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Commentary

Dynamics and function of proteins: The search for general concepts

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> For an understanding of the phenomena, the first condition is the introduction of adequate concepts; only with the help of the correct concepts can we really know what has been observed. When we enter a new field, very often new concepts are needed, and these new concepts usually come up in a rather unclear and undeveloped form. Later they are modified, sometimes they are almost completely abandoned and are replaced by better concepts which then, finally, are clear and well defined.
>
> Werner Heisenberg (1)

Proteins execute and control essentially all functions in living organisms, and they do it elegantly and efficiently, with designs honed by billions of years of evolution. For each protein, we can ask how it performs its specific function. Because there are at least 100,000 different proteins, all important for life, the task to study, characterize, and understand all proteins will take a very long time. There may, however, be a complementary approach to the problem, namely to find general concepts, properties, and laws that characterize proteins and other biomolecules and that may lead to shortcuts in understanding newly discovered systems. The work of Réat and collaborators in this issue of the *Proceedings* (2) adds new information that bears on both aspects, the detailed function of a particular protein and insight into general concepts.

Réat and coworkers study the dynamics of bacteriorhodopsin (BR) with neutron scattering and hydrogen-deuterium exchange. BR is a protein that converts light into energy by pumping protons across a membrane (3). It consists of seven closely packed, essentially parallel, α helices that tightly enclose a retinal. Light is absorbed by the retinal and, through conformational changes, transports a proton (p) from the cytosolic to the extracellular side of the membrane. This process cannot be static; the proton transport requires motions of the protein and the retinal. Spectroscopic studies show that the transport occurs through a number of increasingly slower steps, $BR + h\nu \leftrightarrow J \leftrightarrow K \leftrightarrow L \leftrightarrow M_1 \leftrightarrow M_2 \leftrightarrow N \leftrightarrow O \leftrightarrow BR + p$. Réat and coworkers study the early fast steps with elastic incoherent neutron scattering (EINS). EINS provides information about the motion of individual nuclei as a function of time. In this experiment, it characterizes $<u^2>$, the average mean square amplitude of the nuclear motion faster than 2 nanoseconds. Previous work had used EINS to explore the global dynamics of a few proteins; such studies indicated that proteins are dynamic, moving systems. To get deeper insight into function, global data are not enough; the information must be site-specific. Réat and colleagues achieve this goal by replacing selected hydrogen atoms by deuterium, $^1H \rightarrow {}^2H$. The EINS cross-section for hydrogen is much larger than for deuterium and selective labeling of parts of the protein thus permits conclusions about $<u^2>$ in specific parts of the protein. The EINS experiments show that $<u^2>$ is proportional to T below a temperature T_g, implying harmonic motion. Above T_g, a dynamical transition occurs and $<u^2>$ increases faster than linearly with T, implying nonharmonic motions. T_g is about 150 K in the samples where EINS explores the global motions, but somewhat above 200 K for the selectively labeled sample that characterizes the region near the retinal. The data indicate that the mean-square amplitudes of atomic motion in the retinal pocket and the extracellular side of the protein are smaller than those of the rest of the protein.

Réat *et al.* compare their results with similar data on myoglobin (Mb). Mb is a globular protein; seven α helices surround a heme group with an iron atom at its center (3). Its main function is to store and transport dioxygen in muscles. The dynamics of Mb have been studied in detail (4) and the first EINS measurements on a protein were performed on Mb (5). Already the early x-ray studies demonstrated that there is no obvious pathway for dioxygen to enter Mb (6); motions are necessary (7). Information on these motions has, for instance, been obtained from x-ray diffraction experiments, where the intensities of the diffraction spots (Debye-Waller factor) yield the mean square displacements for all nonhydrogen atoms (8–10). In contrast to EINS, these mean-square displacements are not functions of time, but correspond to averages, snapshots of the protein ensemble. Such x-ray data, discussed by Réat and collaborators, demonstrate that the mean-square displacement of the active center in Mb is smaller than that of the protein overall. The results obtained on BR and Mb thus are similar.

X-ray light sources, detectors, and computers have improved dramatically since the early Mb data were taken. As a result, the resolution has improved and the Debye-Waller factors are determined more reliably. Fig. 1 presents the structure of sperm-whale Mb, with O_2 bound at the iron atom in the heme center. The colors denote the $<x^2>$ ranges, where $<x^2> = <u^2>/3$. The figure shows that $<x^2>$ generally is smaller at the center than toward the outside of the protein, but it also indicates that there are directions that appear to be less rigid than others, thus suggesting where entrance and exit channels could exist (7). The data and the figure refine the notion introduced earlier that the center of Mb can be called an aperiodic solid, whereas the outside is semiliquid (8). The average backbone values of the mean-square displacements for MbO_2 and deoxyMb at 80 K are shown in Fig. 2 versus residue number. The arrows in Fig. 2 indicate the five conserved residues (Leu-29, Phe-43, His-64, Val-68, and Ile-107) that surround the bound oxygen molecule, and His-93, which is bound to the iron atom on the proximal side of the heme group. These residues have small values of $<x^2>$, allowing specific control at the active site. Fig. 2 also shows that most residues on the proximal side of the heme group or far from the heme center have large mean-square displacements, just as was found for BR. The two figures demonstrate how the improvements in technique have led to a deeper insight into where the protein is rigid and where it is flexible.

