FEBS Letters xxx (2015) xxx–xxx

FEBS Letters

journal homepage: www.FEBSLetters.org

Unusual effects of crowders on heme retention in myoglobin

Jayanta Kundu, Uddipan Kar, Saurabh Gautam, Sandip Karmakar, Pramit K. Chowdhury *

Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi 110016, India

ARTICLE INFO

Article history:
Received 21 September 2015
Revised 5 November 2015
Accepted 10 November 2015
Available online xxxx

Edited by Stuart Ferguson

Keywords:
Macromolecular crowder
Glucose and sucrose
Excluded volume effect
Heme retention
BSA
Lysozyme
Soft interaction

ABSTRACT

Myoglobin (Mb) undergoes pronounced heme loss under denaturing conditions wherein the proximal histidine gets protonated. Our data show that macromolecular crowding agents (both synthetic and protein based) can appreciably influence the extent of heme retention in Mb. Interestingly, glucose and sucrose, the monomeric constituents of dextran and ficoll-based crowders were much more effective in preventing heme dissociation of Mb, albeit, at much higher concentrations. The protein crowders BSA and lysozyme show very interesting results with BSA bringing about the maximum heme retention amongst all the crowding agents used while lysozyme induced heme dissociation even in the native state of Mb. The stark difference that these protein crowders exhibit when interacting with the heme protein is a testament to the varied interaction potentials that a test protein might be exposed to in the physiological (crowded) milieu.
© 2015 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

1. Introduction

The heme prosthetic group is one of the most diverse cofactors found in nature, catering to a wide range of essential biological functions viz. oxygen transport, storage, catalysis and electron transfer, etc. Of the different heme-based metalloproteins that exist, myoglobin (Mb), involved in oxygen storage, is one of the most extensively studied biomolecules and has long been considered a paradigm for understanding the structural and functional characteristics of such proteins [1–3]. One of the biggest obstacles/deterrents in the study of Mb has been the loss of heme under denaturing conditions, with the cofactor known to impart significant stability to the apo-protein [4–7]. Moreover, a majority of such studies have been carried out in dilute buffer medium [4–11]. However the physiological fluid is vastly different and is congested with a large concentration (50–400 g/L) of high molecular weight components namely DNA, RNA, enzymes, lipids and other proteins, that exhibit substantial intracellular volume occupancy [12–15]. These so-called macromolecular crowding agents are known to influence protein structure, stability and dynamics primarily by the excluded volume effect, the latter arising from steric repulsions between the crowder molecules and proteins of interest [16–24].

In this communication, we report the effect of both synthetic [Ficoll 70 (F70), Dextran 70 (D70), Dextran 40 (D40) and Dextran 6 (D6), PEG8000 (P8) and PEG35000 (P35)] and protein based crowding agents (BSA and lysozyme) on the heme dissociation of Mb under denaturing conditions by monitoring primarily the Soret band absorbance changes. To check whether the effects so observed are macromolecular, the influence of the monomeric components of the crowding agents, namely, sucrose (F70), glucose (Dextran based crowders) and ethylene glycol (PEGs) were also examined. Our results reveal that the crowding agents have a significant effect on heme stability with bovine serum albumin (BSA) showing unusually high heme retention while lysozyme having the reverse influence, that is, exhibiting enhanced heme dissociation. Moreover glucose and sucrose were also quite effective in increasing heme retention, albeit, at much higher concentrations than the macromolecular crowders.

2. Materials and methods

Myoglobin (Mb) of equine skeletal muscle and bovine serum albumin (BSA) were purchased from Sigma Aldrich Private Ltd. (USA) while Lysozyme of Chicken Egg White was purchased from USB corporation (USA) and were used without further purification. Urea, guanidinium hydrochloride (GdmCl), Ficoll 70, Dextran

Author contributions: JK and PKC conceived and designed the study; JK and UK carried out the bulk of the experiments while JK carried out the data analyses; SG helped with the aggregation studies and native page analyses along with SK. JK and PKC wrote the manuscript.

