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Research Article

Interdomain electron transfer in flavohaemoglobin from *Candida norvegensis* with antibiotic azole compounds

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Abstract

Flavohaemoglobins (FlavoHbs) function as nitric oxide dioxygenases, oxidizing nitric oxide with nitrite and shuttling electrons from NAD(P)H via FAD and O_2. Here, using pulse radiolysis, we investigate intramolecular electron transfer between FAD and haem *b* in FlavoHbs. We found that reduction of FlavoHb with hydrated electrons proceeded via two phases: an initial fast phase and a second slower process. Absorbance measured at 600 nm revealed fast flavin reduction followed by a slower decrease corresponding to reoxidation of FAD. The slower process was partially lost in FlavoHbs lacking FAD. These results suggest that the slower phase is attributable to intramolecular electron transfer from FAD to the haem iron. The rate constant in the absence of azole compound ($3.3 \times 10^3\ s^{-1}$) was accelerated ~ 10-fold ($2.7 \times 10^4\ s^{-1}$) by the binding of econazole, reflecting a conformational change in the open-to-closed transition.

Conflict of interest

The authors declare no conflict of interests.

Filename	Description
feb214327-sup-0001-FigS1-5.docx Word 2007 document , 640.3 KB	**Fig S1**. (A) Kinetic difference-spectra at 1 μs and 50 ms after pulse radiolysis of FlavoHb. (B) Difference-spectrum between Fe^{2+} and Fe^{3+}-FlavoHb. **Fig S2**. O_2 concentration dependence of rate constants observed after pulse radiolysis. **Fig S3**. (A) Absorption spectral changes after rapid mixing of 10 μM oxy FlavoHb with a 0.5 mM CO solution in the presence of 3 mM dithionite. (B) Absorption changes from the oxy form to CO form of FlavoHb, measured at 419 nm. **Fig S4**. Absorption changes after pulse radiolysis of FlavoHb, measured at 405 nm. Samples contained 11 μM protein and 1 μM CO. All samples contained 0.1 M *tert*-butyl alcohol in 10 mM phosphate buffer (pH 7.4). **Fig S5**. Absorption spectral change of Fe(III) econazole and miconazole complexes formation (A, B) and those of Fe(II) complexes (C, D) were shown. Dissociation constants of econazole and miconazole did not change upon reduction (E).

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