EFFECT OF INOSITOL HEXAPHOSPHATE ON THE AZIDE BINDING REACTIVITIY OF MOUSE METHAEMOGLOBIN

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ABSTRACT

Inositol Hexaphosphate (IHP) is observed to increase the reactivity of mouse Methaemoglobin binding to azide ions. The thermodynamic reactivity of the azide binding to mouse methemoglobin in solution shows that IHP shifts the characteristic pH (pHch) to a lower pH. The pHch has previously been defined as the pH at which conformational transition occurs in the methemoglobin protein moiety. A shift to a lower pHch by IHP suggests that IHP modifies the conformation of mouse methemoglobin at the pHch.

Key words: *Methaemoglobin, Binding reactivity, Conformational transition +Corresponding author*

INTRODUCTION

Methemoglobin (metHb) reacts with neutral and negative ligands because of the availability of a net positive charge to form complexes. This reaction involves the replacement of the water molecules at the sixth coordinated position (Lemberg *et al*, 1949). The equilibrium constant K_L in terms of concentration for the reaction is written as

$$K_L = \left(HbL \right) \left(Hb^{+}OH_2 \right)^{-1} L \qquad \ldots\ldots\ldots\ldots\ldots \qquad (1)$$

For the reaction which is represented by the equation

$$Hb^{+}OH_2 + L \rightleftharpoons HbL + H_2O$$

Perutz, 1970 proposed that two primary events occur in the reaction between ligands and the haem in Haemoglobin; a change in the occupancy of the distal ligand site of the iron and a change in stereochemistry of the haem and the reactivity differences emanating from electrostatic interactions are reflected to a great extent in enthalpy. The pH-dependent enthalpy of azide binding reactions to the solution of all the metHb studied was found to pass through a maximum at pH characteristic

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of each of these methemoglobins (Bailey *et al,* 1970). The characteristic pH of metHb is defined as the pH at which conformational changes occur in the protein moiety. Proton NMR studies on the binding of azide ions to the haem of ferricytochrome c at pH 7 have indicated that conformational changes are obvious on the methionine 80 side of the haem cavity in the environment of the axial ligand (Ma.D *et al,* 1998).

Inositol hexaphosphate (IHP) is an organic phosphate known to induce conformational changes in hemoglobins. It binds favorably to metHb at the B-termini of the B-chain of the amino acid identified as valine-1, histidine-2 and 143, lysine-82 (Tan & Noble, 1973, Perutz et al, 1974). Coletta *et al,* 1999, have shown that the T quaternary structure is stabilized at low oxygen pressures and the R structure at high oxygen pressures when IHP and bezafirate (BZF) is coupled simultaneously to human oxyhaemoglobin. This study investigates the effect of IHP on the conformational changes in solution and evaluates the extent of IHP induced conformational change on the characteristic pH that is observed in mouse metHb towards azide ions.

EXPERIMENTAL PROCEDURES

The binding of azide ions to mouse metHb in the presence and absence of IHP was carried out as a function of pH and temperature. 2.0, 5.0 and 10.0 x 10^{-3} M concentration of azide solution was prepared from dry recrystallized sodium azide. 5.0 mL aliquot of buffered mouse metHb suitably diluted to give absorbance of approximately 0.7 at 405nm is filled into eighteen flasks.

Three flasks were left without azide solution, to nine flasks were added incremental volumes 0.01-0.09mL of the azide solution, and few crystals of recrystallized potassium cyanide were added to three flasks. The flasks were allowed to equilibrate for four hours at 27°C, five hours at 20°C and twelve hours at 13°C in a thermostated bath. Absorbances reading for all the flasks were measured at 405nm except for the solution containing KCN which was measured at 420nm using the appropriate buffer at each pH of experiment as blank (LMHR, U.I).

To correct for ionization of metHb at pH 8.0 (Keilin and Hartree, 1952), prepared solutions of metHb absorbance @ 0.7 at 405nm were pipetted into 5mL flasks and thermostated in water bath for thirty minutes at pH 6.0 and between pH 8.0 and 9.2 at 13°C, 20°C and 27°C.The absorbance of the solutions were measured at 405nm. The formation constant of azide metHb in the presence of IHP was carried out by adding 0.1mL of 10mM IHP solution (pH 6.5) to 5.0mL aliquots of metHb pipetted into eighteen flasks containing nine flasks of incremental volume of azide solution, three flasks of azide crystals, three flasks of KCN crystals and

three flasks were left without azide crystals or solutions. All the flasks were equilibrated as earlier stated.

