

Tertiary Conformational Transition In Horse Hemoglobin Induced By Inositol hexakisphosphate

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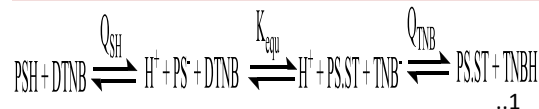


BACKGROUND

The CysF9[93]β sulfhydryl group of hemoglobin exists in two conformations (1). The equilibrium between these conformations is coupled to the transition, $r \rightleftharpoons t$, between two tertiary structures of hemoglobin (1,2). The reaction between 5,5'-dithiobis(2-nitrobenzoate) – DTNB – and CysF9[93]β is a reversible process and is coupled to the tertiary structure transition (3,4). The current wisdom in hemoglobin research is that the binding of O₂ to hemoglobin, especially in the presence of allosteric effectors, is better understood in terms of the effect of tertiary, rather than quaternary, transitions (5,6). For this reason we have developed a method for determining the equilibrium constant, K_{rt} , between the two tertiary structures (7,8). This involves studying the equilibrium constant of the DTNB reaction as a function of pH.

PURPOSE AND HYPOTHESIS

Horse hemolysate contains two hemoglobins. We plan to determine K_{rt} for each of these hemoglobins to check the fraction of r and t structures in each and to later relate this information to their affinities for O₂. The reaction of CysF9[93]β with DTNB may be depicted as:



In Eq. (1) PSH is hemoglobin with its CysF9[93]β in its protonated form, which does not react with DTNB; K_{equ} is the equilibrium constant for the DTNB reaction. It is readily shown from Eq. (1) that K_{equ} is given by the equation:

$$K_{equ} = \frac{[TNB^-]^2 \left\{ 1 + \frac{[H^+]}{Q_{TNB}} \right\} \left\{ 1 + \frac{[H^+]}{Q_{SH}} \right\}}{\left\{ [P]_{total} - [TNB^-] \right\} \left\{ 1 + \frac{[H^+]}{Q_{TNB}} \right\} \left\{ [DTNB]_{total} - [TNB^-] \right\} \left\{ 1 + \frac{[H^+]}{Q_{TNB}} \right\}} \quad ...2$$

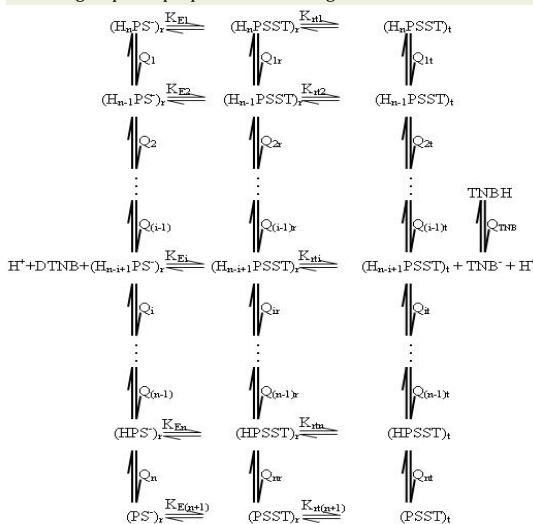
From the absorbance changes recorded at the wavelength of maximum absorption of TNB⁻, it is possible to obtain K_{equ} from Eq. (2) From the variation of K_{equ} with pH we can determine K_{rt} based on the fact that the $r \rightleftharpoons t$ equilibrium is coupled to the DTNB reaction.

MATERIALS AND METHODS

Horse blood was obtained from the jugular vein of domestic horses (*Equus ferus caballus*) at the University of Abeokuta, Ogun State. This was collected into heparinised bottles. Hemoglobin was prepared as described previously (4). The horse hemoglobins were separated as described for sheep hemoglobins. The method employed for the determination of K_{equ} has been described in detail elsewhere (8).

