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, $K_{equ}$, for the reaction of 5,5′-dithiobis(2-nitrobenzoate) — DTNB — with the CysF9β sulfhydryl group of each haemoglobin was determined at 25°C as a function of pH. The reactivity of CysF9β is affected by allosteric effectors such as the proton (H⁺) and inositol hexakisphosphate (inositol-P₆). Between pH 5.6 and 9.0 $K_{equ}$ 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-P₆ also decreased $K_{equ}$ by about two to four orders of magnitude across the experimental pH range. CysF9β 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 $0.143 \pm 0.05$ and $0.446 \pm 0.22$ for the fast and slow stripped horse haemoglobins respectively. In the presence of inositol–P₆, $K_{rt}$ for the fast and slow were $2.219 \pm 0.79$ and $2.214 \pm 0.83$ respectively. The results show that inositol–P₆ increases the relative population of the $t$ tertiary conformation. So, it increases the affinity of CysF9β by changing the relative distribution of two protein conformations.