Allostery of a Dimeric Insect Haemoglobin (CTT II). The Influence of pH on the O_2-binding Equilibria and Kinetics

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The haemolymph of larvae of the insect Chironomus thummi thummi contains at least nine different haemoglobins. Three of these haemoglobins (CTT I, III, and IV) are monomeric under all conditions. The other haemoglobins are dimeric at low pH (<pH 5.5), whereas at high pH (>pH 8.5) they are monomeric.

The dimeric haemoglobins have been separated from the monomeric haemoglobins by gel-filtration on Sephadex G50 in 40 mM phosphate buffer pH 5.6. The first step of separation of CTT II, which is composed of homologous chains, has been performed by ion exchange chromatography on SP-Sephadex C50 by stepwise elution with 5 mM bis-Tris buffer pH 5.6 (fraction I), 5 mM bis-Tris buffer pH 7.6 (fraction II) and 50 mM bis-Tris buffer pH 7.6 (fraction III). Fraction III which contains CTT II is further fractionated on DEAE-Sephadex A50 (6 x 55 cm) in a linear salt gradient at pH 9.0 (5 l 15 mM Tris-HCl plus 5 l 15 mM Tris-HCl in 200 mM NaCl). The homogeneity of CTT II has been checked by disk electrophoresis.

The O_2-binding curves of CTT II are hyperbolic (n = 1.0). The O_2 half-saturation pressure, $p_{1/2}(O_2)$, is pH-dependent. Therefore, the allostery of CTT II is characterized by a Bohr-effect (heterotropic interactions),whereas cooperativity between the two subunits (homotropic interactions) are apparently not observable in equilibrium studies. The Bohr-effect curve is characterized by an inflection point at pH 7.3, an amplitude $\Delta\log p_{1/2}(O_2)$= 1.7, and a Bohr proton release of $-\Delta\log p_{1/2}/\Delta pH$ = 0.77 mol H^+ per mol O_2.

The O_2 dissociation kinetics measured by dioxygen pulse experiments demonstrates between pH 7.0 and pH 9.5 two apparently successive time courses, i.e. in the <90 ms- and >100 ms-ranges. The fast and the slow reactions are attributed to dimeric and monomeric fractions respectively. The off-rate constants of both reactions are pH-dependent, with an inflection point at pH 6.8 for the fast reaction. The exact accordance of the pH dependences of the off-rate constants of monomeric CTT IV and dimeric CTT II indicates, that the tertiary structure-linked heterotropic interactions are the same in the subunit and in the oligomer.

The on-rate constants have been calculated on the basis of equilibrium and off-rate constants. While the on-rate of the monomeric haemoglobin CTT IV is independent of pH, that of the dimeric CTT II is pH-dependent with an inflection point at pH 7.8. It is assumed that the pH dependence of the on-rate in CTT II originates from the monomer-dimer aggregation equilibrium.

A possible cooperativity between the subunits of CTT II postulated on the basis of polysteric linkage theory and kinetic results could not be observed. Formation of met haemoglobin during the O_2-binding experiment leads to valency hybrids with only one O_2-binding site per dimer. Therefore, experiments demonstrating the polysteric linkage behaviour of CTT II must be performed with the redox-inactive ligand CO.