J Therm Anal Calorim (2011) 104:339–342
DOI 10.1007/s10973-010-1104-2

Boron increases the transition temperature and enhances thermal stability of heme proteins

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Received: 8 April 2010 / Accepted: 11 October 2010 / Published online: 26 October 2010

Abstract Transition temperature and thermal stability of proteins were studied in the presence and absence of boron. The observed midpoint of thermal denaturation (T_m) of cytochrome *c* (Cyt *c*) at pH 9.2 was 68.8 °C, which in the presence of boron increased to 71.0 °C. For metmyoglobin, T_m increased from 79.7 °C in the absence of boron to 83.5 °C in the presence of boron. Boron caused an increase of 10% in the reversibility of thermal denaturation of cytochrome *c* when compared with control. Activity measurements of the heat treated proteins and T_m suggest an increased thermal stability toward inactivation and denaturation of heme proteins in the presence of boron.

Keywords Boron · Heme proteins · Thermal denaturation · Transition temperature

Introduction

Maintenance of protein structure and function is of prime importance from both physiological and industrial point of view. High temperature is one of the physical conditions that causes denaturation and irreversible inactivation of proteins. Ligand binding, amino acid composition, hydrophobic interactions, disulfide bonds, salt bridges, and hydrogen bonding influence the protein stability. Recently, boron has been reported to stabilize the structure of heme proteins in the presence of peroxide [1]. Heme protein (or hemoprotein) is a metalloprotein that contains heme-prosthetic group. Heme (or haem) consists of an iron atom contained in the center of a large heterocyclic organic ring called porphyrin. It is either covalently or noncovalently bound to the protein. The proteins that contain the heme group have diverse biological functions including the detection and transportation of diatomic gases, electron transfer, and chemical catalysis. This study shows that boron increases the transition temperature of heme proteins, and enhances their thermal stability.

Experimental

Following heme proteins were used in the study: myoglobin (Mb) and cytochrome *c* (Cyt *c*) from horse heart. RNase A from bovine pancreas was used as a nonheme control protein. The proteins were purchased from Sigma Chemical Co, USA. Other reagents were of high purity grade purchased from standard commercial sources in India. The proteins were oxidized with 0.1% potassium ferricyanide, and excess of salt was removed by dialysis. Metmyoglobin (metMb) and Cyt *c* were determined in the respective stock solutions spectrophotometrically at 409 nm using molar absorption coefficient values of 171,000 M^{-1} cm^{-1} for metMb [2] and 106,000 M^{-1} cm^{-1} for Cyt *c* [3]. RNase A concentration was determined at 287 nm using the absorption coefficient of

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9,800 M^{-1} cm^{-1} [4]. Stock solution of borax (40 mM) was prepared in glycine NaOH buffer at pH 9.2.

Jasco V-560 UV/vis spectrophotometer equipped with a peltier type temperature controller (ETC-505T) with a heating rate of 1 °C/min was used to study thermal denaturation. This heating rate was adequate to reach equilibrium. Thermal denaturation of Cyt *c* and metMb were followed by observing changes in absorption at 405 nm and that of RNase A at 287 nm in the range of 20–85 °C. After completing the denaturation, the sample was cooled down, and reversibility of the reaction was measured. Solution blanks were subtracted before data analysis. Raw absorbance data were converted into molar absorption coefficient (M^{-1} cm^{-1}). The heat-induced transition curve was analyzed for T_m and ΔH_m (enthalpy change at T_m) using non-linear square analysis according to the relation:

$$y(T) = \frac{y_N(T) + y_D(T)\exp[-\Delta H_m/R(1/T - 1/T_m)]}{1 + \exp[-\Delta H_m/R(1/T - 1/T_m)]}. \quad (1)$$

In the equation, $y(T)$ is the optical property at temperature T, $y_N(T)$, and $y_D(T)$ are the optical properties of the native and denatured protein molecules at temperature T, measured in Kelvin, and R is the gas constant. The absorption spectra of each sample were recorded at 20 °C before and after heating at 85 °C.

