International Journal of Biological Macromolecules 47 (2010) 109–115

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Boron stabilizes peroxide mediated changes in the structure of heme proteins

Shakir Ali[a,*], Humaira Farooqi[a,b], Ram Prasad[a], Mohammad Naime[a], Indusmita Routray[a], Savita Yadav[c], Faizan Ahmad[d]

[a] Department of Biochemistry, Faculty of Science, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062, India
[b] Department of Biotechnology, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India
[c] Department of Biophysics, All India Institute of Medical Sciences (AIIMS), New Delhi 110029, India
[d] Center for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, New Delhi 110025, India

ARTICLE INFO

Article history:
Received 15 April 2010
Received in revised form 13 May 2010
Accepted 17 May 2010
Available online 31 May 2010

Keywords:
Boron
Protein stabilization
Peroxide
Cytochrome *c*
Metmyoglobin

ABSTRACT

Boron is reported in this study to stabilize the structure of heme proteins exposed to peroxides. The oxidized heme protein (15 μM) was treated with H_2O_2 (10 mM) in 1 M glycine–NaOH buffer (pH 9.2) at 25 °C in absence/presence of boron, and characterized by visible absorption spectroscopy, gel exclusion chromatography, native PAGE, HPLC and DLS. Spectral analysis of exposed heme proteins revealed a decrease in absorbance in the Soret region, which was stabilized by boron. The native PAGE analysis of exposed heme proteins showed high molecular weight products; the band intensity was lesser in presence of boron. Further, elution profile of the exposed heme proteins on Sephadex G-200 column and HPLC revealed more than one peak (aggregate formation) when compared to the respective untreated proteins. DLS, which measures the hydrodynamic radius (R_H), was used to ascertain whether the peaks correspond to monomer, dimer or aggregate forms. The R_H of boron pretreated heme proteins was close to R_H of the respective heme protein. Non-heme protein RNase did not show any change when exposed to peroxide. Taken together, results conclude that boron stabilizes the structure of heme proteins, which might be due to specific sites on heme proteins that can bind to borate ions.

1. Introduction

Folded structure of a protein, which is largely stabilized by covalent and non-covalent interactions, is thermodynamically more stable and active than its unfolded form. However, under stress conditions such as the oxidative stress, the 3-D folded structure is perturbed, and leads to the loss of protein function. Oxidative stress is produced by the excessive production of partially reduced oxygen moieties such as the superoxide anion radical ($O_2^{\bullet -}$) and hydrogen peroxide, and has been implicated in large number of pathological conditions such as Alzheimer's disease and various chronic inflammatory disorders [1–3]. The partially reduced chemical species (ex: peroxides) are produced from both the exogenous and endogenous sources as inescapable by-product of normal aerobic metabolism, and may inflict damage to nucleic acids, lipids, sugars and proteins [4–8]. Reactions of H_2O_2 with heme proteins have been extensively investigated and reported [9–19].

In the cell, alterations in protein structure are usually counteracted by specific chemical chaperones capable of correcting the misfolded conformations, thus preventing the loss of protein stability and biological activity. In this study, we have investigated the effect of boron on peroxide mediated alterations in the structure of heme proteins. Boron is a dynamic trace element with multiple effects. It is important in mineral metabolism, brain function and performance and prevention of osteoporosis and osteoarthritis [20]. Boron strengthens the body's immune system [21], and prevents the progression of tissue injury [22]. We hypothesize these effects to be due to the ability of boron to preserve the three dimensional structure of protein, and in the current study, demonstrated that boron in the form of borax stabilizes the structure of oxidized heme proteins, cytochrome *c* (Cyt *c*) and metmyoglobin (met-Mb) exposed to peroxides *in vitro*. Considering the widespread role of iron-containing proteins, this study has both clinical and industrial implications.

2. Materials and methods

2.1. Materials

Horse heart cytochrome *c* and metmyoglobin were purchased from Sigma and Sephadex G-200 was purchased from Pharmacia, Sweden. Other reagents used in this study were of high purity purchased from the standard commercial sources in India. Hydrogen peroxide (30%) and methanol was purchased from E. Merck, India, and borax was purchased from CDH.

* Corresponding author. Tel.: +91 11 26059688.
E-mail addresses: ali.alishakir@gmail.com, sali@jamiahamdard.ac.in (S. Ali).

