4.2: Biological Oxygen Carriers

As noted earlier, three solutions to the problem of dioxygen transport have evolved: hemoglobin (Hb), hemocyanin (Hc), and hemerythrin (Hr). Their remarkable distribution over plant and animal kingdoms is shown in Figure 4.8.[15]

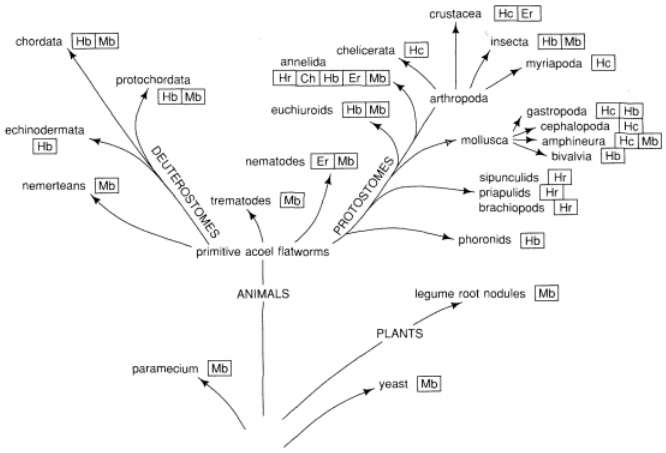

Figure 4.8 - Phylogenetic distribution of oxygen-carrier proteins: Hb, hemoglobin; Mb, myoglobin; Er, erythrocruorin; Ch, chlorocruorin; He, hemocyanin; Hr, hemerythrin.[15a] Reproduced with permission from K. E. van Holde and K. I. Miller, Quart. Rev. Biophys. **15** (1982), 1-129.

The hemoglobins and myoglobins found in plants, snails, and vertebrates all appear to share a common, very ancient ancestor. There is some evidence now for a common ancestral hemocyanin.[42c] The appearance of hemerythrin in a few annelid worms is an evolutionary curiosity. These few words and the diagram will suffice to give some hints about how respiratory proteins evolved, a subject that is outside the scope of this book.

The Hemoglobin Family

Hemoglobins are the most evolutionarily diverse family of dioxygen carriers. They are found in some plants (e.g., leghemoglobin in the nitrogen-fixing nodules of legumes), many invertebrates (including some insect larvae), crustaceans, molluscs (especially bivalves and snails), almost all annelid worms, and in *all* vertebrates with one possible exception, the Antarctic fish *Cyclostomata*.

With few exceptions the monomeric and oligomeric hemoglobins all share a basically similar building block: a single heme group is embedded in a folded polypeptide with a molecular weight of about 16 kDa (see Figure 4.2), and is anchored to the protein by coordination of the iron center to an imidazole ligand from a histidine residue. Mammalian myoglobin is often taken as the archetypical myoglobin (see Table 4.1). Sperm whale, bovine, or equine myoglobin are specific examples; the muscle tissue from which they may be extracted is more available than that from *Homo sapiens*. The archetypical oligomeric hemoglobin that shows cooperative binding of O_2 is the tetrameric hemoglobin A. It is readily available from the blood of human donors.* In some invertebrate hemoglobins, especially those of annelids, aggregates may contain as many as 192 binding sites, to give a molecular weight of about 3 x 10^6 Dalton. These and other high-molecular-weight hemoglobins of arthropods are often referred to as erythrocruorins (Er). In a few annelid worms, the otherwise ubiquitous heme b or protoheme is replaced by chloroheme (see Figure 4.2) to give chlorocruorins (Ch), which tum green upon oxygenation (*chloros*, Greek for green). Some organisms, for example the clam *Scapharca equivalvis*, feature a dimeric hemoglobin.

The only known anomalous hemoglobin is Hb *Ascaris*, which comes from a parasitic nematode found in the guts of pigs. It has a molecular weight of about 39 kDa per heme; this value is not a multiple of the myoglobin building block.[31] Moreover, presumably in response to the low availability of O_2 in pigs' guts, Hb *Ascaris* has an extraordinarily high affinity for dioxygen, in large part owing to an extremely slow rate of dioxygen release.[32] Leghemoglobin is another carrier with a high affinity for dioxygen, in this case because of a high rate of O_2 binding. Since O_2 is a poison for the nitrogenase enzyme, yet the nodules also require dioxygen, diffusion of O_2 is facilitated, but the concentration of free dioxygen in the vicinity of nitrogen-fixing sites is minimized.[33]

