as a molecular capsule for a functional DDS. The influence of pH, the concentration of ferritin and the organic supporting electrolyte, and the effect of repetitive sweeps on the voltammetric response were investigated.

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2. Experimental

2.1. Reagents

Ferritin from equine spleen (F4503) was purchased from Aldrich and used as received. The concentration of ferritin was calculated by using the molecular weight of the peptides (*ca.* 440 kDa). 1.0×10^{-2} mol dm^{-3} LiCl and 5.0×10^{-3} mol dm^{-3} bis(triphenylphosphoranylidene)ammonium tetrakis(pentafluorophenyl)borate (BTPPATPFB) were used as supporting electrolytes for the aqueous and organic phases, respectively. BTPPATPFB was prepared by metathesis of bis(triphenylphosphoranylidene)ammonium chloride (BTPPACl) (Aldrich > 97%) and lithium tetrakis(pentafluorophenyl) borate (LiTPFB) ethyl ether complex (TCI > 70%). The organic solvent, 1,2-dichloroethane (DCE), was of HPLC grade (Nacalai Tesque > 99.7%). The aqueous solutions were prepared with purified water from a Milli-Q system (Millipore Simplicity UV). The pH of the aqueous phase was adjusted by the addition of HCl, 5.0×10^{-3} mol dm^{-3} LiH_2PO_4/LiOH or LiOH.

2.2. Apparatus

The structure of the electrochemical cell is represented in Fig. 1. The water|DCE interface with a geometrical area of 0.50 cm^2 was polarized by a four-electrode potentiostat (Hokuto Denko HA1010 mM1A). Platinum wires were used as counter electrodes in both aqueous and organic phases. Luggin capillaries were provided for the reference electrodes (Ag/AgCl) in both phases. The Galvani potential difference ($\Delta_o^w\phi \equiv \phi^w - \phi^o$) was estimated by taking the formal transfer potential ($\Delta_o^w\phi^{o'}$) of the tetraethylammonium cation as 0.02 V [27].

3. Results and discussion

Typical cyclic voltammograms (CVs) measured for ferritin under various pH conditions are shown in Fig. 2a. CVs obtained under acidic conditions varied gradually depending on the repetitive sweeps (discussed later). Unless otherwise noted, therefore, the CVs obtained after 5 repetitive sweeps are used for the following discussion. No obvious voltammetric responses were observed in the potential window under neutral and alkaline conditions. However, at pH 2.1, anodic and cathodic peaks were obtained at 0.30 and 0.15 V, respectively, and the current increased at the edge of the positive potential window. The reported isoelectric point (pI) of ferritin is between 4.1 and 5.1 [28]. Therefore, ferritin should be the cationic species at pH 2.1 when ferritin is also disassembled into subunits [3]. By contrast, the voltammetric responses were not clearly observed at pH 11.8, where ferritin should be negatively charged due to the deprotonated carboxyl groups. It could be considered that ferritin could respond beyond the potential window measured in this study, since the current response was slightly increased in the negative potential region. In addition, the peak-to-peak separation was much larger than the theoretical value ($\approx 59/z$ mV), suggesting the occurrence of multi-interfacial mechanisms. In order to study the electrochemical behavior under acidic conditions in more detail, AC voltammograms (ACVs) were measured for ferritin at pH 2.1 (Fig. 2b). It should be noted that the ACVs were drastically different

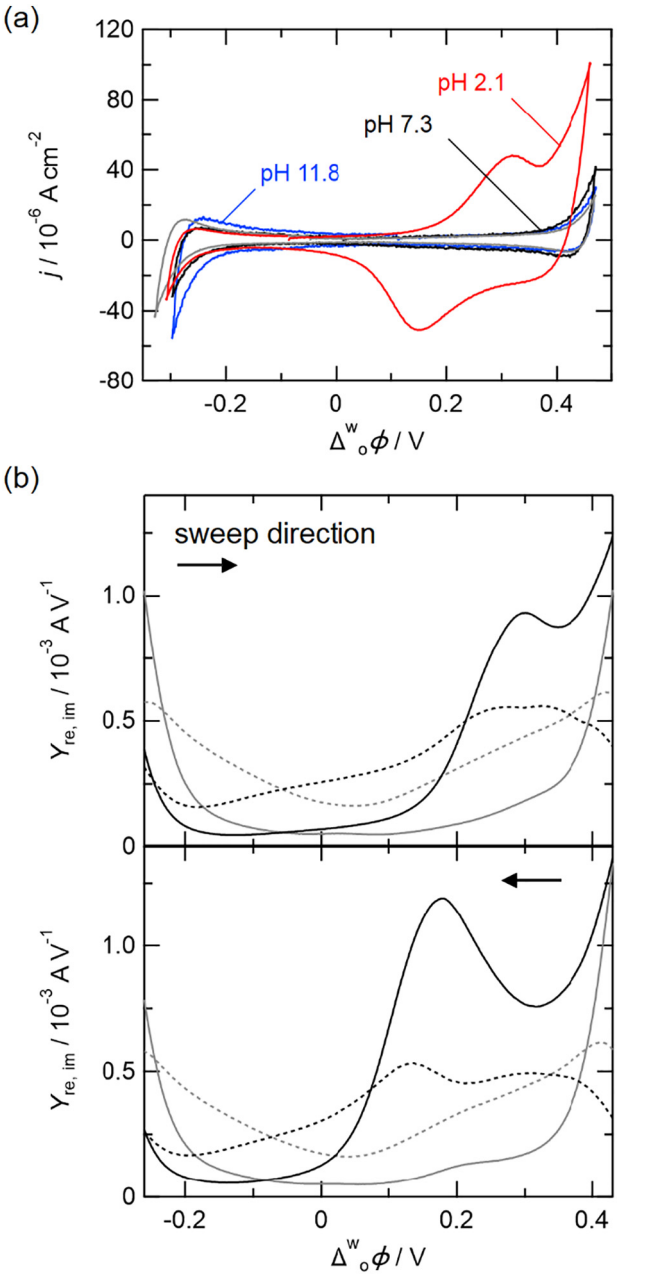

Fig. 2. (a) Typical CVs measured for ferritin at various pHs. The potential sweep rate was 100 mV s^{-1}. The concentration of ferritin was 1.0×10^{-6} mol dm^{-3}. (b) ACVs measured for ferritin at pH 2.1. The solid and dashed lines are the real (Y_{re}) and imaginary (Y_{im}) components of the admittance, respectively. The gray lines indicate the admittance in the absence of ferritin at pH 2.1. The potential sweep rate was 5 mV s^{-1}. The amplitude of AC potential modulation was 10 mV at 7 Hz. The concentration of ferritin was 1.0×10^{-6} mol dm^{-3}.

Ag	AgCl	1.0×10^{-3} mol dm^{-3} BTPPACl 1.0×10^{-2} mol dm^{-3} LiCl (aq)	5.0×10^{-3} mol dm^{-3} BTPPATPFB (DCE)	**ferritin** 1.0×10^{-2} mol dm^{-3} HCl 1.0×10^{-2} mol dm^{-3} LiCl (aq)	AgCl	Ag

Fig. 1. Composition of the electrochemical cell.