0141-8130/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijbiomac.2010.05.013

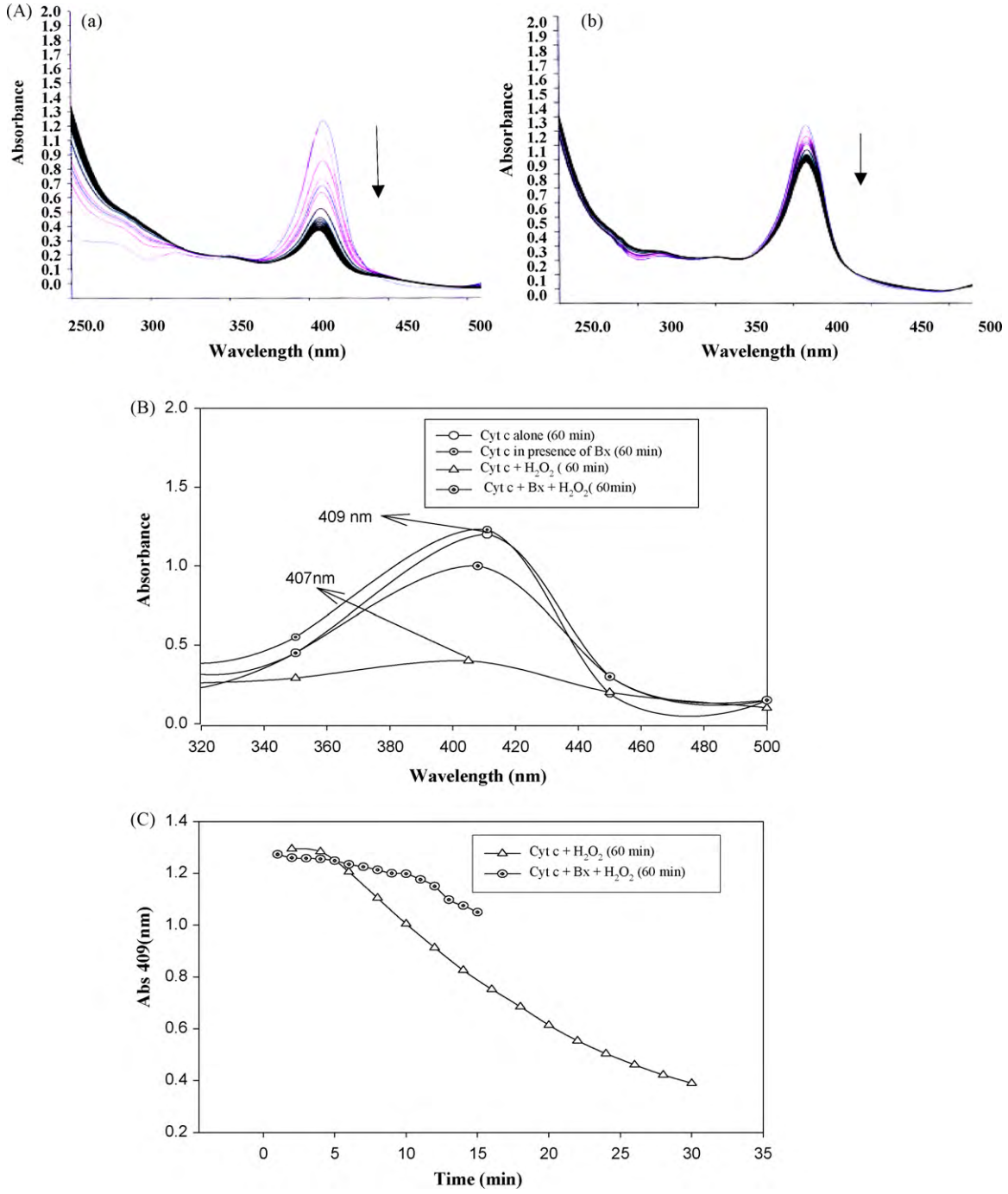

Fig. 1. (A) Spectroscopic measurements of Cyt *c* (15 μM) in presence of H_2O_2 (10 mM) and absence (a) or presence (b) of borax (40 mM). Arrows indicate the direction of change in absorbance with time. (B) Spectra of Cyt *c* monitored after 1 h incubation with hydrogen peroxide in absence or presence of borax. Note a 2 nm shift to the left of Soret absorbance peak in H_2O_2 treated samples both in presence and absence of boron. (C) Time-scan of change at A_{409} for cytochrome *c* in absence or presence of borate ions. The spectra were taken as function of time for 1 h.

2.2. Preparation of protein stock solutions

To ensure that the proteins used in the experiments were all in the ferric form, each sample was oxidized in 0.1% potassium ferricyanide. Excess of this salt was removed by dialysis.

2.3. Preparation of H_2O_2-activated metMb and oxidized Cyt c system

The commercial solution of H_2O_2 (10 M) was diluted to 10 mM and mixed with the oxidized Cyt *c* and metMb to initiate the oxidation *in vitro*. These systems were used as positive control. Absorption spectra of the respective proteins were also taken, which served as control.

2.4. Preparation of borax stock solution (133 mM)

The stock solution of borax was prepared by dissolving 1.268 g borax in 20 ml water or glycine–NaOH buffer (1 M, pH 9.2). pH of the mixture was adjusted to 9.2, and the final volume was made up to 25 ml in distilled water or the desired buffer. Molarity of borax in the final reaction mixture containing the protein was 40 mM, which