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Focussed review

Structure and function of heme proteins in non-native states: A mini-review

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ABSTRACT

Heme proteins perform various biological functions ranging from electron transfer, oxygen binding and transport, catalysis, to signaling. Although adopting proper native states is very important for these functions, progresses in representative heme proteins, including cytochrome *c* (cyt *c*), cytochrome b_5 (cyt b_5), myoglobin (Mb), neuroglobin (Ngb), cytochrome P450 (CYP) and heme-based sensor proteins such as CO sensor CooA, showed that various native functions, or new functions evolved, are also closely associated with non-native states. The structure and function relationship of heme proteins in non-native states is thus as important as that in native states for elucidating the precise roles of heme proteins in biological systems.

1. Introduction

As pointed out recently by Tokuriki and Tawfik [1], the traditional view that proteins adopt a single structure and possess a specific function conflicts with their marked ability to acquire new structures and functions. And thus an "avant-garde view" of proteins was proposed by the authors [1], in which proteins possess inherent conformational variability and hence functional diversity. It was revealed that a folded protein can also exist in multiple conformational substates with a distinct structure, and each substrate reflects a local minimum on the free-energy landscape [2–4]. These substates may represent the non-native states of the folded protein, although the distinction between the conformational energy landscapes for native and non-native states varies in different proteins [5]. Conformational diversity in proteins may range from fluctuations of side chains and movements of active-site loops to secondary structure exchanges and rearrangements of the entire protein fold, which confers new functions for the proteins in the non-native states [1]. Understanding the intrinsic mechanisms responsible for the transitions from the native protein to its functional non-native conformers presents numerous challenges, which ultimately uncovers the secrets of how the proteins execute a fascinating variety of functions in biological systems [6].

Metalloproteins accounts for nearly half of all proteins in nature [7], in particular, heme proteins are intensively investigated due to their large array of biological functions, including electron transfer (*e.g.* cytochrome *c*, cyt *c* and cytochrome b_5, cyt b_5), oxygen binding and transport (*e.g.* myoglobin, Mb and hemoglobin, Hb), catalysis (*e.g.* cytochrome P450, CYP) and signaling (*e.g.* CO sensor CooA) [8]. Although different heme proteins generally perform different biological functions, the same heme protein may also exhibit diverse functions, for example, the electron transfer protein cyt *c* was also found to carry out functions beyond respiration because of its conformational diversity [9]. The structure and function of heme proteins in non-native states have attracted much attention in recent years, which is as important as that in the native states for elucidating their precise roles in biological systems, as they experience a variety of conformational changes *in vivo*, and such transitions are essential for their biological functions [10]. In this mini-review, we focus mainly on some representative heme proteins, including cyt *c*, cyt b_5, Mb, neuroglobin (Ngb), CYP and heme-based sensor protein CooA. Recent progresses on these heme proteins provide valuable information for understanding their delicate structure and function relationship in non-native states.

2. Cytochrome *c*

A representative heme protein is the well-known cyt *c*, which has a six-coordinated heme (Met/His) group attached covalently to the protein polypeptide chain through one, and in most cases, two thioether linkages [11] (Fig. 1A). In the past decade, in addition to the function of electron transfer in mitochondria, cyt *c* was also found to trigger caspase activation and to be involved in cell death [12]. It was found that during the early phase of apoptosis, cyt *c* forms a complex with cardiolipin (CL), a mitochondria-specific phospholipid, and acts as a peroxidase that selectively oxidizes CL peroxidation, which decreases the strength of CL–cyt *c* interactions, promoting the release of the pro-apoptotic factors from the mitochondrial membrane and initiating the apoptosis [13]. The heterogeneous CL-bound cyt *c* ensemble is difficult

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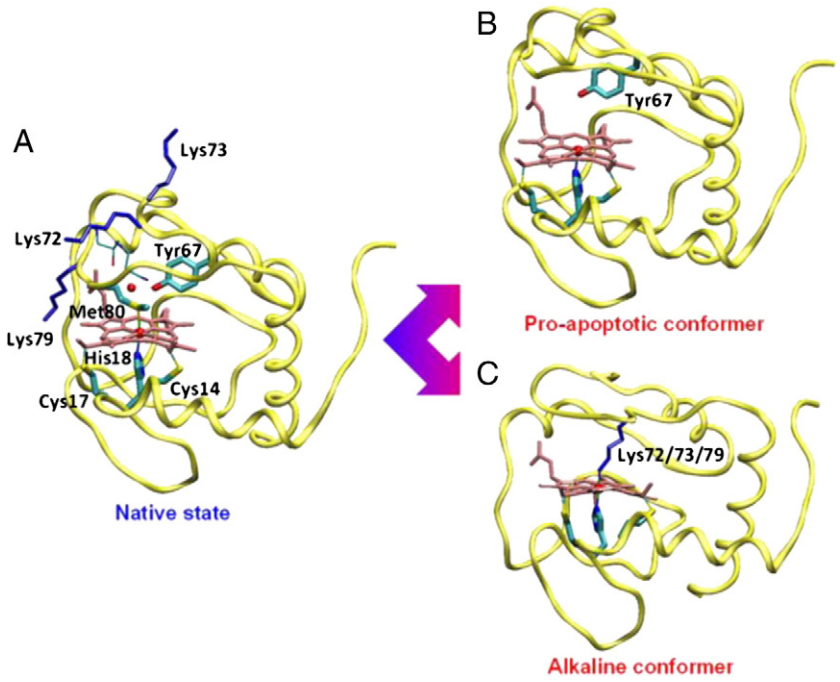

