Novel Mechanisms of pH Sensitivity in Tuna Hemoglobin

Takeshi Yokoyama[a], Khoon Tee Chong[b], Masahiro Watanabe[a], Daniel T.-B. Shih[c], Jeremy R.H. Tame[a], Sam-Yong Park[a], [a]*Graduate School of Integrated Science, Yokohama city university, Japan.* [b]*Department of Bioengineering, Osaka university.* [c]*Graduate Institute, Taipei Medical University, Taiwan.* E-mail: takeshi@tsurumi.yokohama-cu.ac.jp

The crystal structure of fish hemoglobin (Hb) has been known for several years, yet various features of the molecule remain unexplained or controversial. Fish Hbs are well known for their widely varying interactions with heterotropic effector molecules and pH sensitivity. Some fish Hbs are almost completely insensitive to pH, whereas others show extremely low oxygen affinity under acid conditions, a phenomenon called the Root effect. We have solved the crystal structure of tuna Hb in the deoxy form at low and moderate pH and in the presence of carbon monoxide at high pH. In the T state a novel salt salt bridge is formed between His69β and Asp72β. This salt bridge is broken in the R state structure, releasing a proton. Additional proton binding to the T state occurs through a pair of carboxyl groups, Asp96α1 and Asp101β2. The dramatic change between the two T state models is found at His60 of one α subunit. At low pH, this residue swings out the of the heme pocket. Removal of His60α from the heme pocket will significantly reduce the α subunit affinity for oxygen.

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