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Tertiary Conformational Transition In Horse Haemoglobin Induced By Inositol Hexakisphosphate

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The red blood cell of the domestic horse contains two haemoglobin types. The two haemoglobins were separated on a column of carboxymethylcellulose. The equilibrium constant, Kequ, for the reaction of 5,5'-dithiobis(2-nitrobenzoate) — DTNB — with the CysF9[93]β sulfhydryl group of each haemoglobin was determined at 25°C as a function of pH. The reactivity of CysF9[93]β is affected by allosteric effectors such as the proton (H^+) and inositol hexakisphosphate (inositol-P_6). Between pH 5.6 and 9.0 Kequ decreased by about two to four orders of magnitude, demonstrating that H^+ is a heterotropic allosteric effector of haemoglobin with respect to its reaction with DTNB. Inositol-P_6 also decreased K_{equ} by about two to four orders of magnitude across the experimental pH range. CysF9[93]β exists in two tertiary conformations, r and t, in dynamic equilibrium.

K_{rt}, the equilibrium constant for the r $\leftrightarrow$ t conformational transition, was determined for each of the two horse haemoglobins from an analysis of the pH dependence of K_{equ}. The calculations from the pH dependence of K_{equ} showed that the pK_a values of the ionisable groups coupled to the DTNB reaction vary between 5.0 and 8.9. The equilibrium constants, K_{rt}, for the r $\leftrightarrow$ t tertiary structure transition, were 0.143 ± 0.05 and 0.446 ± 0.22 for the fast and slow stripped horse haemoglobins respectively. In the presence of inositol-P_6, K_{rt} for the fast and slow were 2.219 ± 0.79 and 2.214 ± 0.83 respectively. The results show that inositol-P_6 increases the relative population of the t tertiary conformation. So, it increases the affinity of CysF9[93]β by changing the relative distribution of two protein conformations.