Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Colloid and Interface Science 297 (2006) 561–569
JOURNAL OF Colloid and Interface Science
www.elsevier.com/locate/jcis

Effect of *n*-alkyl trimethylammonium bromides on folding and stability of alkaline and acid-denatured cytochrome c: A spectroscopic approach

J. Chamani [a,*], A.A. Moosavi-Movahedi [b]

[a] *Department of Biology, Faculty of Science, Islamic Azad University-Mashhad Branch, Mashhad, Iran*
[b] *Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran*

Received 8 October 2005; accepted 12 November 2005
Available online 9 December 2005

Abstract

The molten globule (MG) state can be an intermediate in the protein folding pathway; thus, its detailed description can help understanding protein folding. Alkyl trimethylammonium bromides including dodecyl trimethylammonium bromide, DTAB; tetradecyl trimethylammonium bromide, TTAB; and hexadecyl trimethylammonium bromide, HTAB; cationic surfactants that are commonly used to mimic hydrophobic binding environments such as cell membranes, are known to denature some native state proteins, including horse cytochrome c (cyt c). In this article, refolding of alkaline and acid-denatured cyt c are studied under the influence of *n*-alkyl trimethylammonium bromides to form MG-like states at both low concentration (pH 11) and above the critical micelle concentration (pH 2) using ultraviolet and visible absorption, fluorescence and circular dichroism (CD). The addition of *n*-alkyl trimethylammonium bromides to the unfolded state of cyt c in alkaline and acidic condition appears to support the stabilized form of the MG state. The *m*-values of the refolded state of cyt c by DTAB, TTAB and HTAB showed substantial variation. The enhancement of *m*-values as the stability criterion of the MG state corresponded with increasing chain length of the cited *n*-alkyl trimethylammonium bromides. Based on the results obtained, the merits of two models of the protein-surfactant structure are discussed for various *n*-alkyl trimethylammonium bromides concentration in inducing the MG state at two different pH conditions. Therefore, hydrophobic interactions play a dominant role in stabilizing the MG state.

Keywords: Cytochrome c; Cationic surfactants; Molten globule-like state; Protein folding; Hydrophobic interaction; Stabilization

1. Introduction

Cytochrome c (cyt c) plays an important role in the biological electron transfer system and has been extensively studied [1–3], including having its fully resolved three-dimensional structure determined by X-ray and nuclear magnetic resonance (NMR) [4–6]. For cyt c, several probes (IR, UV–vis, CD, fluorescence) can be used to monitor the structural changes needed to obtain the variety of states accessible under different solution perturbations (GuHCl, urea, pH, temperature, etc.) [7–15]. When acidified, cyt c is denatured to a primarily random coil structure, destabilized due to the electrostatic repulsion between positively charged residues. A molten globule (MG) state of cyt c can be achieved by adding salt to this acid-denatured state, whereby the electrostatic repulsion is reduced, which is believed to drive the protein to become more compact [16]. That state is characterized by high helical content in its secondary structure, but with little evident tertiary structure [17,18].

It is important to elucidate the structure and stabilizing mechanism of the MG state, as an intermediate between the native and denatured states, in order to understand the principles for constructing a three-dimensional protein structure X-ray small angle scattering studies have shown that the MG states of various proteins have a wide range of structures from relatively disordered to highly ordered [19–21]. This implies that the MG state is a largely fluctuating ensemble of various energy minimums. Moreover, the stability of the MG state is determined by a delicate balance of interactions, such as electrostatic repulsion between charged residues and opposing forces such

* Corresponding author. Fax: +98511 8435050.
E-mail address: chamani@ibb.ut.ac.ir (J. Chamani).

0021-9797/$ – see front matter
doi:10.1016/j.jcis.2005.11.035

as hydrophobic interaction. A significant influence of salts or charges on the stability of the MG state reveals that the main driving force of the MG state is the reduction of electrostatic repulsion between charged residues, which favors unfolded conformations [17,22,23]. However, there is a lack of substantial evidence regarding the contribution of hydrophobic interactions to the stability of the MG state. However such interactions have been suggested for the positive heat capacity changes of the thermal unfolding of the MG of apomyoglobin [24,25] and cyt c [26–28].

