Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 96 (2012) 365–369

Contents lists available at SciVerse ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Observation of heme transfer from cytochrome b_5 to DNA aptamer

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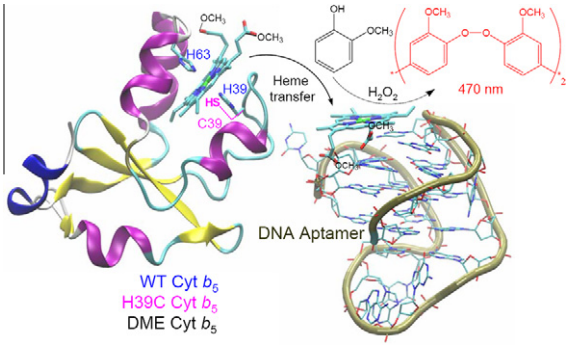
HIGHLIGHTS

- Heme transfers from WT cyt b_5, H39C cyt b_5, and DME cyt b_5 to heme–aptamer, PS2.M.
- Order of heme transfer rate constants: k(H39C cyt b_5) ≫ k(WT cyt b_5) > k(DME cyt b_5).
- Cyt b_5–PS2.M system exhibits peroxidase activity as a result of heme transfer.
- Heme transfer provides insights into both cyt b_5–heme and PS2.M–heme interactions.
- Heme transfer to DNA can be used to evaluate the relative stability of heme proteins.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 February 2012
Received in revised form 12 May 2012
Accepted 25 May 2012
Available online 4 June 2012

Keywords:
Heme proteins
Cytochrome b_5
DNA aptamer
Heme transfer
Peroxidase

ABSTRACT

Heme transfer is commonly observed from one heme protein to the other such as from cytochrome b_5 (cyt b_5) to apo-myoglobin. In this study, instead of to another heme protein, we observed the heme transfer from wild-type (WT) cyt b_5, H39C cyt b_5 with heme axial ligand His39 mutated to Cys39, and DME cyt b_5 with heme replaced by protoporphyrin IX dimethyl ester, to a heme DNA aptamer, PS2.M, respectively, with a different rate constant. The heme transfer was further confirmed by the enhancement of peroxidase activity of the cyt b_5s–PS2.M system due to the formation of catalytic PS2.M–heme complex. This study provides valuable insights into both cyt b_5–heme and PS2.M–heme interactions and shows that heme transfer from heme protein to heme–aptamer can be used to evaluate the relative stability of heme proteins. In addition, this study sheds light on the maturation of heme proteins *in vivo* by interacting with DNA/RNA enzymes.

Introduction

Heme, Fe-protoporphyrin IX, can be used by various heme proteins to perform diverse biological functions, such as myoglobin (Mb) and hemoglobin (Hb) (oxygen carrier), cytochrome b_5 (cyt b_5) and cytochrome c (cyt c) (electron transporter), and horseradish peroxidase (HRP) and cytochrome P450 (cyt P450) (biocatalyst) [1–5]. The diverse functions of heme proteins can be attributed to the diverse interactions between heme group and protein polypeptide chain. For example, cyt b_5, an electron transporter, has the heme group coordinated by two axial histidines (His39 and His63) (Fig. 1A) [6], whereas biological enzymes such as HRP has a five-coordinate heme group with an open site for substrate binding and oxidation [5].

Moreover, unlike in cyt c where the heme group was attached to the polypeptide chain via one or two thioether linkages [7], it was commonly associated with heme proteins such as Mb, Hb, cyt b_5, HRP and cyt P450 non-covalently, major through coordination interactions, hydrogen interactions as well as hydrophobic interactions. Due to the different interactions responsible for the stabilization, heme proteins have different heme binding stabilities. For

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http://dx.doi.org/10.1016/j.saa.2012.05.065

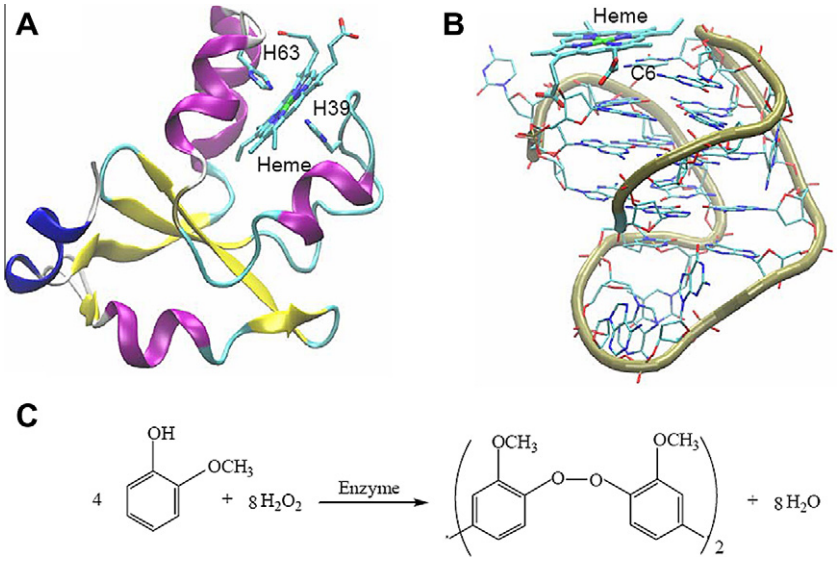

Fig. 1. (A) Crystal structure of cyt b_5 (PDB entry 1CYO [6]), highlighting the heme group and two axial ligands, His39 and His63; (B) a model of DNA aptamer–heme complex, as referenced from a recent report [10]. (C) Chemical reaction of guaiacol oxidation.

example, Mb has a higher binding affinity for heme than microsomal cyt b_5, and as a result, apo-Mb with heme removal can attracts the heme from microsomal cyt b_5, resulting in holo-Mb and apo-cyt b_5 [8].

