Journal of Molecular Structure 1073 (2014) 18–23

Contents lists available at ScienceDirect

Journal of Molecular Structure

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

EPR investigation of libration motion of spin labeled hemerythrin

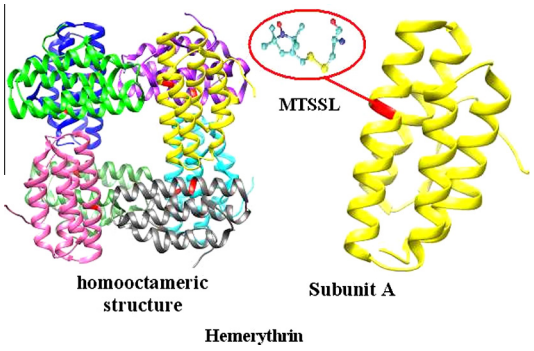
Istvan Mihaly Takacs [a], Augustin Mot [b], Radu Silaghi-Dumitrescu [b,*], Grigore Damian [a,*]

[a] "Babes-Bolyai University", Faculty of Physics, 1 Mihail Kogalniceanu St., RO-400084 Cluj-Napoca, Romania
[b] "Babes-Bolyai University", Faculty of Chemistry and Chemical Engineering, 11 Arany Janos St., RO-400028 Cluj-Napoca, Romania

HIGHLIGHTS

- Room-temperature X-band EPR spectra of site directed spin labeled hemerythrin, reported for first time.
- Viscous media (glycerol, PEG4000, BSA) affect the the mobility (rotational correlation time τ_c) differently.
- Effects on multimeric organization noted.
- This study is as part of efforts to design a hemerythrin-based blood substitute.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Available online 4 February 2014

Keywords:
Spin labeling
EPR spectroscopy
Hemerythrin

ABSTRACT

Reported here are room-temperature continuous wave X-band Electron Paramagnetic Resonance (EPR) spectra of the non-heme di-iron protein hemerythrin (Hr), spin labeled at position 51C in different viscous media, illustrating the mobility and oligomeric recombination tendency of the *Phascolopsis gouldii* Hr. The mobility of a spin labeled Hr depends on the local viscosity and its connectivity to the nature of the molecular environment (glycerol, PEG4000 and BSA). This provides the basis for a tool useful in directly monitoring Hr in ex vivo samples upon injection within the bloodstream of test animals, for blood substitute research.

1. Introduction

Proteins can adopt a large number of structures according to their role and environment and can fluctuate through various conformations according to environmental changes. These structural changes usually happen in a time range of picoseconds to milliseconds. Electron Paramagnetic Resonance (EPR) coupled with site-directed spin labeling (SDSL) can be a useful tool for investigating such conformational flexibility.

Hemerythrin (Hr) is a respiratory protein found in the blood of *Phascolopsis gouldii* (Peanut worm). It is responsible for oxygen transport in marine invertebrates using a non-heme di-iron site [1–3]. Due to the oxygen binding capability [4], Hr was proposed as an alternative solution to hemoglobin-based blood substitutes [5–8]. Hr has a homooctameric structure with a mass of 108 kDa, (Fig. 1a). Each subunit consists of a four-helix bundle protein backbone (Fig. 1b), containing 114 aminoacids and with a mass of 13.6 kDa. Each subunit contains a single native cysteine at the 51C position.

In this work we examine the Hr dynamics by a combined method of site directed spin labeling (SDSL) and EPR analysis [11], using the spin label methanethiosulfonate (MTSSL) which is the label of choice for the majority of SDSL studies [12–14]. The

* Corresponding authors. Tel.: +40 264593833; fax: +40 264590818 (R. Silaghi-Dumitrescu).
E-mail addresses: rsilaghi@chem.ubbcluj.ro (R. Silaghi-Dumitrescu), grigore.damian@phys.ubbcluj.ro (G. Damian).

http://dx.doi.org/10.1016/j.molstruc.2014.01.074
0022-2860/

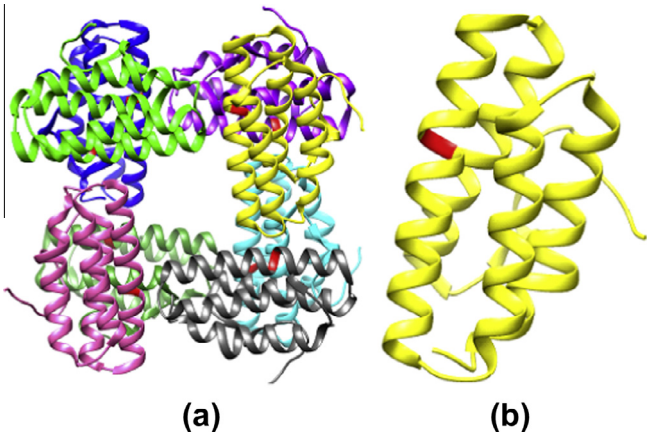


Fig. 1. (a) *Phascolopsis gouldii* Hr homooctameric structure (b) *Phascolopsis gouldii* Hr Subunit A. The cysteines in the 51C position of each subunit are marked in red. Drawing was generated with the visualization software UCSF Chimera [9] using the coordinates from 1I4Y [10] entry in the Protein Data Bank. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

EPR spectroscopy of site-directed spin-labeled biomolecules is particularly useful in the studies of larger proteins and membrane proteins, where other methods like X-ray or NMR are less accessible [15–20]. The continuous wave (cw) EPR spectroscopy combined with SDSL gives information about, the nitroxide side chain dynamics and its protein environment and solvent accessibility of the label [21–23].