It is well known that ionic surfactants can interact very strongly with oppositely charged globular proteins [22–32]. The interaction of cationic surfactants (especially *n*-dodecyl trimethylammonium bromide, DTAB; *n*-tetradecyl trimethylammonium bromide, TTAB; and *n*-hexadecyl trimethylammonium bromide, HTAB) with biomacromolecules has been studied earlier [30]. Cationic surfactants often destabilize the protein. The presence of DTAB causes the destabilization of protein and results in a decrease in the temperature of unfolding with an increase in the DTAB concentration [30,31]. The interaction of DTAB with calf thymus DNA has also been studied using various methods, such as microcalorimetry and potentiometry [33]. Studies of such interactions between proteins and surfactants have been carried out for half a century; however, the mechanism by which the surfactants influence protein structure is still not well defined. Information concerning the structure of protein–surfactant complexes has been derived from rheological [34], spectroscopic [34,35], electrophoretic [36], binding [37,38] and scattering studies [39,40]. Several models have been proposed for the structure of protein–surfactant complexes over their critical micelle concentration (CMC): (1) a correlated "necklace and bead" model in which clusters with a micelle-like structure are stabilized by protein (Fig. 1) [40–42]; (2) "rod-like" prolate ellipsoidal surfactant aggregate with a semi-minor axis of ∼18 Å, corresponding to the surfactant chain length [34,41]; and (3) a flexible capped helical cylinder micelle with the protein wrapping around the micelle [42]. A small-angle neutron scattering study of BSA and ovalbumin complexes with SDS led to the conclusion that a necklace and bead structure, compose of protein–surfactant aggregates, accounted for the scattering behavior of these systems [39,40], which was confirmed by NMR experiments by Turro et al. [42].

In this article, the interactions between alkaline and acid-denatured cyt c and various kinds of *n*-alkyl trimethylammonium bromides are studied. MG-like states can be achieved at very low concentrations and above the CMC of *n*-alkyl trimethylammonium bromides at alkaline and acidic conditions, respectively. Recently, other workers also showed the formation of MG-like states of cyt c induced by low concentration *n*-alkyl sulfates [43]. Those authors suggested that hydrophobic interactions play an important role in stabilizing the MG state. Another paper on cyt c in surfactant appeared [44], which focused on urea unfolding and refolding with SDS. In this present work, by comparing the results of different kinds of cationic surfactant-induced MG-like states, we propose that the primary driving force for the formation of cyt c–alkyl trimethylammonium bromides induced MG-like states is the reduction of electrostatic repulsion, although the hydrophobic effect dose remains a factor.

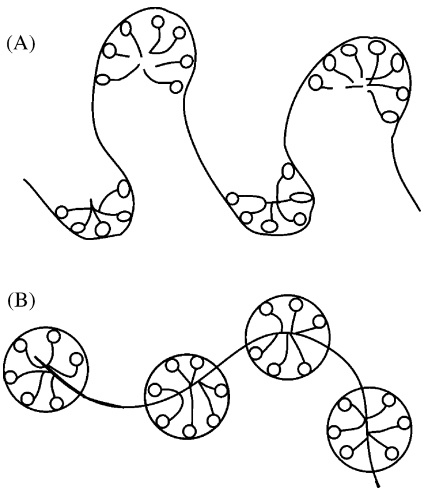

Fig. 1. The "necklace and bead" structure of protein–surfactant complexes and its two possibilities. (A) The protein wraps around the micelle. (B) The micelles nucleate on the protein hydrophobic sites.

2. Materials and methods

2.1. Materials

Horse cytochrome c (type IV), in the oxidazed form, was purchased from Sigma and used without further purification. Alkyl trimethylammonium bromides (DTAB, TTAB and HTAB) were also obtained from Sigma. All the pH values represent apparent pH meter readings.

2.2. Solution preparation

The protein solution was dialyzed against buffers (20 mM HCl, pH 2; 25 mM phosphate buffer in pH 7 and 50 mM Glycine-NaOH buffer, in pH 11). The extinction coefficients were used to calculate the concentration of the native protein at pH 7 and the denatured protein at pH 2 and 11. If the initial concentration and volume of the protein solution are $[P]_0$ and V_0, respectively, and the stock ligand concentration is $[L]_0$, then the total concentration of protein ($[P]_t$) and ligand ($[L]_t$) can be obtained by accounting for the total volume of the aliquot (V_c) added during the titration experiment [45]:

$$[P]_t = [P]_0 V_0/(V_0 + V_c), \quad [L]_t = [L]_0 V_0/(V_0 + V_c).$$

Aliquots of *n*-alkyl trimethylammonium bromides were injected into the cyt c solution at 5 min interact to allow for equilibration; each experiment was repeated three times.

2.3. Methods

2.3.1. Circular dichroism measurements

Far-UV experiments were performed on a Jasco-810 spectropolarimeter equipped with a Jasco 2-syringe titrator. Spectra