Results and discussion

Effect of various chemicals including osmolytes and metal ions on the stability of proteins in stress has been studied [5–7]. Recently, DSC scans to monitor the thermal stability of tumor suppressor protein p53 have shown that the binding of metal ions to p53 increases its thermal stability [7]. However, the role of ions such as borate has not been investigated, in spite of its established essentiality in plants. This study on heme proteins reports the results of thermal denaturation parameters (T_m and reversibility) in the presence and absence of boron. The values, summarized in Table 1, were measured by following the changes in $\Delta\varepsilon 405$ as a function of temperature. T_m of Cyt *c* in the absence of boron was 68.8 °C, which increased to 71.0 °C in the presence of boron (Fig. 1a). As shown in Fig. 1a, thermal denaturation of Cyt *c* followed a complete transition measurable in the range 20–85 °C. On the other hand, the transition was incomplete for metMb (Fig. 2a), which is consistent with earlier reports, indicating an incomplete transition at alkaline pH [8]. No significant change in the thermal denaturation curve was found in RNase, and the T_m remained around 66 °C (Fig. 3). The comparison assumes significance, since RNase A, which was used as a control protein, does not contain heme moiety, indicating the role of heme-prosthetic group.

Table 1 The transition temperature (T_m) and thermal reversibility of various heme containing proteins observed in the presence and absence of boron

Protein	T_m		Thermal reversibility/%	
	Boron (+)	Boron (−)	Boron (+)	Boron (−)
Cytochrome c	71.0	68.8	80	90
Myoglobin	83.5	79.7	80	80
RNase A	67.0	66.0	No change	No change

Boron (+) in the presence of borax, *Boron (−)* in the absence of borax

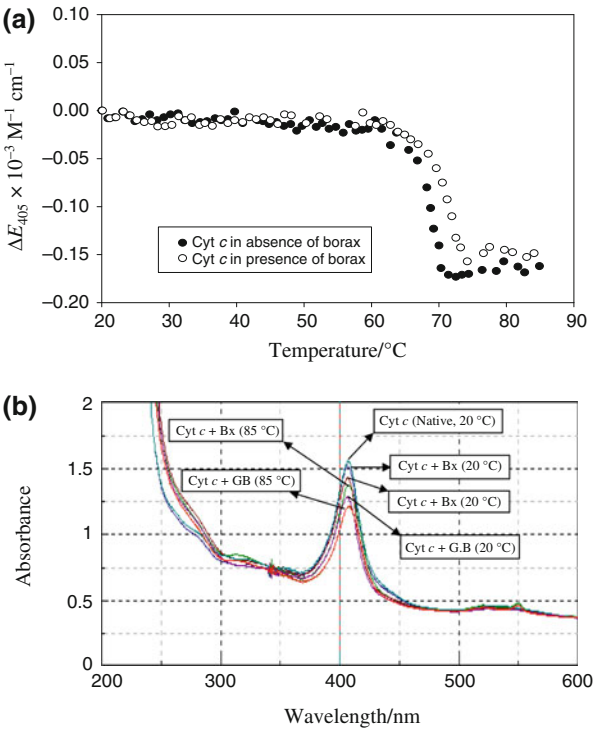

Fig. 1 **a** Thermal denaturation profile of Cyt *c* in the absence and presence of boron (40 mM) at pH 9.2. Thermal denaturation of Cyt *c* at a fixed concentration of boron was followed by measuring changes in absorbance at 405 nm in UV–visible spectrophotometer using a scan rate of 1 °C/min. **b** Thermal spectra of Cyt *c* showing the effect of boron on the structural stability of proteins at higher temperature, determined by monitoring the reversibility of proteins before (20 °C) and after heating (85 °C). The reversibility increased by 10% in the presence of boron. *Bx* borax, *GB* glycine buffer