Kinetic and thermodynamic data for dioxygen binding and release from a variety of hemoglobins are summarized in Table 4.2.[9,10,31,34-36] Notice that for the hemoglobin tetramer, which comprises two pairs of slightly dissimilar subunits, the α and β chains bind O_2 with significantly different affinities and rate constants, especially in the T state. Isolated chains behave like monomeric vertebrate hemoglobins, such as whale myoglobin, which have affinities close to those of R-state hemoglobin. The

chlorocruorins have a low affinity compared to other erythrocruorins. Especially for proteins that bind O_2 cooperatively, a range of values is specified, since affinities and rates are sensitive to pH, ionic strength, specific anions and cations (allosteric effectors), and laboratory. For example, as we noted above, the O_2 affinity of hemoglobin A is sensitive to the concentration of 2,3-DPG and to pH (Bohr effect). Trout hemoglobin I is insensitive to these species, whereas a second component of trout blood, trout hemoglobin IV, is so sensitive to pH (Root effect) that at pH < 7 trout hemoglobin IV is only partially saturated at $P(O_2)$ = 160 Torr.[4] Note that O_2 affinities span five orders of magnitude. Since heme catabolism produces carbon monoxide, and since in some environments CO is readily available exogenously, selected data for CO binding are also presented.

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* Blood from human donors is also a source for a variety of abnormal hemoglobins, the most famous of which is HbS, the hemoglobin giving rise to sickle-cell anemia, It was Pauling and coworkers[30] who first found that HbS differs from HbA through the *single* substitution of valine for glutamic acid in each of two of the four subunits comprising Hb, Sickle-cell anemia was the first condition to be denoted a "molecular disease."

The Hemocyanin Family

Hemocyanins (Hc), the copper-containing dioxygen carriers, are distributed erratically in two large phyla, *Mollusca* (for example, octopi and snails) and *Arthropoda* (for example, lobsters and scorpions). The functional form of hemocyanin consists of large assemblies of subunits.[14,15,37] In the mollusc family the subunit has a molecular weight of about 50 kDa and contains two copper atoms. From electron-microscopic observations, hemocyanin molecules are cylindrical assemblies about 190 or 380 Å long and 350 Å in diameter comprising 10 or 20 subunits, respectively, for a molecular weight as high as 9 x 10^6 Dalton. In the arthropod family, the subunit has a molecular weight of about 70 kDa with two copper atoms. Molecular aggregates are composed of 6, 12, 24, or 48 subunits. Upon oxygenation the colorless protein becomes blue (hence cyanin from *cyanos*, Greek for blue). Spectral changes upon oxygenation, oxygen affinities, kinetics of oxygen binding (Table 4.2),[4.5,14,15,38] anion binding, and other chemical reactions show that the active site in the phylum *Arthropoda* and that in *Mollusca*, although both containing a pair of copper atoms, are not identical.[4,14]

No monomeric hemocyanins, analogous to myoglobin and myohemerythrin (next section), are known. For some hemocyanins the binding of dioxygen is highly cooperative, if calcium or magnesium ions are present, with Hill coefficients as high as n ~ 9. However, the free energy of interaction per subunit can be small in comparison with that for tetrameric hemoglobin; 0.9 to 2.5 kcal/mol compared to 3.0 kcal/mol. Allosteric effects, at least for a 24-subunit tarantula hemocyanin, can be separated into those within a dodecamer (12 subunits)—the major contributor to overall allostery—and those between dodecamers.[39c] This has been termed *nested allostery*. In contrast to the hemoglobin family, isolated chains have affinities typical of the T-state conformation for hemocyanin. The binding of CO, which binds to only one copper atom, is at best weakly cooperative.[39]

As alluded to above, the distribution of hemocyanins is striking, Among the molluscs exclusive use of hemocyanin as the respiratory protein occurs only with the cephalopods (squid, octopi, and cuttlefish), and in the arthropods only among the decapod (ten-footed) crustaceans (lobsters, shrimp, and crabs). The bivalve molluscs (for example, oysters and scallops) all use small dimeric or octameric hemoglobins. The edible gastropod (snail) *Helix p*omatia uses hemocyanin, whereas the apparently closely related fresh-water snail *Planorbis* uses a high-oligomer hemoglobin. Both use a myoglobin as the oxygen-storage protein. The structure of the active site has been extensively probed by EXAFS methods,[40,41] and the x-ray crystal structure of a hexameric deoxyhemocyanin is known.[42] Each copper atom is coordinated to three imidazole groups from histidine residues. The pinwheel arrangement of the six subunits, the domain structure of a single subunit, and the domain containing the active site are shown in Figure 4.9.

