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Cytochrome c in sodium dodecyl sulfate reverse micelle nanocage: From a classic electron carrier protein to an artificial peroxidase enzyme

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ABSTRACT

Cytochrome c (Cyt c) is a hemoprotein involved in shuttling electrons in the mitochondrial electron transport chain. Under oxidative stress conditions, the interaction of Cyt c with anionic phospholipids results in significant enhancement of its peroxidase activity that is essential during apoptosis. Reverse micelles are convenient membrane mimetic nanostructures and were used here to simulate Cyt c peroxidase activity. Cyt c peroxidase activity was markedly enhanced in sodium dodecyl sulfate (SDS) reverse micelles. This was dependent on buffer concentration of water pool and mass percentage of buffer in reverse micelles. Fluorescence intensity based on tryptophan residue, electronic absorption curves, and circular dichroism measurements indicated partial unfolding and cleavage of axial methionin 80-Fe bond of Cyt c in reverse micellar medium resulting in the formation of a peroxidase-like artificial enzyme.

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1. Introduction

Cytochrome c (Cyt c) is a small hemoprotein (110 residues) of about 30 Å in diameter and exists in the intermembrane space of mitochondria [1]. The Cyt c's essential roles in energy metabolism and shuttling of electrons between mitochondrial respiratory complexes III and IV have been known for decades. Moreover, the antioxidant function of Cyt c has been recently described [2]. The central iron in Cyt c has tetra-coordinate association with porphyrin and two coordinated bonds with methionin-80 (Met-80) and histidine-18 (His-18). Thus, the six coordinated iron center is designed for electron donor/acceptor function and, peroxidase activity of solubilized Cyt c is very low [3]. Cyt c also plays an important role during apoptosis through oxidizing cardiolipin (CL) in the mitochondria and phosphatidylserine (PS) in the plasma membrane [4]. Jemmerson et al. [5] showed that the structure of Cyt c changes during apoptotic reaction cascade and, that these changes can be mimicked by interactions of Cyt c with the membrane. Salamon and Tollin [6] showed that Cyt c redox potential, through interactions with lipid bilayer membrane, shifts to positive values resulting in decreased rate of electron transfer by Cyt c. Furthermore, Cyt c peroxidase activity has been reported upon its interactions with a bilayer membrane, and can be induced by surfactant assemblies [7,8].

Reverse micelles are spherical nanodroplets of water surrounded by a monolayer of closely packed surfactant molecules dispersed in a solvent of low polarity [9] in which the surfactant non-polar tails are in the oil, while the polar head groups are in direct contact with the centered water. The size of nanodroplets can be varied by simple addition of water at a constant surfactant concentration. The [water]/[surfactant] (w_0) controls the size of nanodroplets [9]. Reverse micelles can be host of hydrophilic and membrane bound proteins, keeping low viscosity and clarity of solution and, in most cases protein integrity [9]. Reverse micelles can be used as nanoreactors to produce either well-defined nanosized crystallites or chemically modified enzymes [10].

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Interaction of Cyt c with sodium bis(2-ethylhexyl) sulfosuccinate (AOT) reverse micelles has been previously investigated [1]. X-ray scattering results showed a decrease in the droplet radius upon addition of Cyt c to the isooctane/AOT/water system [1]. The electrostatic interactions between the cationic surface of Cyt c and the anionic sulfonate groups of AOT were involved in the conformational perturbation of Cyt c as well as the lipid bilayer membrane [1]. The catalytic activity of Cyt c hosted in AOT reverse micellar solution was higher than that in water [11]. Sodium dodecyl sulfate (SDS) is also a convenient anionic surfactant whose interactions with Cyt c can mimic binding of the protein to the mitochondrial membrane as occurs during apoptosis [12]. SDS is spectroscopically silent for various techniques and has been previously used for studying the folding/unfolding of Cyt c [12–16]. SDS micelles induce and stabilize secondary structures, particularly α-helixes [17]. In fact, SDS monomers and micelles act both as a denaturant and a recovery reagent [18]. Interaction of Cyt c with SDS promotes its conversion to the same conformational state as binding to liposomes or Cyt c oxidase [16,19]. It is expected that the Cyt c solubilization in SDS reverse micelles enhances its peroxidase activity accommodated by structural changes upon its interactions with the surfactants.

Peroxidases catalyze oxidation of a wide variety of organic substrates and are promising biocatalysts for specific organic synthesis [11,20]. Oxidative polymerization of phenol, aniline, and their derivatives generates environmentally friendly resins [21]. Production of a chiral organic molecule through region and enantioselective oxidation [22] and decolorization of azo and antraquinone dyes [23] are examples of peroxidase applications in organic medium. The high cost, decreased activity, and low stability and solubility of peroxidases in organic medium have largely limited their use as biocatalysts in industry. Cyt c with covalently bound heme prosthetic group is active over a wide range of pH [24], high concentration of organic solvents, and high temperatures making it a good candidate for use as a biocatalyst [25]. There are a few reports of interaction of aqueous solutions of SDS in micellar and sub-micellar concentrations with Cyt c, but the study of SDS reverse micelles with Cyt c has not been previously reported. Here we determined the catalytic efficiency of Cyt c in SDS reverse micelles as an artificial enzyme.