On the other hand, it was reported that an 18-nucleotide DNAzyme, PS2.M, can bind heme and exhibits peroxidase activity [9], as well as catalyzes oxygen transfer reactions [10]. In essence, PS2.M has a guanine-rich consensus sequence and folds into a G-quadruplex (Fig. 1B) [10]. It has a submicromolar heme-binding affinity and was known as a heme–aptamer [11]. Although heme transfer between heme proteins such as from cyt b_5 to Mb was observed for more than ten years [8], no observation of heme transfer between heme protein and DNA aptamer was attempted to date. Meanwhile, protein expression is closely linked to the interaction between both RNA and DNA [12]. In particular, the maturation of heme protein, with a process of heme assembly [13], is thus likely to be affected by competition of heme binding with DNA/RNA. It is thus of importance to test whether there is a possibility for heme transfer from a heme protein to a heme-binding DNA aptamer.

As shown in this study, it was really found to be the case, which was exemplified by the observation of heme transfer from wild-type (WT) microsomal bovine cyt b_5, its axial His39 to Cys39 mutant (H39C cyt b_5) [14,15], and its derivative (DME cyt b_5) with the heme replaced by protoporphyrin IX dimethyl ester [16], to the heme–aptamer, PS2.M. The heme transfer was further confirmed by the enhancement of peroxidase activity of the cyt b_5–PS2.M system due to the formation of catalytic PS2.M–heme complex.

Materials and methods

Protein and DNA aptamer preparation

Bovine liver microsomal cyt b_5 and its H39C mutant were expressed and purified as described previously by Funk et al. [17] and Wang et al. [15], respectively. Apo-cyt b_5 was prepared by the method of Teale [18], and DME cyt b_5 was prepared by reconstitution of apo-cyt b_5 with protoporphyrin IX dimethyl ester, according to the method described by Reid et al. [16]. The concentrations of WT cyt b_5, H39C cyt b_5 and DME cyt b_5 were determined with an extinction coefficient of $\varepsilon_{413} = 117\ mM^{-1}\ cm^{-1}$ [19], $\varepsilon_{423} = 90\ mM^{-1}\ cm^{-1}$ [15], and $\varepsilon_{280} = 10.5\ mM^{-1}\ cm^{-1}$ [19], respectively, in the ferric state. DNA aptamer, PS2.M, with a sequence of 5′-GTG GGT AGG GCG GGT TGG-3′, and another oligonucleotide, ADNA, with a sequence of 5′-CAC ACA CAC ACA CAC ACA CAC-3′ as used for control in a recent study [20], were synthesized by Shanghai Sangon Biological Engineering Technology and Services Co. Ltd., (Shanghai, China).

Heme transfer studies

Kinetic analysis of heme transfer from WT cyt b_5, H39C cyt b_5 or DME cyt b_5 (2.5 μM) to DNA aptamer, PS2.M (5 M) in 20 mM Tris–HCl, 75 mM KCl buffer (pH 7.0) was carried out on an Agilent 8453 spectrometer using a tandem mixing cell with path length of 2×0.438 cm. The temperature was controlled at 25 °C with a circulating bath instrument. The change in absorbance due to heme transfer was monitored at 404 nm due to the formation of PS2.M–heme complex [9]. The kinetic trace can be described mathematically by the equation of $\Delta A_t = \Delta A_{eq}(1 - e^{-kt})$, where ΔA_t is the increase in absorbance at time t, ΔA_{eq} is the increase in absorbance at equilibrium, and k is the rate constant for heme transfer [8].

Peroxidase activity studies

The peroxidase activity of the PS2.M–heme complex formed at equilibrium in WT cyt b_5–, H39C cyt b_5–, or DME cyt b_5–PS2.M system was estimated with oxidation of guaiacol (0.5 mM). The reaction was initiated by the addition of hydrogen peroxide, H_2O_2, with a final concentration of 5 mM, and followed by monitoring the absorbance change at 470 nm of the product, tetraguaiacol (Fig. 1C). The initial rate was calculated for the first 60 s using an extinction coefficient of $\varepsilon_{470} = 26.6\ mM^{-1}{\cdot}cm^{-1}$ [21]. Control experiments were carried out for cyt b_5, cyt b_5 H39C and DME cyt b_5 under the same conditions in the presence of ADNA or in the absence of both PS2.M and ADNA.

Results and discussion

Observation of heme transfer

To test the possibility of heme transfer from a heme protein to a heme-binding DNA aptamer, we chose microsomal bovine cyt b_5