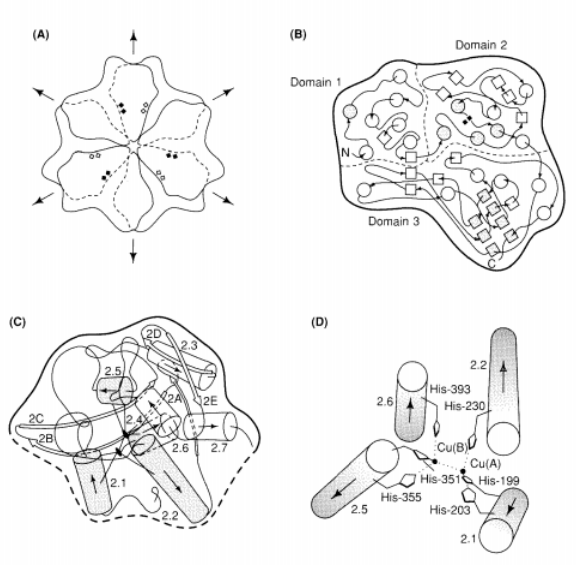

Figure 4.9 - Diagram of the structure of deoxyhemocyanin from Panulirus interruptus at 3.2 Å resolution:[42c] (A) The hexameric arrangement of subunits; (B) The domain structure of one subunit; (C) The tertiary structure of domain 2, which contains the pair of copper atoms: α-helices are represented by cylinders; β-strands by arrows, and copper atoms by diamonds; (D) The active site and its histidine ligands. Reproduced with permission from B. Linzen, Science **229** (1985), 519-524.

The Hemerythrin Family

The biological occurrence of hemerythrins (Hr in Figure 4.8), the third class of dioxygen carriers, is relatively rare, being restricted to the sipunculid family (nonsegmented worms), a few members of the annelid (segmented worm) family, a couple of brachiopods (shrimps), and a couple of priapulids. The oxygen-binding site contains, like hemocyanin, a pair of metal atoms, in this case, iron. Upon oxygenation the colorless protein becomes purple-red. Monomeric (myohemerythrin), trimeric, and octameric forms of hemerythrin are known; all appear to be based on a similar subunit of about 13.5 kDa. When hemerythrin is extracted from the organism, its oxygen binding is at best only weakly cooperative, with Hill coefficients in the range 1.1 to 2.1.[18] In coelomic cells (the tissue between the inner membrane lining the digestive tract and the outer membrane of the worm—analogous to flesh in vertebrates), oxygen apparently binds with higher cooperativity (n ~ 2.5).[43] Perchlorate ions have been observed to induce cooperativity: since ClO_4^- has no biological role, it appears that in protein purifications the biological allosteric effector is lost. No Bohr effect occurs. Dioxygen binding data are accumulated in Table 4.2.[36,44]

The structure of hemerythrin in a variety of derivatives (oxy, azido, met, and deoxy) is now well-characterized. With three bridging ligands, a distinctive cofacial bioctahedral stereochemistry is seen (Figure 4.10).[45-48]

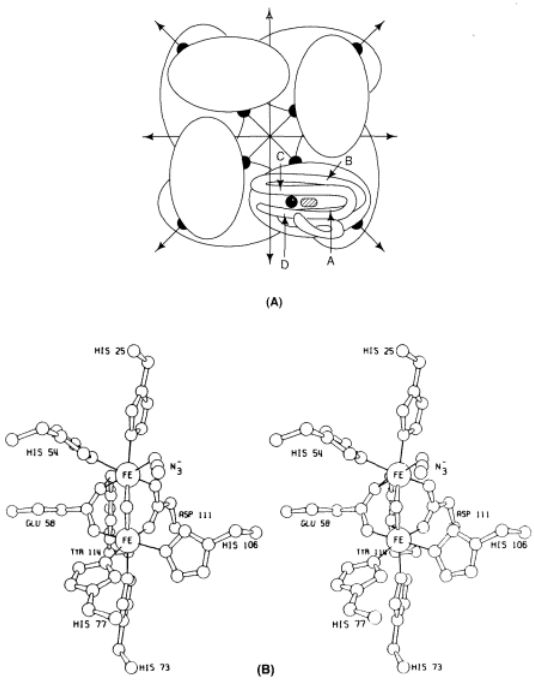

Figure 4.10 - Structure of hemerythrin: (A) The tertiary structure of octameric hemerythrin[46b] with four (α-helices (A, B, C, D) of one of the eight subunits. The filled half-circles denote anion binding sites (e.g., ClO_4^-); the filled circle the Fe_2 site; and the cross-hatched oval the N_3^- and SCN^- binding sites $(Fe^{III})_2$ and the O_2 binding sites $(Fe^{II})_2$. Reproduced with permission from R. E. Stenkamp, L. C. Sieker, and L. H. Jensen, J. Mol. Biol. **126** (1978), 457-466. (B) The structure of the active site of metazidomyohemerythrin,[48] showing the cofacial bioctahedral stereochemistry. The structure of oxyhemerythrin is very similar, including the orientations of the $(H)O_2{}^{II}$Iigand.[45] Reproduced with permission from S. Sheriff, W. A. Hendrickson, and J. L. Smith, J. Mol. Biol. **197** (1987), 273-296.