* Corresponding author. Fax: +91 1126581102.
E-mail address: pramitc@chemistry.iitd.ac.in (P.K. Chowdhury).

http://dx.doi.org/10.1016/j.febslet.2015.11.015
0014-5793/© 2015 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Please cite this article in press as: Kundu, J., et al. Unusual effects of crowders on heme retention in myoglobin. FEBS Lett. (2015), http://dx.doi.org/10.1016/j.febslet.2015.11.015

(70, 40 and 6), Poly Ethylene Glycol (35 and 8), ethylene glycol, sucrose, glucose, ANS (8-anilino-1-naphthalenesulphonic acid ammonium salt) and thioflavin T (ThT) were also purchased from the Sigma Aldrich Private Ltd. (USA) and used as received. Sodium phosphate dibasic (Na_2HPO_4) anhydrous, monobasic (NaH_2PO_4) dihydrate, sodium acetate (anhydrous) and acetic acid were purchased from Merck Specialties Private Limited (Mumbai, India).

2.1. Preparation of solutions

Phosphate buffer solution (50 mM) of pH = 7 was prepared by dissolving weighed amounts of mono sodium phosphate and disodium phosphate while the acetate buffer solution (50 mM) of pH = 4 was made by dissolving weighed amounts of sodium acetate followed by the addition of desired amount of glacial acetic acid in Millipore water (Elix 3 UV; Millipore, Molsheim, France). Mb, ANS and ThT containing stock solutions were prepared in the aforesaid phosphate buffer (pH = 7), centrifuged (Centrifuge 5415R, Eppendorf) and diluted as required before carrying out the measurements. The concentrations of Mb, ANS and ThT were measured using an UV–Vis spectrophotometer (Model UV-2450, Shimadzu) in the range of 200–700 nm. The molar extinction coefficients used for Mb are as follows: 13 980 M^{-1} cm^{-1} at 280 nm and 188 000 M^{-1} cm^{-1} at 409 nm (the Soret band) [25] while those for ANS and ThT are 5000 M^{-1} cm^{-1} [26] and 26 620 M^{-1} cm^{-1} respectively [27]. For acid denaturation experiments of Mb, the urea solutions were prepared in acetate buffer (pH = 4) and for chemical denaturation experiments urea and GdmCl solutions were prepared in phosphate buffer (pH = 7). The concentrations (of chemical denaturants) were determined by measuring the corresponding refractive indices using a refractometer (KRUSS, A Kruss Optronic, Germany). pH of the solutions was maintained using a pH meter (Hanna HI3220) followed by the addition of NaOH, H_3PO_4 and acetic acid as per requirement. For the crowding experiments, different concentrations (100, 200, 300 and 400 g/l) of the macromolecular crowders were dissolved in phosphate and acetate buffers after weighing out the appropriate amounts using an analytical balance (Precisa XB 120A) to get the desired concentration. For the chemical denaturation experiments it was difficult to dissolve the crowders beyond 300 g/L while for PEG35 we were able to dissolve only up to 200 g/L of the crowder (further increase of crowder concentration resulted in turbidity). For the protein crowders, the maximum concentration for which reliable and reproducible data could be obtained was 200 g/L.

Specific details of methods have been provided in the figure captions and the rest including experimental techniques are given in the Supporting information for further reference.

3. Results and discussion

The absorption spectrum of native myoglobin in the visible region (Fig. 1A), is characterized by an intense peak at 409 nm (the Soret band or B-band) and two low-lying peaks at ~506 and ~637 nm (the Q-bands). The Soret band is very sensitive to the heme environment and hence has commonly been used to monitor heme pocket disruption in presence of external perturbations. Prior to subjecting the protein to denaturing environments, our initial goal was to probe whether the crowding agents were able to bring about any changes in the heme pocket of native Mb. F70, D70, D40, P35 and BSA gave rise to little or no change of the band in the Soret region; however in presence of D6, P8, and lysozyme, significant distortions and/or modulations could be seen (Fig. 1 and Supplementary information Fig. 1). For example, D6 (Fig. 1B) brought about a substantial red-shift in the Soret maximum (from 409 nm in absence of the crowder to 418 nm in 400 g/l of D6) while in presence of P8 (Fig. 1C), the absorption intensity decreased to a large extent (without any wavelength shift) signifying a concomitant reduction in the probability of the electronic transition. One of the most notable effects on Mb was that of the protein lysozyme (Fig. 1D and E) wherein the spectrum not only became broad but showed a significant blue-shift akin to water exposure of heme that can either happen on its dissociation (as a result of Mb denaturation) or the heme being squeezed out of the pocket at such high concentrations of the crowder protein. On the other hand, the small molecules (glucose, sucrose and EG) had no visible effect on the Soret profile of Mb.