Further, the denaturation of Cyt *c* and metMb in their native states was not completely reversible at alkaline pH. Cyt *c* reported a 10% increase in reversibility (Fig. 1b); reversibility was 80% under native condition, and increased to 90% in the presence of boron. Weser and Kaup [9] have reported an increase in the stability of alkaline phosphatase in the presence of sodium tetraborate at 45 °C. The effect has been attributed to borate bridging between the carbohydrate residues, which are good candidates for

the formation of borate complexes. Interpretation of results in Figs. 1a and 2a suggests that boron increases the thermal transition temperature by shifting the denaturation equilibrium (N ↔ D) toward left. However, boron could not increase the reversibility of metMb, although increased its T_m (Fig. 2b).

Taken together, the results suggest that boron stabilized the native structure of heme proteins against thermal denaturation. The exact mechanism of action, however, needs to be worked out. Boron might act by interacting with the positively charged side chains of certain amino acids. Chen et al. [10] have reported that the positively charged side chains of amino acids at specific locations are capable of forming hydrogen bonds with three of the four borate oxygen and stabilize the negative charge on borate. We do assume that boron effect might be attributed to its property of acting as a ligand to the proteins or due to the general preferential hydration effect of borax [11]; negatively charged borate ions form ionic bonds (the salt bond or salt bridge) with the positively charged Cyt *c* (pI 10.6). Hyper-thermophilic analogues of certain proteins have been reported to have increased number of salt bridges [12]. However, the role of heme moiety cannot be denied because boron did not increase the T_m, or enhanced the thermal stability of RNAse, the nonheme protein, under similar conditions (Fig. 3).

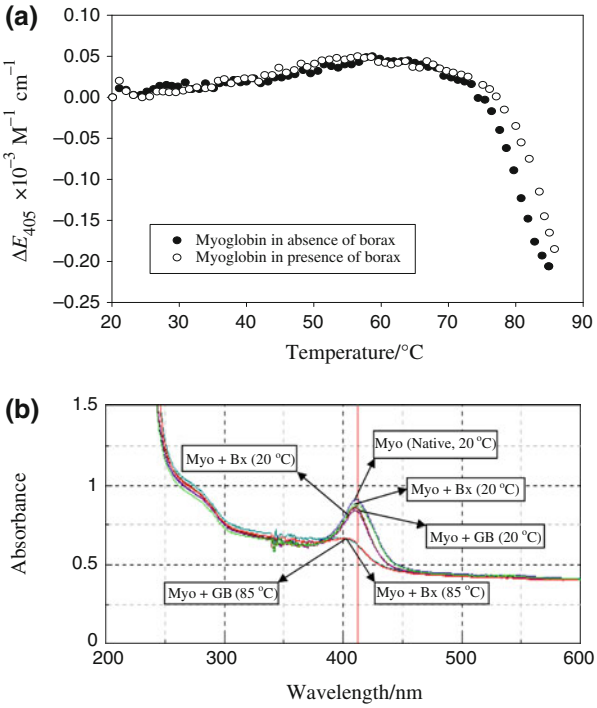

Fig. 2 **a** Thermal denaturation profile of metMb in the absence and presence of boron (40 mM) at pH 9.2. Thermal denaturation of metMb at a fixed concentration of boron was monitored by measuring the increase in absorbance at 405 nm in a UV–visible spectrophotometer using a scan rate of 1 °C/min. **b** Thermal spectra of metMb showing the effect of boron on the structural stability of protein at higher temperature, determined by monitoring the reversibility of the protein before (20 °C) and after heating (85 °C). No change in reversibility in presence of boron was observed. *Bx* Borax, *GB* Glycine buffer