Fig. 1. Native state (A, PDB entry 2YCC [11]) and two non-native states, pro-apoptotic (B) and alkaline (C) conformers of yeast iso-1 cyt *c*, as obtained by MD simulation (unpublished data).

to characterize with traditional structural methods and ensemble-average probes. Through a combination of magnetic circular dichroism (MCD) spectroscopy and potentiometric titrations, Cheesman, Butt, and coworkers [14] showed that both the ferric and ferrous forms of CL–cyt *c* complex exist as multiple conformers at pH 7.4. In the ferric state, Fe(III) is coordinated by His/Lys or His/OH ligands, whereas in the ferrous state, Fe(II) is predominantly high-spin and coordinated by a single His ligand. Recently, Pletneva and co-workers [15] employed time-resolved fluorescence resonance energy transfer (TR-FRET) technique to evaluate the structural properties of the CL-bound protein in several dansyl-labeled variants of horse heart cyt *c*, which uncovered two distinct types of CL-bound cyt *c* conformations, a largely unfolded one (*i.e.*, extended form), and another with native-like fold. Although the extended form of cyt *c* is very open, it likely preserves some native interactions such as residual α-helicity. The loss of tertiary contacts in the extended form thus contributed to the associated increase in the cyt *c* peroxidase activity. It was shown that the two conformations of CL-bound cyt *c* are not independent but undergo conformational exchange related to the break-up and reestablishment of contacts between the protein's N- and C-terminal helices [16]. Further study showed that one major component of mitochondria, ATP, weakens CL–cyt *c* interactions [17]. Moreover, Groves and co-workers revealed very recently that cyt *c* causes pore formation in CL-containing membranes and itself leakage across the membrane, which are significantly reduced in the presence of ATP [18].

To provide insights into the structure and function of cyt *c* in the non-native state of "pro-apoptotic conformer" (Fig. 1B), Huang and co-workers [19] developed two mutants of yeast iso-1 cyt *c*, Y67H and Y67R, and found that these two mutants represent a state which resembles an unfolded state in cyt *c* exhibiting high peroxidase activity. In these mutants, the hydrogen bond network which connects two less stable loops, residues 40–57 and 71–85, in cyt *c* was damaged, and the Fe–S (Met80) bond was weakened (in the Y67H mutant) or broken (in Y67R mutant). Consequently, Y67H and Y67R mutants exhibited a significantly increased peroxidase activity at 42 °C, which is ~70-fold and ~130-fold higher than that of wild-type cyt *c* under the same condition, respectively. Since cyt *c* could bind to mitochondrial membrane through electrostatic interactions with the anionic CL molecules, probably by its surface Lys72 and Lys73 residues [20], and Tyr67 is just located in this segment of cyt *c* (residues 65–72), thus the interaction of this domain with CL could lead to a damage of the inner hydrogen bond network associated with Tyr67, triggering the conformational transition and conferring the protein high peroxidase activity, which is important in the initial stages of apoptosis.

Moreover, Spiro and co-workers [21] recently found that His26 protonation in cyt *c* triggers exposure of the heme center to exogenous ligands, thereby inducing peroxidase activity. The unfolding mechanism for generating the non-native state of cyt *c* may play a role in CL-peroxidation by cyt *c* during apoptosis. On the other hand, Santucci and co-workers [22] showed that the replacement of His26 by a tyrosine frees two loops (20s and 40s loops) in cyt *c*. Consequently, the H26Y mutant adopts a molten globule structure, even at neutral pH, with enhanced protein flexibility and weakened Fe–S (Met80) bond. The non-native H26Y mutant was found to exhibit a 4.4-fold increased peroxidase activity compared to that of native cyt *c* [23].

In addition to a "pro-apoptotic conformer" with an increased peroxidase activity, cyt *c* can adopt an "alkaline conformer" in which the heme axial ligand Met80 is replaced by Lys72, Lys73 or Lys79 at alkaline pH (Fig. 1C) [24]. The peroxidase activity as well as the redox potential of cyt *c* is significantly reduced during this transition [24]. A great deal of effort has been devoted to the identification of the "trigger group" for alkaline transition, whose deprotonation is a key requirement to initiate the conformational change of cyt *c*, and much progress has been made in recent years [24–30]. Bowler et al. [25–28] in a series of studies suggested that the deprotonation of lysine itself (Lys72/73/79) may trigger the alkaline transition. Moreover, several other protonable groups were also suggested, such as Tyr67, His18 and one of the heme propionate groups [24,25]. Huang and co-workers showed that Tyr67 is a probable trigger for the pro-apoptotic conformational transition in cyt *c* [19], and they further showed that it is not the trigger for the alkaline transition, based on the observations of alkaline conformational transitions for Y67H, Y67H/M80V and Y67H/M80D mutants of cyt *c* [30]. To address the mechanistic relevance of the two distinct conformational transitions in cyt *c*, *i.e.*, the pro-apoptotic and alkaline transitions (Fig. 1), Huang and co-workers proposed that the alkaline conformational transition may serve as a protective mechanism against cyt *c* abnormal enhancement