In the present work we examine the effects of several environmental factors on the *P. gouldii* hemerythrin (Hr), such as different concentrations of glycerol and polyethylene glycol 4000 (PEG-4000) for investigating the osmolyte effect, and various concentration of BSA in the proteins environment for studying the crowding effect around our sample.

2. Materials and methods

The purification and expression of the *P. gouldii* Hr is described in a previously published paper [7]. The spin label methanethiosulfonate, MTSSL, from Enzo Life Sciences, was dissolved in DMSO (100 mM, stock solution). The spin labels carrying a free electron will bind selectively to the sulfhydryl group from the cysteine at the 51st position of each subunit of the Hr. The side chain after labeling with MTSSL will hereafter be denoted as R1 [24]. The length of MTSSL side chain is around 8 Å, due to the flexibility of the linkage the spin label has a minimal to no disturbance in the structural and functional properties of the investigated protein [25,26].

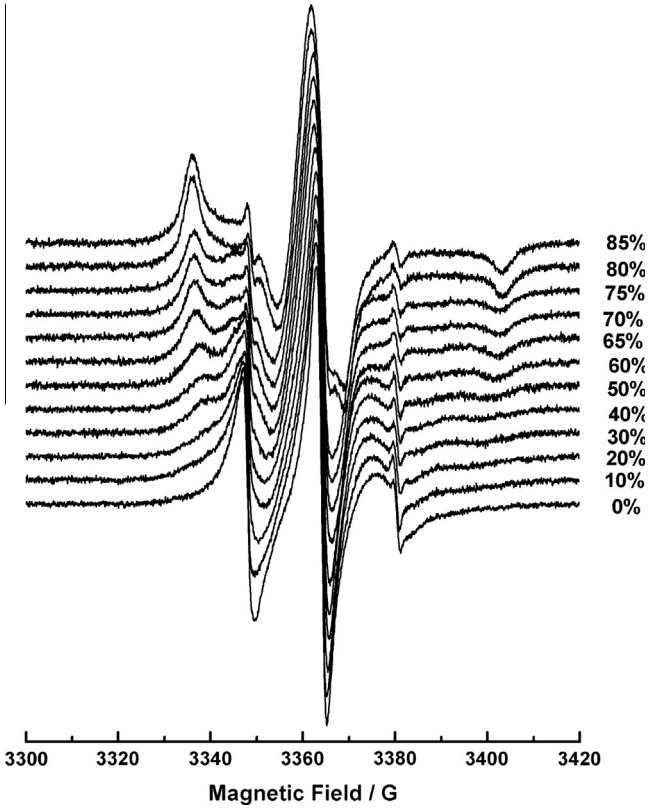


Fig. 3. EPR spectra of of Hr51R1 with different concentrations glycerol.

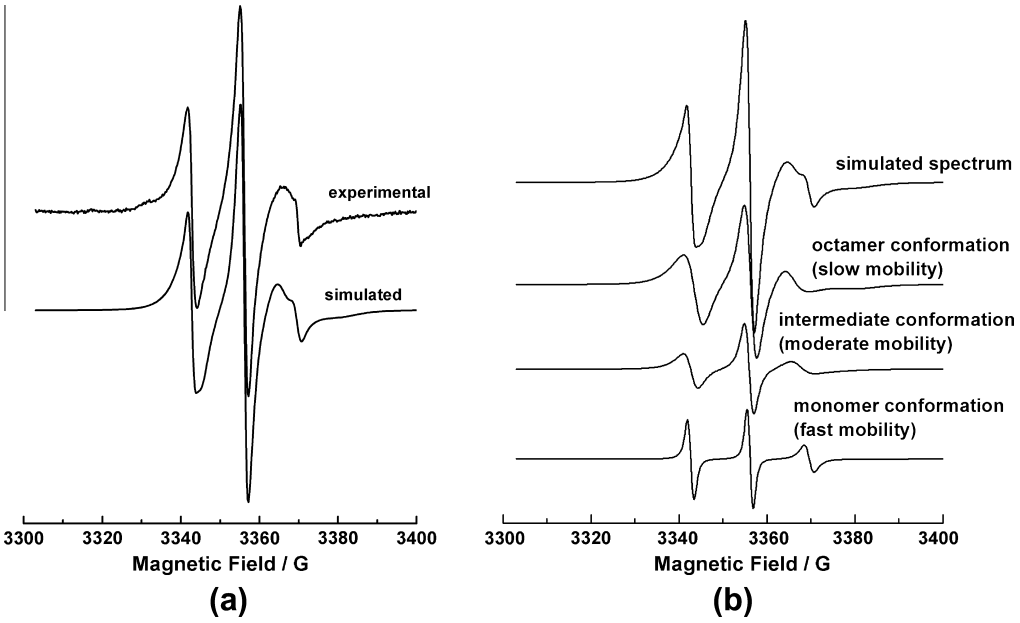


Fig. 2. The experimental and simulated EPR spectrum of the Hr51R1 (a). Conformational states of which the EPR spectrum is composed represented individually (b).