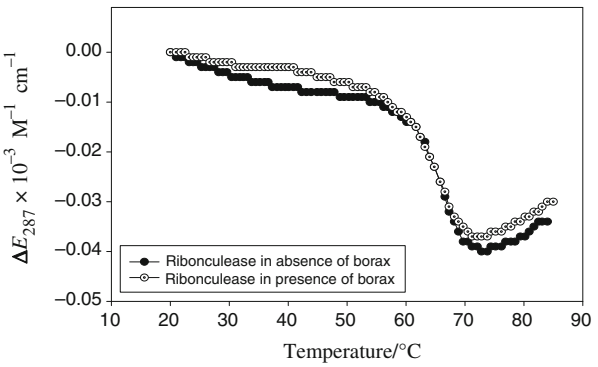

Fig. 3 Thermal denaturation profile of RNAse A in the absence and presence of boron (40 mM) at pH 9.2. Thermal denaturation of RNAse A at a fixed concentration of boron was monitored by measuring increase in absorbance at 287 nm in a UV–visible spectrophotometer using a scan rate of 1 °C/min

Conclusions

This study concludes that boron in the form of borax enhances the thermal stability of heme proteins either by increasing the T_m or by increasing the percentage of thermal reversibility toward native form, and in some proteins by increasing both, the T_m and the reversibility. Boron increased the T_m as well as reversibility of Cyt *c*, and increased the T_m of metMb. Boron could not stabilize the structure of RNase, a nonheme protein, under similar conditions, indicating the role of heme-prosthetic group toward increasing the thermal stability of proteins.

Acknowledgements Authors acknowledge Dr. GN Qazi, the Vice Chancellor, JH for his interest and support. The grant-in aid provided by UGC, DBT, and FIST (DST) to augment the infrastructure of the Department of Biochemistry is acknowledged.

References

1. Ali S, Farooqi H, Prasad R, Naime M, Routray I, Yadav S, Ahmad F. Boron stabilizes peroxide mediated changes in the structure of heme proteins. Intl J Biol Macromol. 2010;47:109–15.
2. Puett D. The equilibrium unfolding parameters of horse and sperm whale myoglobin. Effects of guanidine hydrochloride, urea and acid. J Biol Chem. 1973;248:4623–34.
3. Margoliash E, Frohwirth N. Spectrum of horse-heart cytochrome c. Biochem J. 1959;71:570–2.
4. Sinha A, Yadav S, Ahmad R, Ahmad F. A possible origin of differences between calorimetric and equilibrium estimates of stability parameters of proteins. Biochem J. 2000;345:711–7.

5. Foglia F, Carullo P, Vecchio PD. The effect of trimethylamine N-oxide on RNase A stability. A DSC study. J Therm Anal Calorim. 2008;91:67–72.
6. Yancey PH. Organic osmolytes as compatible metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J Exp Biol. 2005;208:2819–30.
7. Xue Y, Wang S, Feng X. Effect of metal ion on the structural stability of tumour suppressor protein p53 DNA-binding domain. J Biochem. 2009;146:193–200.
8. Kumar R, Prabhu NP, Rao DK, Bhuyan AK. The alkaline molten globule state of horse ferricytochrome c: observation of cold denatuartion. J Mol Biol. 2006;364:483–95.
9. Weser U, Kaup Y. Borate, an effective mummification agent in Pharaonic Egypt. Z Natuturfosch. 2002;57b:819–22.
10. Chen X, Schauder S, Potier N, Dorsselaer AV, Pelczer I, Bassler BL, Hughson FM. Structural identification of a bacterial quorum-sensing signal containing boron. Nature. 2002;415:545–9.
11. Taler G, Navon G, Becker M. The interaction of borate ions with cytochrome c surface sites: a molecular dynamics study. Biophys J. 1998;75:2461–8.
12. Priyakumar UD, Ramakrishna S, Nagarjuna K, Reddy SK. Structural and energetic determinants of thermal stability and hierarchical unfolding pathways of hyperthermophilic proteins, Sac7d and Sso7d. J Phys Chem B. 2010;114:1707–18.