FA1-MS02-O1

Hemoglobin - Then and Now. Jeremy Tame. *Graduate School of Arts and Sciences, Yokohama City University, Tsurumi, Japan.*
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The determination of the molecular structures of myoglobin and hemoglobin by John Kendrew and Max Perutz is a landmark in the progress of science comparable to putting a man on the moon. Like the Apollo missions, announced by President Kennedy a few months before Kendrew and Perutz received the Nobel Prize in 1962, it was done not because it was easy but because it was hard. It also took longer. The hemoglobin structure was the first to be used to direct drug design, the first to explain genetic disorders and molecular evolution, and the first to demonstrate allostery. Since the 1980s however, when the two-state model matured into a standard text-book form, the protein has attracted far less attention. A brief renaissance occured as hemoglobin-based artificial blood substitutes became a popular research topic in the early 1990s. The difficulties encountered with nitric oxide binding led to new progress in the understanding of ligand binding at the heme, and modern high-resolution crystallography shows the ligand interactions in atomic detail. New crystal structures have tested the limits of the two-state model, causing considerable discussion of more complicated analyses. Just within the last few years it has been found that drug binding to hemoglobin may reduce its oxygen affinity by three orders of magnitude while preserving the “high affinity” R type structure. This realization throws new light on the properties of many animal hemoglobins, which remain poorly understood, and overthrows entirely the basis used earlier for the design of drugs to treat sickle cell anemia. New efforts are being made through crystallography to address this widespread disease, which kills roughly 100,000 children every year in Nigeria alone.

Keywords: molecular disease; drug design; allostery

FA1-MS02-O2

Archaeal Protoglobin: Novel Ligand Diffusion Paths and Heme Reactivity. Martino Bolognesi. *Dept. Biomol.Sciences and Biotechnology, U. of Milano-Italy.*
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Protoglobin (Pgb) from *Methanosarcina acetivorans* C2A, a strictly anaerobic methanogenic Archaea, is the latest entry in the hemoglobin superfamily. Our crystallographic studies have shown that protoglobin-specific loops and a N-terminal extension completely bury the heme within the protein matrix [1]. Access of O_2, CO, and NO to the heme is granted by specific apolar tunnels reaching the heme distal site from the B/G and B/E helix interfaces. Functionally, *M. acetivorans* dimeric protoglobin displays a selectivity ratio for O_2/CO binding to the heme that favours O_2 ligation. Here we report structural and kinetic data on Pgb mutants engineered to probe the protein structural/functional properties. Six crystal structures (Pgb mutants: Δ20 (missing 20 N-ter residues), Y(B10)61→A, Y(B10)61→W, F(B12)63→W, F(G7)145→W, I(G11)149→F) show that the mutations engineered essentially restrict access to ligand_tunnel_1. Closure of tunnel_1 correlates well with decreased CO association/dissociation rates. CO-dissociation kinetics is heterogeneous, displaying at least 2 well separated components, being slowed down when the tunnel_1 is obstructed. A structural analysis of such effects in relation to conventional Hbs shows dramatic functional changes coded within a modified, but still quite recognizable, globin fold.

[1] Nardini, et al. **2008**. *EMBO Rep* 9, 157-63.

Keywords: hemoglobin; globin fold; ligand channeling

FA1-MS02-O3

Combined Raman and X-ray Crystallography Reveals Multiple Coordinations and Quaternary States of Fish Hemoglobins Alessandro Vergara[a,b], Luigi Vitagliano[b], Antonello Merlino[a,b], Filomena Sica[a,b], Cinzia Verde[c], Guido di Prisco[c], Lelio Mazzarella[a,b]. [a]*Dept of Chemistry, University of Naples ’Federico II’, Napoli, Italy.* [b]*Biostructures and Bioimages Institute, C.N.R, Napoli, Italy.* [c]*Institute of Protein Biochemistry, CNR, Naples, Italy.*
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Many fish hemoglobins (Hbs) are endowed with Root effect, that produces a decrease in oxygen affinity at low pH, associated with complete loss of cooperativity. Over the years, several hypotheses on the structural determinants of the Root effect have been suggested. Up to now, all the structural explanations of the Root effect have been based on the two-state model, in which the Root effect is related to an increase of the allosteric equilibrium between the R and T state at acidic pH [1-3]. Here, we report the crystal structures of the deoxy and carbomonoxy forms of the non-Root-effect major component Hb 1 isolated from the Antarctic fish (Af) *Trematomus newnesi* (Hb1Tn). Surprisingly, in the deoxy state of the non-Root effect Hb1Tn, the inter-aspartic hydrogen bond between Asp95α and Asp101β at the α1β2 interface, which is believed to be important in Root effect [2], is observed. A combined Resonance Raman / x-ray crystallography of this AfHb has revealed heterogeneity in the deoxy coordination. Novel ligated states are observed in both a T-like state and a R/T intermediate quaternary state. Three of four independent CO coordination states are not assisted by the hydrogen bond of the distal histidyl, that swings out of the heme pocket. These un-assisted CO coordination states (supported by FT-IR spectroscopy solution studies) are associated with unusually small thermal fluctuations which characterise both α and β CD corners. The accessibility of ligated states within three different quaternary structures (T, R and R/T intermediate [4]) suggests a novel structural explanation of protein allostery, based on a three-state Edelstein’s model [5]. Grant Sponsors: PRIN , PNRA.