RESULTS & DISCUSSION

Figs. 1 and 2 show the dependence of K_{equ} on pH for DTNB reacting with various derivatives of the *slow* hemoglobin. It is seen in each case that K_{equ} decreases by about 3 orders of magnitude between pH 5.6 and 9. Similar results were obtained for the *fast* hemoglobin (not shown). These strong pH dependences suggest that the DTNB reaction is linked to the ionisation of groups on the protein. To determine the number and the nature of these groups we propose the following scheme 1:



The relationship between the experimentally determined K_{equ} (Eq. (2)) and the parameters of Scheme 1 is Eq. (3). The data in Figs. 1 & 2 were fit with Eq. (3) using programmes written on a MicroMaths Scientist software (Salt Lake City, UT). The lines through the experimental points are the best fit lines, obtained with an n value of 2 in each case. Table 1 reports the best-fit parameters for stripped hemoglobin and the corresponding parameters in the presence of inositol-P₆.

Table 1: Mean equilibrium parameters for slow hemoglobin

Parameters	Mean ± SE	
	Stripped Hb	Hb + inositol-P ₆
pQ _{1R}	5.48 ± 0.3	6.38 ± 0.6
pQ _{2R}	7.34 ± 0.3	7.56 ± 0.3
pQ _{1T}	6.62 ± 0.3	7.04 ± 0.7
pQ _{2T}	8.54 ± 0.2	7.78 ± 0.3
K _{E3} /K _{E2}	0.13 ± 0.06	0.67 ± 0.1
K _{rt3}	0.49 ± 0.2	2.21 ± 0.8

Table 1 shows that inositol-P₆ increases the pQ values of the ionisable groups; but the most notable result is the change in the mean value of K_{rt3} from 0.49 0.2 to 2.21 0.8. This represents a change in t isomer population from (32.9 8) % to (68.8 7) %. Calculations show that with decreasing pH the value of K_{rt} increases, suggesting that the proton (H⁺) increases the t isomer population.

$$K_{equ} = \frac{\left\{ 1 + \sum_{j=1}^n [H^+]^{-n+j} \left(\prod_{j=1}^n Q_{jr} \right)^{-1} + K_{rt(n+1)} \left\{ 1 + \sum_{j=1}^n [H^+]^{-n+j} \left(\prod_{j=1}^n Q_{jt} \right)^{-1} \right\} \right\}}{\left\{ 1 + K_{E(n+1)} \left\{ \sum_{j=1}^n [H^+]^{-n+j} \left(\prod_{j=1}^n Q_{jr} \right)^{-1} K_{rt} \right\} \right\}} \quad ...3$$

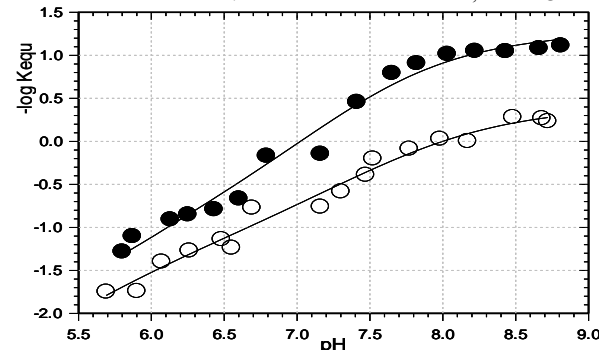


Fig. 1: Horse HbO₂ (● Hb + Inositol -P₆, ○ Stripped Hb)

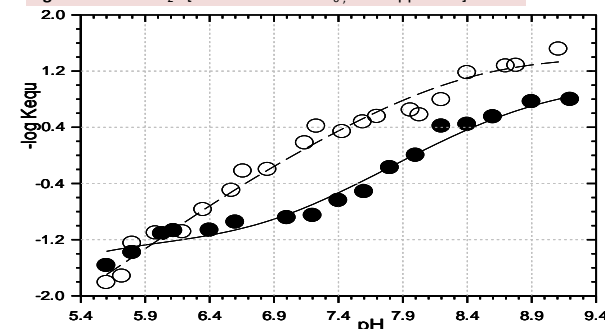


Fig. 2: Horse HbCO (● Hb + Inositol -P₆, ○ Stripped Hb)

CONCLUSION

The evidence presented indicates that inositol-P₆ and H⁺ increase the population of the t tertiary isomer of hemoglobin. This strongly suggests that both act as allosteric effectors of hemoglobin in the DTNB reaction.

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