Subsequently heme dissociation studies in presence and absence of the crowding agents were carried out by subjecting Mb to three different denaturing conditions: (i) pH = 4 with 1 M urea (ii) 1.9 M GdmCl and (iii) a mixture of 1.4 M GdmCl and 1.4 M urea. Before we proceed further, we would like to provide a brief rationalization with regards to why such denaturation conditions have been chosen. At low pH (pH < 4) Mb is known to undergo facile heme loss because of protonation of the proximal histidine [4]; however under such conditions crowding agents might not remain stable. Hence to mimic the low pH environment, at pH = 4, urea (1 M) was added. The other two denaturing conditions were chosen such that the extent of heme loss was very similar to that of the low pH, as determined by ratio of the absorbance of the bound to that of dissociated heme (explained below). Moreover, a recent simulation study has shown that use of mixed denaturants induces non-native collapsed states in proteins, with urea acting as a potential crowding agent [28]. The crowder concentrations were varied over an appreciable range, reaching as high as 400 g/l for the present study. For native myoglobin, the intact heme shows an absorption maximum at 409 nm (A_{409}) while the broad absorption band with a maximum at ~360 nm (A_{360}) has often been attributed to that of dissociated heme remaining loosely attached (non-covalently and/or out of the heme pocket) to the protein matrix [10]. Decrease in pH or increase in the concentrations of GdmCl leads to significant changes in the Soret band. Under conditions wherein heme dissociation is minimal (in presence of urea), the Soret band shifts to the blue with concomitant broadening, signifying extensive water coordination as the heme pocket opens up (SI Fig. 2A). A convenient and informative means of monitoring the effect of crowding agents on heme stability/loss, is therefore to plot the A_{409}/A_{360} ratio as a function of the crowder concentration. For example, for native myoglobin, at pH = 7, the ratio of bound to free heme (A_{409}/A_{360}) is ~4.85, the same undergoing a considerable decrease as a function of chemical (SI Fig. 2A and B) and pH-induced denaturation (SI Fig. 2C).

Shown in SI Fig. 3 are some representative spectra of the Soret band at pH = 4 (and 1 M urea) for the different crowding agents used in this study. As evident (Fig. 2A), the A_{409}/A_{360} ratio increases for almost all the synthetic macromolecular crowders with P35 showing the maximum heme retention (amongst the synthetic crowders) by having the highest absorbance ratio of ~4.5 at a concentration of 200 g/l. Here, it should be noted that this ratio is very close to that observed for native Mb (as mentioned above). P8 also showed a steep initial rise in the ratio but beyond 200 g/l this crowding agent adversely affected the heme stability as observed by the decrease in absorbance of the bound heme. On the other hand, the dextran based crowding agents showed a gradual increase in the heme retention as the crowder concentration was increased, with D6 showing the least among these while the profiles for D70 and D40 were quite similar. F70, having the same average molecular weight as that of D70, however was much less effective, reaching a final ratio of ~2.80 as compared to that of ~4.20 observed for D70. Fig. 2B (and SI Fig. 4) shows the guanidine hydrochloride induced denaturation of Mb where GdmCl concentration was kept at 1.9 M to reach the absorbance ratio

Please cite this article in press as: Kundu, J., et al. Unusual effects of crowders on heme retention in myoglobin. FEBS Lett. (2015), http://dx.doi.org/10.1016/j.febslet.2015.11.015

Download English Version:

https://daneshyari.com/en/article/8383971

Download Persian Version:

https://daneshyari.com/article/8383971

Daneshyari.com