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Control of catalysis in globin coupled adenylate cyclase by a globin-B domain

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ABSTRACT

The globin coupled heme containing adenylate cyclase from *Leishmania major* (HemAC-Lm) has two globin domains (globin-A and globin-B). Globin-B domain (210–360 amino acids) may guide the interaction between globin-A and adenylate cyclase domains for the regulation of catalysis. We investigated the role of globin-B domain in HemAC-Lm by constructing a series of mutants namely Δ209 (209 amino acids deleted), Δ360 (360 amino acids deleted), H161A, H311A and H311A-Δ209. Spectroscopic data suggest that the Δ209 and H311A-Δ209 proteins to be Fe^{2+}–O_2 form and apo form, respectively, indicating that His311 residue in the globin-B domain is crucial for heme binding in Δ209 protein. However, the H311A mutant is still of the Fe^{2+}–O_2 form whereas H161A mutant shows the apo form, indicating that only His161 residue in the globin-A domain is responsible for heme binding in full length enzyme. cAMP measurements suggest that the activities of Δ360 and Δ209 proteins were ~10 and ~1000 times lesser than full length enzyme, respectively, leading to the fact that globin-B domain inhibited catalysis rather than activation in absence of globin-A domain. These data suggest that the O_2 bound globin-A domain in HemAC-Lm allows the best cooperation of the catalytic domain interactions to generate optimum cAMP.

Introduction

Cyclic AMP, a universal second messenger, which is used by diverse organisms to control processes ranging from chemotaxis to cell differentiation and apoptosis [1]. In mammals, cAMP is synthesized from ATP by two different types of adenylate cyclases (ACs)[1] those are membrane bound and soluble ACs. Adenylate cyclases are mainly regulated through binding of extracellular ligands to GPCRs (G-protein-coupled receptors), pH, bicarbonate and calcium [2–4]. In addition, a globin-coupled heme containing AC has been described in trypanosomatid parasite *Leishmania major* (HemAC-Lm) [5]. Like other globin-coupled oxygen sensors [6] (HemAT-Bs [7], *Av*GReg [8], *Bpe*GReg [9], HemDGC [10], and YddV [11–13]), the catalytic activity of soluble HemAC-Lm is significantly enhanced by the binding of O_2 to the heme iron [5]. Gene knockdown studies suggest that the O_2 dependent cAMP signaling via protein kinase A plays a key role in cell survival through the suppression of oxidative stress under hypoxia. Inhibitor of soluble AC (KH7) as well as activator of membrane bound AC (forskolin) is insensitive to this parasite specific AC activity suggesting that the HemAC-Lm appears to be biochemically different from human host ACs.

On the basis of primary structure of HemAC-Lm, it is composed of two distinct types of domains: one that binds to ATP (adenylate cyclase domain) at the C-terminal and the other that binds to heme (globin domain) at the N-terminal. O_2 binds directly to the distal site of the ferrous form of heme iron by the displacement of the distal site axial ligand and stimulates AC activity [14]. The displacement of the distal site axial ligand by O_2 in the globin domain may involve dynamic conformational changes in which the AC domain switches from inactive state to active state. Many studies have revealed that the gaseous ligands such as O_2, CO, or NO binding causes a conformational change in the environment of heme that regulates the activity of diverse proteins including soluble guanylate cyclase [15], FixL [16], CooA [17], HemAT [18], AxPDEA1 [19], NPAS2 [20], EcDOS [21], DosC [12], and diguanylate cyclase [11]. We have recently shown that the heme-free form as well as the ferric form of wild type of HemAC-Lm has low AC activity [14]. In contrast to hemoglobin/myoglobin, a key feature of HemAC-Lm is its six-coordinate heme structure in the ferrous states, with the proximal histidine (His161) and an endogenous distal unknown residue. Additionally, HemAC-Lm enzymes have another ~150 amino acids containing globin like domain (210–360 amino acids) at the middle position between the globin (globin-A) and the AC domains (Fig. 1). However, the detailed

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[1] *Abbreviations used:* HemAC-Lm, heme containing adenylate cyclase from *Leishmania major*; Δ209, 209 amino acids deleted from N-terminal; Δ360, 360 amino acids deleted from N-terminal; H360, 1–360 amino acids.

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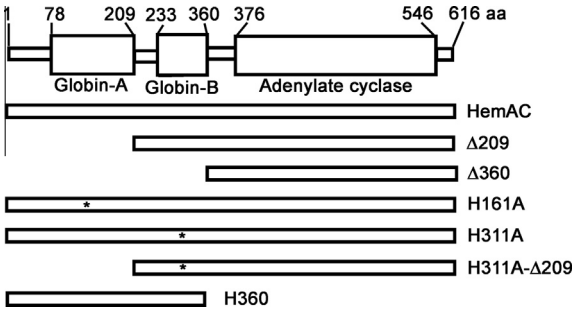

Fig. 1. Schematic presentation of domain structures of the wild-type and mutant enzymes generated in the present study. *Indicates position of mutation.

functions of the second globin like domain (globin-B) in the HemAC-Lm have not been studied.

