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Hemoglobin: Some (Dis)Assembly Required

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The remarkable chemical properties of hemoglobin have fascinated researchers for more than 150 years. Historical accounts remind us of the work of Lavoisier and proceed from one seminal contribution to the next as they paint the dioxygen carrier found in vertebrate blood as a star at the origin of several modern fields of natural sciences. As one of the most extensively studied proteins, human hemoglobin is a staple of biochemistry textbooks. Its essential features, such as three-dimensional structure in different conformational and ligation states and the mode of action of allosteric effectors, are by now extensively characterized. Yet fundamental questions remain. How is hemoglobin assembled, and how does it fall apart? These aspects have so far resisted quantitative description.

The challenge of drawing models of assembly and disassembly is indeed considerable. Human adult hemoglobin (HbA) is a dimer of $\alpha\beta$-heterodimers. Two types of interfaces are formed, $\alpha_1\beta_1$, which is held by sturdy packing contacts, and $\alpha_1\beta_2$, which forms the weaker sliding contacts responsible for quaternary changes linked to dioxygen binding. Although the study of protein subunit interactions may not seem particularly noteworthy, the hemoglobin system has a truly problematic trait: it contains heme groups. This cofactor, which associates tightly in a 1:1 stoichiometry with each of the α- and β-chains (Fig. 1), is redox active and practically insoluble in water. As such, it complicates experimental work designed to unravel the mechanistic features of functional tetramer dismantling. In this issue of *Biophysical Journal*, Samuel et al. (1) present an equilibrium model of ferric hemoglobin denaturation. The difficulty of their task is presaged by decades of unfolding studies of myoglobin (2,3), the monomeric counterpart of hemoglobin. The new hemoglobin study builds on the myoglobin example and a prior analysis of apohemoglobin unfolding (4) to propose a comprehensive view of reversible Hb disassembly.

Oxygenated hemoglobin is prone to iron oxidation, a reaction that leads to the "met" state. Not only is methemoglobin nonfunctional, but it is also relatively unstable, and its formation marks the onset of hemoglobin disassembly. Thus, Samuel and co-workers chose to map out the fate of methemoglobin in detail, and to do so, they performed chemical denaturation experiments with recombinant HbA wild-type and variants and recombinant fetal hemoglobin (HbF). Guanidinium chloride is the denaturant of choice to maintain hemin (the Fe(III) version of heme) in solution and in the monomeric state. Circular dichroism and electronic absorption were selected to monitor secondary structure and hemin throughout the transitions. With spectral deconvolution, to tease out hemin coordination, and global fitting, to integrate all available data, Samuel et al. were able to reconstruct the energy landscape visited by HbA as it breaks down into its components. The spectroscopy results, enhanced by small-angle x-ray scattering, support that the heme cofactor is integral to the stability and compaction of the tetramer.

As myoglobin announced, a number of partially folded states, some resembling molten globules, are necessary to account for the denaturation path of Hb. One particular type of intermediate, the hemichrome, stands out in the analysis. Hemichromes coordinate hemin with the proximal histidine and a second protein side chain, a scheme that conveys distinctive electronic absorption signatures. For some proteins, HbA included, hemichrome states occur through distortions of the functional conformation (Fig. 1, *C* and *D*), whereas for some others, hemichromes are the native, resting state (5). This last observation has renewed interest in the properties of endogenously hexacoordinated species. To appreciate the significance of hemichrome intermediates in HbA, we must take an in vivo detour.

In the erythrocyte precursor stages, hemoglobin assembly requires precise orchestration of several events, each an opportunity for mishap. Chaperones (6,7), α-chains, and β-chains must be expressed in the correct proportions; subunits must fold and incorporate the toxic heme in a timely manner; the tetramer must be built; and all the while, the cell must keep reactive oxygen