Table 4.2 - Thermodynamics and kinetics of ligand binding to biological oxygen carriers (at 20-25 °C and buffered at pH 6.5-8.5).

Solubility of O2 in water: 1.86 x 10^6 M/Torr

Solubility of CO in water: 1.36 x 10^{-6} M/Torr

a) 10 mM Ca^{2+} added: necessary for cooperativity

b) CO binding at pH 9.6.

Carrier	Dioxygen binding					Carbon Monoxide Binding				
	$P_{1/2}(O_2)$ Torr	ΔH kcal/mol	ΔS eu	k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}	$P_{1/2}(O_2)$ Torr	ΔH kcal/mol	ΔS eu	k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}
Hemoglobins										
Hb *Ascaris*	0.0047	—	—	1.5	0.0041	0.063	—	—	0.21	0.018
Leg Hb	0.047	-18.9	—	156.	1.	0.00074	—	—	13.5	0.012
whale Mb	0.51	-14.9	—	14.	12.	0.018	-13.5	—	0.51	0.019
Whale Mb	—	—	—	140.	1600.	—	—	—	—	—
HbA isolated chains - α	0.74	-142	-21.	50.	28.	0.0025	—	—	4.0	0.013

Carrier	Dioxygen binding					Carbon Monoxide Binding				
	$P_{1/2}(O_2)$ Torr	ΔH kcal/mol	ΔS eu	k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}	$P_{1/2}(O_2)$ Torr	ΔH kcal/mol	ΔS eu	k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}
HbA isolated chains - β	0.42	-16.9	-29	60.	16.	0.0016	—	—	4.5	0.008
HbA R α chain	0.15-1.5	-18.	-30.	29.	10.	0.001-0.004	—	—	3.2	0.005
HbA R β chain	0.15-1.5	-18.	-30.	100.	21.	0.001-0.004	—	—	9.8	0.009
HbA R $\alpha_{E7His \rightarrow Gly}$				220.	620.				19.	0.007
HbA R $\beta_{E7His \rightarrow Gly}$				100.	3.				5.0	0.0013
HbA T α chain	9-160	-12	-35	2.9	183.	0.10-2.8	—	—	0.099	0.09
HbA T β chain	9-160	-12	-35	11.8	2500.	0.10-2.8	—	—	0.099	0.09
Chironimus Mb	0.40	—	—	300.	218.	0.0019	—	—	27.	0.095
Glycera Mb	5.2	—	—	190.	1800.	0.00089	—	—	27.	0.042
Aplysia Mb	2.7	-13.6	—	15.	70.	0.013	—	—	0.49	0.02
Spriographis chlorocruorin	16-78	-4.5	—	—	—	—	—	—	—	—
Hemocyanins[a]										
Molluscan Hc										
Helix pomatia R	2.7	-11.5	-12.6	3.8	10.	10.	-13.5	-24	0.66	70.
Helix pomatia T	55.	-15.4	-31.1	1.3	300.	CO	binding	noncooperative	since not	measureable
Levantina hierosohimia R	3.8	-7.5	-1.8	—	—	—	—	—	—	—

Carrier	$P_{1/2}(O_2)$ Torr	ΔH kcal/mol	Dioxygen ΔS eu	binding k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}	$P_{1/2}(O_2)$ Torr	Carbon ΔH kcal/mol	Monoxide ΔS eu	Binding k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}
Levantina hierosohimia T	18.	+3.1	+31.	—	—	—	—	—	—	—
Arthropod Hc										
Panulirus interruptus R[b]	1.0	—	—	31.	60.	720.	-6.0 CO	-2.7 binding	4.1 noncooperative	8100.
P. interruptus monomer	9.3	—	—	57.	100.	—	—	—	—	—
Leirus quinquestris R	1.7	-7.4	0.	—	—	—	—	—	—	—
Leirus quinquestris T	117.	+3.1	+27.	—	—	—	—	—	—	—
Hemerythrins										
Phascolopsis gouldii	2.0	-12.4	-18	7.4	56	not	known	to	bind	CO
Themiste zostericola 8-mer	6.0	—	—	7.5	82					
T. zostericola monomer	2.2	—	—	78.	315.					