The fact that BR and Mb, two proteins with very different structures and functions, show similarities in their dynamic behavior leads back to the question asked at the beginning,

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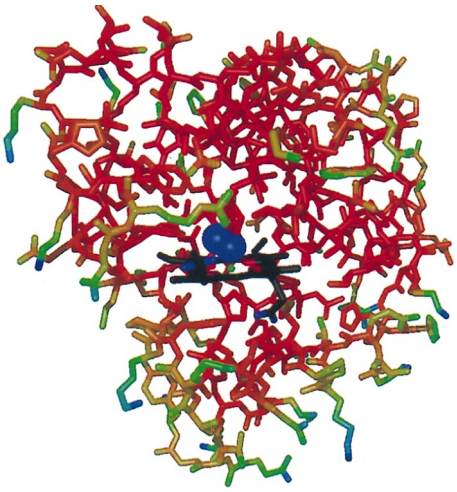

FIG. 1. Structure of myoglobin, with regions of lowest mean square displacement shown in red. Regions of successively higher mean square displacement are shown in yellow, green, and blue. The blue spheres near the center represent the oxygen molecule, which is bound to the heme iron atom.

namely do general concepts describe biomolecules? The results of the paper by Réat and coworkers, combined with results from many earlier studies imply that such concepts do exist. The notion of a rugged energy landscape (11, 12) permits a unified description of many phenomena observed in biomolecules: A biomolecule can assume a very large number of nearly isoenergetic conformations that are close to, but differ in detail from, the average conformation. The energy landscape describes the energy of all possible protein structures for a given primary sequence in a space of $3N$ dimensions, where N is the number of atoms. Each individual conformation is represented by a valley in this energy hypersurface, called a conformational substate (CS). Well below the glass temperature T_g, each protein remains "frozen" in a particular CS. Above T_g, atoms can jump from CS to CS, and the protein experiences motions. Evidence for the existence of the energy landscape and the CS comes from a large number of experiments (4, 12). Fig. 2 provides a striking example: Mb proteins in different CS have slightly different structures and the overall structure determined by x-ray diffraction therefore has values of the mean-square displacements that are far larger than expected if the atoms were only vibrating. At temperatures below T_g, x-ray diffraction shows the frozen distribution,

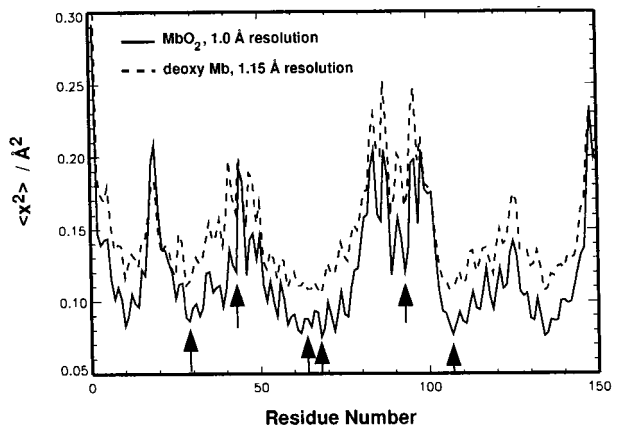

FIG. 2. Mean square displacement as a function of amino acid residue at 80 K of sperm-whale Mb with oxygen bound, and the deoxy form. Arrows indicate the five conserved distal residues that line the heme pocket and His-93, which is covalently bound to the heme iron atom on the proximal side.

whereas EINS gives no information about the existence of CS. Above T_g, proteins can access CS that are higher in energy and are not occupied below T_g. X-ray diffraction takes snapshots of the expanded distribution, whereas EINS follows the jumping nuclei from CS to CS. Both therefore see a broader distribution above T_g. The concept of an energy landscape is not only useful in the discussion of the dynamics of the folded protein, it also enters the folding problem (13).

The preceding paragraph oversimplifies the description of the energy landscape. It is actually organized in a hierarchy of a number of tiers (14). Different tiers are characterized by different heights of the average enthalpy barriers separating the individual CS. Each CS in a given tier contains a number of CS in the next lower tier, and so on. The functional importance of the hierarchical organization is not yet known. The existence of a hierarchy implies that each tier has a different T_g (15). This phenomenon is seen in figure 4 of the paper by Réat *et al.* (2): the global T_g is about 150 K, whereas T_g for the region near the active center is about 220 K.

In a normal glass, T_g is essentially independent of the medium that surrounds the glass. In a protein, however, T_g depends on the surrounding, as observed in Mb (16–19) and in BR (20). If, for instance, Mb is embedded in a glass, the interconversion of the CS in some tiers is suppressed (18, 21). The transitions in some of the tiers are "slaved glass transitions;" their T_g is closely linked to that of the solvent (22). This linkage provides a mechanism for the control of protein reactions by changes in its surrounding.

The concepts of a rugged energy landscape, conformational substates, and slaved glass transition are recognizable in the proteins studied in detail over broad temperature ranges and with many tools. Are they, echoing Heisenberg, still unclear and undeveloped or are they already clear and well defined? More work of the type described by Réat and coworkers (2) on more proteins is needed before a final answer will be available. In particular, much work will be needed to establish a clear connection between structure, dynamics, and function. Figs. 1 and 2 suggest that a study of the mean-square displacements in mutants could lead to a better understanding of how to design proteins with desired properties, for instance high-temperature enzymes (23).

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