Functional Analysis of Carp Hemoglobin According to the Theories of Adair; of Monod, Wyman & Changeux (MWC), and of Koshland, Némethy & Filmer (KNF).

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The properties of Carp hemoglobin (Cyprinus carpio) add a new dimension to the functional versatility of the hemoglobin molecule. The affinity of Carp hemoglobin for oxygen shows an extreme pH dependence, 160 times higher at alkaline pH than at acid pH. In order to analyse possible functional mechanisms of Carp hemoglobin, precise equilibria were measured from approximately 1% to 99% oxygen saturation at 15°C in bis-tris buffer and in phosphate buffer in the presence and absence of P6-inositol (IHP)(1). Adair constants, k_i, (2) were estimated by non-linear least square fit of Hill plots. Parameters of the Monod, Wyman & Changeux (MWC) model (3) were estimated by a similar non-linear least square procedure and by iterated computer calculations from the Adair parameters. The parameters of the simple, sequential model of Koshland, Némethy & Filmer (KNF) (4) were calculated from the Adair parameters. From the Adair constants, one can conclude that Carp hemoglobin, in the cooperative regime, undergoes an allosteric transition after oxygenation of the third heme; lowering of cooperativity by changes in pH seems to shift conformational transitions to earlier stages of oxygenation. The MWC parameters of K_R, c, and L were found to be 8.2 $mmHg^{-1}$, 1.3 x 10^{-2} and 4.2 x 10^6, respectively, at pH 7.2 (maximal cooperativity in phosphate buffer). Changes in pH in either direction increase the values of K_R and L, and decrease the value of c. Large values of L at reduced cooperativity are unreasonable in the context of this model. The simple, sequential KNF model, as interpreted here, seems to predict a symmetry conserved mode of action in regions of high, positive cooperativity, and a symmetry non-conserved mode of action in regions of low, non-, or negative cooperativity. Therefore, when regions of high, positive cooperativity are encountered, an almost concerted conformational transition occurs upon ligand binding. In this case, the KNF model extrapolates to the MWC model for all intents and purposes.

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