Protein blast analysis of the sequence of globin-B with those of other proteins suggests that the globin-B domain in the HemAC-Lm displays only 6% identity and 34% similarity with that of cytoglobin/neuroglobin (Fig. 2). Furthermore, SWISS-MODEL protein modeling also predicts that the His311 is the proximal iron-coordinating ligand. The most significant feature is that the globin-B domain sequence lacks the distal His residue, crucial residue for the endogenous distal axial ligand in the ferrous state of neuroglobin. Although the structure of the globin-B domain in the HemAC-Lm is similar to neuroglobin yet it lacks the entire D-helix. Based on the homology alignment data, we have speculated that the globin-B domain may have heme binding capacity. To investigate the role of the connecting globin-B domain, the globin-A domain deleted protein (Δ209) was constructed and compared with full length (HemAC-Lm) as well as catalytic domain (Δ360 HemAC-Lm) protein. In the present investigation, cloning, overexpression, and purification of the Δ209 HemAC-Lm protein were done and the characterization of its catalytic and physicochemical properties were studied *in vitro* as well as *in vivo* system.

Materials and methods

Materials

Ni^{2+}-nitrilotriacetate resin, 5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) and imidazole were obtained from Sigma-Aldrich. Other reagents sources were described in previous papers [5,22,23].

Cloning of different forms of truncated HemAC-Lm constructs

The coding region of truncated constructs was PCR amplified from *L. major* genomic DNA by using sense and antisense primers (Table S1). The PCR products were purified and cloned into the BamHI and XhoI sites of pTrcHisA (Invitrogen) vector and the corresponding plasmids were separately transformed into JM109 cell. The sequences of all constructs were confirmed at the molecular biology core facility of the Indian Institute of Chemical Biology.

Mutagenesis

Site-directed mutagenesis of full length and truncated version of HemAC-Lm DNA in the pTrcHisA (Invitrogen) expression

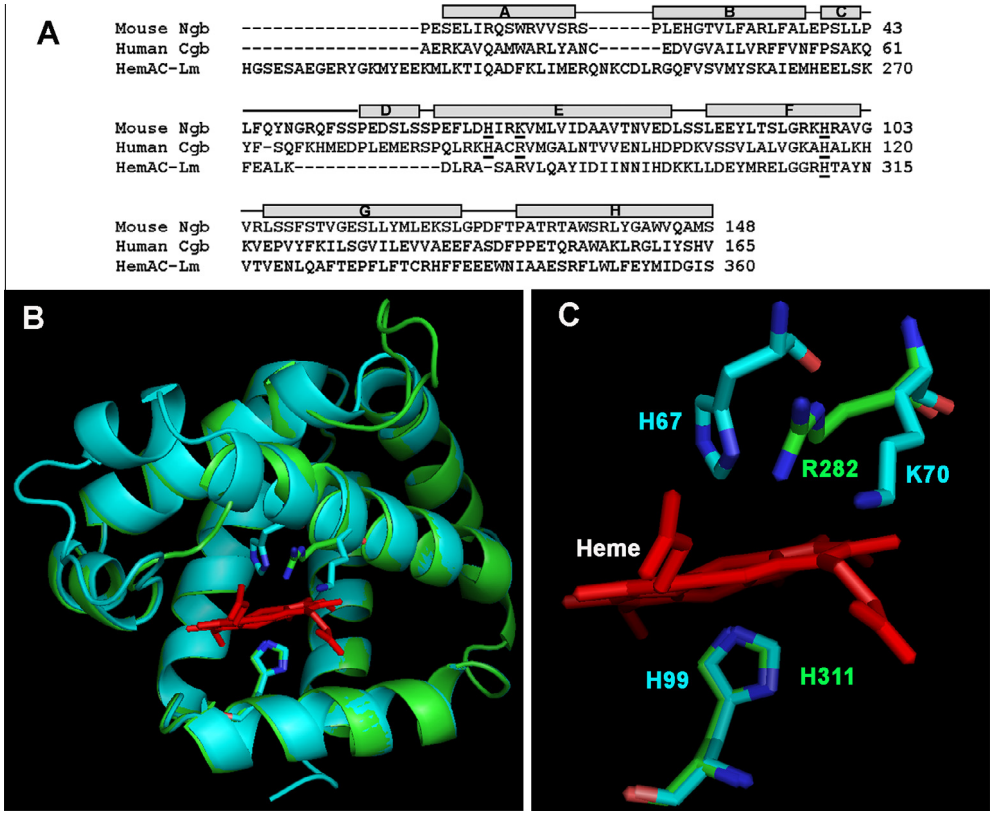

Fig. 2. Sequence alignment and ribbon structural model of globin-B domain of HemAC-Lm. (A) Alignment of the globin-B domain of HemAC-Lm with both neuroglobin (Ngb) and cytoglobin (Cgb). The amino acid residues of the proximal and distal sites of heme are highlighted by underlined letters. (B) The ribbon structural model of globin-B domain of HemAC-Lm with distal site and proximal site residues. Based on the published X-ray crystallographic structures of murine Ngb (cyan color) [39] (PDB entry code: 4O2G.1), we constructed a three dimensional model by homology modeling. Prediction of the three-dimensional structure of the globin-B domain of HemAC-Lm (green color) was done by knowledge-based homology modeling using the SWISS-MODEL [40] and PyMOL software [41]. (C) is the same as (B) except ribbon. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)