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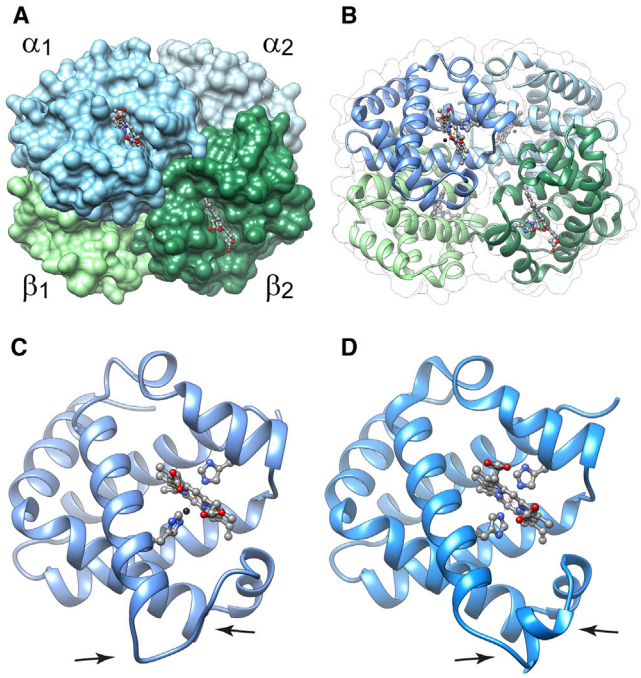

FIGURE 1 (*A*) The molecular surface and (*B*) ribbon diagram of the adult hemoglobin tetramer in the ferric (met) R state with water bound (PDB: 2P5Q (9)). The α-chains are in blue, and the β-chains are in green. The proximal histidine, heme (iron-protoporphyrin IX), distal histidine, and heme-coordinated water molecule are shown with balls and sticks. The $\alpha_1\beta_2$-interface is facing the viewer. Assembling and disassembling the tetramer are complex processes involving four heme molecules and four polypeptide chains capable of adopting multiple conformational and cofactor-bound states. (*C*) The α-subunit of horse methemoglobin is oriented to emphasize the coordination of the Fe(III) heme group by the proximal histidine and a water molecule (*black*) (PDB: 2MHB (10)). (*D*) Shown is the α-subunit of horse methemoglobin in a hemichrome state (PDB: 1NS6 (11)). In this structure, proximal and distal histidines are axial ligands to the iron. The arrows point to noticeable structural distortions. Other hemichromes, using different axial ligands, are also thought to exist. To see this figure in color, go online.

species under check. The α-chain hemichrome has emerged as an assembly intermediate resolved with the help of the chaperone α-hemoglobin stabilizing proteins (AHSP) (6). In the clearance of HbA, hemichromes are formed, first as reversible species, then with further conformational changes, as species committed to precipitation in Heinz bodies. In this role, hemichromes bind to the anion transporter Band 3, promote its clustering, and trigger binding of IgG, which labels the cell for phagocytosis (8). In the in vitro model of Samuel et al., hemichromes are now found to stabilize the $\alpha_1\beta_1$-heterodimer of HbA (and the $\alpha_1\gamma_1$-heterodimer of HbF) and to have a broad involvement in partially folded heterodimeric and monomeric forms.

The usual questions can be asked of the multiparameter unfolding experiments, including the influence of the chosen temperature and pH, and the fact that experimental constraints limit the range of hemoglobin concentrations that can be explored. It is also evident that a higher-resolution description than provided by circular dichroism and electronic absorption will be necessary to understand the attributes of assembly intermediates and the effect of the many mutations naturally encountered in hemoglobin genes. For now, it is finally possible to anticipate with some confidence the behavior of HbA under different conditions; for example, those mimicking adult erythrocytes (~5 mM tetramer) or plasma (submicromolar tetramer). The study provides a much-awaited thermodynamic framework for the formation of the functional tetramer.

Extension of the model to in vivo situations will be the next hurdle. In the cell and in plasma, additional players abound that are expected to affect the partitioning of hemoglobin states and components. These players are dedicated to manage senescence (Band 3), the redox state of the heme (peroxyredoxin 2 and the NADH cytochrome-b_5 reductase system), acellular oxidative damage, and dysregulation of blood pressure caused by seepage from the erythrocyte (haptoglobin and hemopexin), to name a few proteins. An all-encompassing representation of the life of hemoglobin tetramers will also require rate constants so that fluxes can be assessed and a dynamic picture composed. The minimal scheme that we now have is an exciting step toward these goals.

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