RESULTS AND DISCUSSION

The pH and temperature dependence of azide binding to mouse metHb in the presence and absence of IHP is shown in tables 1 and 2. Figures 1and 2 shows the graphical representation Tables 3 and 4 represent thermodynamic parameters obtained from the experiment using the Gibb's-Helmhortz equation. Figures3 and 4 is the graphical representation of the pH – dependent enthalpy of the reaction.

$$\log K_N = -\Delta H\,(2.303RT)^{-1} + \text{constant}$$

The equilibrium constant K_N for the reaction was calculated from the equation

$$K_N = K_{obs}\left(1 + {}_{-H}(\ )^{+-1} K_a\right)$$

The reactivity of mouse met hemoglobin towards azide ions in the presence and absence of inositol hexaphosphate is observed to be pH and temperature dependent (figures 1 and 2). The formation constant decreases with increasing temperature and pH. This observation may be attributed to ionization of some amino acids of the protein molecule at alkaline pH with the result that the net charge is more negative at this pH and this may probably lead to electrostatic repulsion between the azide ion and the molecule. This repulsion is further enhanced in the presence of IHP with six negative charges.

IHP is observed to increase the formation constant of mouse metHb; this affirms the observation of Hensley *et al,* 1975 who have shown that IHP increases the reactivity of azide binding to metHb. The pH – dependent enthalpies of the reactions are characteristic bell-shaped curves (figure 3) and the pH of maximum enthalpy of mouse metHb binding to azide ions in the presence of IHP is 6.8, while in the absence of IHP it is 7.2. Our result shows that IHP shifts the characteristic pH (pH_{ch}) to a lower pH, which suggests that IHP affects the conformation of mouse metHb at the pH_{ch}.

Perutz *et al,* 1974 have argued that IHP affects the conformation of human azidemetHb by modifying its conformation in an unknown manner. Previous researchers (Jayaraman *et al*, 1993, Ascenzi *et al,* 1991) have also shown that IHP favors a switch of R – T conformational equilibrium in favor of the T- state at low pH. From this evidence, we suggest that IHP affects the conformation of the mouse metHb by shifting the pH_{ch} of azide metHb from 7.2 to 6.8. The azide metHb has the same structure as the metHb (Deatherage *et al,* 1979), and metHb exhibit R-T state of equilibrium, it is being suggested that IHP modifies the azidemetHb

conformation in favor of the T-state that is favored by low pH and organic phosphate like IHP.

CONCLUDING REMARKS

Since metHb and azidemetHb has been shown to possess the same structure, and metHb exists in a R-T equilibrium (11) which can be switched depending on conditions, we suggest that IHP affects the R-T equilibrium by shifting the equilibrium in favor of the T-state which is favored by low pH and organic phosphate like IHP.

REFERENCES

Ascenzi, P.; Coletta, M.; Santucci, R.; Amiconi, G. (1991). Ferrous Influence of Allosteric Effectors on the Heme Conformation of Dromedary Ferrous Nitrosyl Hemoglobin Detected by XANES Spectroscopy. *Biochemistry Biophysical Acta, 1080, (2):119 - 25.*

Bailey, J. E.; Beetlestone. J. G.; Irvine, D. H. and **Ogunmola, G. B.** (1970). Reactivity Differences between Hemoglobin. Part XVI. A Correlation between the Amino Acid Composition of Methemoglobin and their Reactivity towards Azide Ions. *Journal of Chemical Society A. 749.*

Coletta, M.G. *et al* (1999). Coupling of Oxygen-linked Interaction for Inositol Hexakisphosphate and Bezafibrate Binding to Human Hb AO. *Journal Biological Chemistry, 274(11):6865-6874.*

Deatherage, J. F.; Obendorf, S. K. and **Moffat, K.** (1979). Structure of Azide Methemoglobin. *Journal of Molecular Biology, 134:419 - 429.*

Hensley, P. Edelstein; Stuart, J.; Wharton, D. C. and **Gibson, Q. H.** (1975). Conformation and Spin State in Methemoglobin. J. Biological Chemistry, 250; 952-960. Jayaraman V. Rodgers K.R., Mukeorji Biochemistry 13; 2174-2186I.