2. Materials and methods

2.1. Chemical reagents

Bovine heart Cyt c, SDS, AOT and histidine were obtained from Sigma. Other chemicals were from Merck products of analytical grade and were used without further purifications. Double distilled water was used throughout these studies.

2.2. Preparation of solutions

Cyt c, H_2O_2, ABTS and guaiacol solutions were prepared fresh in 20 mM sodium phosphate buffer pH 7.0. SDS reverse micelles used in this study comprised aqueous buffer, SDS, 1-hexanol and dodecane. This system has been previously used for study of protein structure and function in reverse micelles [26]. Enzymatic activity in SDS reverse micelles was explored along experimental paths with different water (buffer) to dodecane ratios, but with constant surfactant/alcohol mass percentage. The molar ratio K_m between surfactant and alcohol was 1/6.54 and the mass percentage of SDS + alcohol was $Ps_{SDS+alcohol} = 0.424$. AOT reverse micelles comprised 50 mM AOT in isooctane $w_0 = 10$. Reverse micellar solutions of Cyt c were prepared by direct injection of aqueous solution of Cyt c to reverse micellar medium.

2.3. Electronic absorption measurements

10 μL aqueous solution of Cyt c was added to 2.9 mL aqueous medium (20 mM phosphate buffer pH 7.0) or reverse micellar medium (20% (w/w) water content). After proper time for salvation and reaching the equilibrium, the electronic absorption curves (250–800 nm) were collected with a spectrophotometer (Shimadzu-3100), at room temperature with 1-cm path-length cells equipped with a thermostatic holder. The final protein concentration in cells was 10 μM.

2.4. Circular dichroism measurements

The circular dichroism (CD) measurements were carried out in a model 215 circular dichroism spectrometer (AVIV) at room temperature. Far-UV CD spectra (190–260 nm) were measured using a quartz cell of 1 mm path length. Protein concentration of 10 μM in 20 mM phosphate buffer pH 7.0 or in 20% (w/w) water content reverse micelles were used. The protein helical content was calculated by the method of Chen et al. [27].

2.5. Fluorescence spectra measurements

Tryptophan fluorescence measurements were carried out in a Carry-Eclipse fluorescence spectrophotometer (Varian) at room temperature using excitation at 280 nm and the scan rang of 285–435 nm. Protein concentration of 10 μM in 20 mM phosphate buffer pH 7.0 or in 20% (w/w) water content reverse micelles was used.

2.6. Steady state kinetic measurements

ABTS or Guaiacol was added to reverse micellar solution of Cyt c, subsequently addition of H_2O_2 initiated oxidative reaction. Typical reaction concentrations were as follows: [Cyt c] = 2.4 μM, [H_2O_2] = 0.5 mM, [guaiacol] = 0.05–3 mM, and for ABTS oxidation, [Cyt c] = 0.4 μM, [H_2O_2] = 0.2 mM, [ABTS] = 0.005–0.5 mM. In order to reach the steady state conditions a lag time of 7 s was used. Progress curves of reactions were obtained at 414 nm at various ABTS concentration or 470 nm at various guaiacol concentrations. The obtained initial rates were used to record the Michaelis–Menten curves. The concentration of H_2O_2 was kept high and constant with respect to hydrogen donor during the course of reaction to ensure pseudo-first order kinetics.

3. Results and discussion

In a neutral pH aqueous solution, Cyt c has a charge-transfer band related to the presence of Met-80 as the sixth ligand of the central iron at 695 nm [28]. In addition, the Q bands of the heme at 520–550 nm and the Soret band at 406 nm were observed (Fig. 1). In reverse micelles, the 695-nm band was not observed and the Soret band underwent an increase in intensity and a small blue shift. The Q bands remained unchanged. Cyt c in 2 and 7 mM SDS aqueous solution at pH 7.0 exhibited such characteristics attributed to the alternative low-spin form [13,29].

Cyt c has only one tryptophan residue (Trp-59). A very weak fluorescence band from Trp-59 was observed in Cyt c solutions. This was mainly attributed to quenching of the excited Trp by the heme in aqueous solution [30]. The emission intensity had a maximum at 330 nm indicating Trp-59 as a buried residue [31]. In reverse micelles, the Trp fluorescence yield underwent a large increase and a small blue shift (Fig. 2). The fluorescence intensity was depended on the water content of the reverse micelle (Fig. 3). Similar results were observed in AOT reverse micelles [1]. The increased fluorescence intensity may be indicative of a less efficient Trp quenching

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