Spiro, T. G. (1993). R and T States of Fluoromethemoglobin Probed by Ultra Violet Resource and Raman Spectroscopy. *Biochemistry,32(17) 4517-51.*

Keilin, D. and **Hartree, E. F.** (1952). Effect of Drying upon the Absorption Spectra of Hemoglobin and its Derivatives. *Nature 170: 161-162.*

Laboratory Manual of the Hemoglobin Research Group (1972).University of Ibadan, Ibadan.

Lemberg. R. and **Legge, J. W.** (1949). *In Heamatin Compounds and Bile Pigments, 207-229.* New York: Inter-Science Publishers.

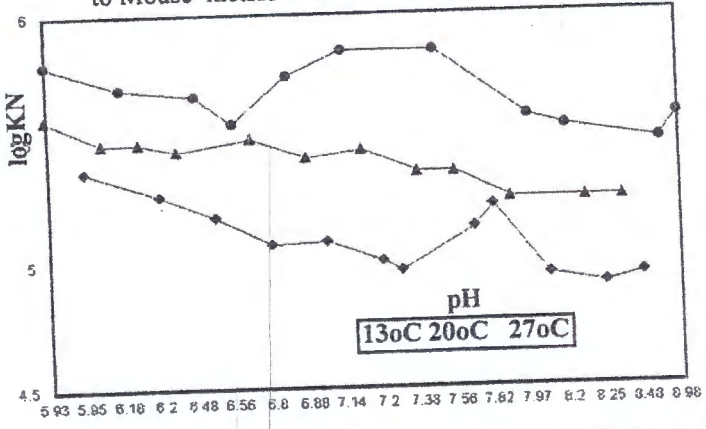
Ma D. Lu J. Tang W. (1998). 1H NMR Studies of Azide Binding to Cytochrome c. *Biochimica et Biophysica Acta, 1384(1): 32-42.*

Perutz, M. F.; Fersht, A. R.; Simon, S. R. and **Roberts, G. C. K.** (1974). Influence of Globin Structure on the State of the Heme.II. Allosteric Transition in Methemoglobin. *Biochemistry 13; 2174-2186.*

Perutz, M. F.; Heidner, E. J.; Ladner, J. E.; Beetlestone, J. G.; Chien, H. O.; and **Slade, E. F.** (1974). Influence of Globin structure on the State of the Heme III. Changes in Heme Spectra Accompanying Allosteric Transition in Methemoglobin and Their Implication for Heme – Heme Interaction. Biochemistry 13; 2187-2200.

Tan, A. L. and **Noble, R. W.** (1973). The Effect of Inositol Hexaphosphate on the Allosteric Properties of Carp Haemoglobin. *J. Biochem. 248; 7412-7416.*

Fig. 1: pH and Temperature Dependence of Azide Binding to Mouse metHb in the absence of IHP



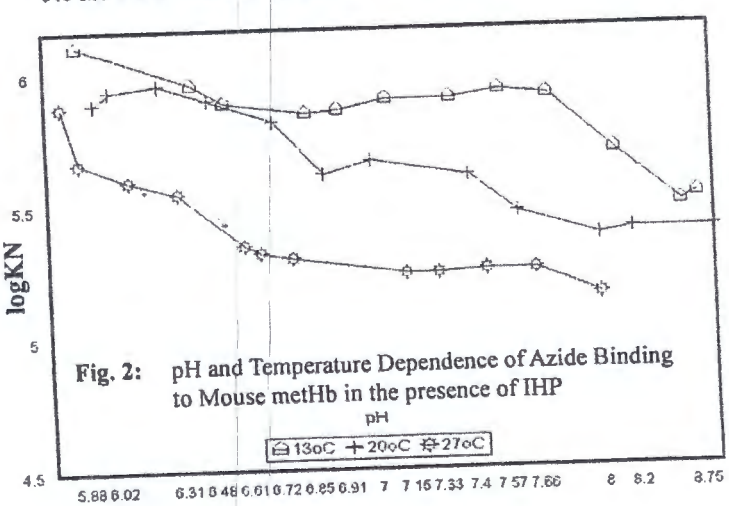


Fig. 2: pH and Temperature Dependence of Azide Binding to Mouse metHb in the presence of IHP