Jaws, Lids & Swinging Gates: Proteins on the Move

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While many areas of biology interest me, molecular biology is my special favorite. This is such a fast-moving area of biology; there is always something new to learn. In graduate school I did research on enzymes, so they've always remained particularly important to me. Twenty-five years ago, the structures of only a few proteins had been worked out, and there was little information on the active sites of enzymes. Today, there is a great deal known about the chemistry of enzyme active-sites, and research journals such as *Nature* and *Science* are filled with computer-generated images of protein structures. What is not apparent from these images is the dynamic nature of protein structure. This is a terrible shame, because proteins are very lively molecules, and their movements are very much related to their functions.

To imagine proteins as the static blobs pictured in research articles is to have a very deficient view of these molecules, one that lacks the excitement stemming from their dynamism. Proteins are not just sitting there, they are "kicking and screaming" as G. Weber notes (Frauenfelder et al. 1991). They are vibrating, and in some cases also undergoing conformational changes involving the whole molecule; in others, only part of the molecule is in motion. But no matter what form their motion takes, they are never sitting still. In this column I'd like to explore protein dynamics, because it's an area that deserves attention as more and more is discovered about the gyrations of these molecules.

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Still Pictures

The first thing to discuss is why the motions of proteins haven't been more extensively treated. The main problem is that proteins move so fast it's difficult to catch them in the act. This problem is similar to one that plagued the early days of photography. Because of the long exposure times, any movement showed up as a blur. The only solution to the problem was to have the subject remain perfectly still. This same approach has been taken with the imaging of proteins, and the situation in which a molecule is most static is when it is part of a crystal. But getting a protein to sit still is only part of the problem. Proteins are too small to be photographed with light waves, so X-rays with their short wavelengths are needed. The "picture" produced when X-rays bombard a protein crystal is not an image in which the protein structure is recognizable; it's a series of dots that must be analyzed mathematically to produce information on the position of atoms within the protein. It took Max Perutz and his colleagues more than 25 years to work out the structure of hemoglobin. The project did not go well until he added a heavy atom-like mercury to the molecule. This caused a shift in the position of the atoms. Calculations involving the displacement of the atoms in this form of the molecule relative to the form with iron made it much easier to determine atomic positions.

After the structure of deoxygenated hemoglobin was worked out, the structure of the oxygenated molecule was also determined. Not surprisingly, the two structures were found to be quite different from each other. However, these "still" pictures of hemoglobin, before and after oxygenation, told little about the movement involved in the conformational change. It took years of work to discover that this change involves not just an alteration of the shapes of the molecule's four subunits [two α and two β chains] and of the bonds between the units, but a global shift of the interfaces between the two $\alpha\beta$ dimers (Ackers et al. 1992). Such an overall change in shape correlated with change in activity is called allosteric.

The understanding of the movement even in this one protein is still incomplete. Recent work indicates that as four oxygen molecules bind to hemoglobin, so do 60 additional water molecules (Rand 1992). This suggests that hydration/dehydration reactions contribute much more to the energetics of conformational change than had been assumed. The water molecules adhering to a protein are usually not pictured in the protein's structural "portrait," but they obviously play a role in maintaining and changing the molecule's form. I found this idea surprising. While I knew that most proteins exist in an aqueous environment and that water molecules are attracted to the hydrophilic amino acid side chains found on outer surfaces of proteins, I had never considered the water as more than a rather inert coating—much like the water that clings to the skin after a shower. But it makes sense that water should be an active

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player in protein dynamics because of the significant energy involved in hydrogen bonds between the water molecules and the surface amino acids.

Myoglobin Motion

Despite all that's known about hemoglobin and its conformational changes, there is still a great deal to be learned. It's difficult to study a molecule as complex as hemoglobin with its four polypeptide chains and its four heme groups, so some researchers are focusing their attention on myoglobin, a simpler relative of hemoglobin that also binds oxygen but has only one polypeptide chain and one heme group. They have taken advantage of the fact that the bond between carbon monoxide and myoglobin's heme group can be broken by light. If this reaction takes place while the myoglobin crystal is bombarded by X-rays, then the conformational change can be caught in the act.

But this only works if the reaction takes place at 40° above absolute zero, a temperature at which a reaction can be slowed by a factor of 10 billion (Petsko 1994). This means that a reaction that normally takes place in less than a microsecond may be stretched over hundreds of seconds. At warmer temperatures, the reaction takes place too rapidly to be detected using X-ray crystallography (Taubes 1994). But cooling the crystals isn't enough to produce X-ray images in fractions of a second. To understand why, recall that in the early days of photography, subjects had to remain still because the photographic plate had to be exposed for a long time in order for an image to be made. Later, exposure times decreased as photographic emulsions became more sensitive to light. With X-ray-produced images of proteins, progress has been made not by making detection more sensitive, but by increasing the amount of radiation bombarding the crystal. Synchrotrons are X-ray sources that produce the extremely intense beams needed to create images in very short exposure times. What this technique, when carried out at very cold temperatures, has revealed is the movement of the heme group as a carbon monoxide molecule is released. For the first time, molecular movements that had been assumed to take place can actually be seen. But so far, the technique has only been used on myoglobin, a molecule that is made up of a single polypeptide chain and one which has been very extensively studied so that the subtle movements occurring in the X-ray "motion pictures" can be appreciated.

Jaws & Hinges

Though it will probably be quite some time before large, more complex proteins will be appearing in movies or even in computer-generated images of movement, more traditional techniques have already revealed a good deal about protein movement. For example, ribonuclease inhibitor, the protein inactivating the enzyme that breaks down RNA, is a horseshoe-shaped molecule in which the two ends of the horseshoe move substantially relative to each other (Kobe & Deisenhofer 1993). This elasticity is important in binding the molecule's substrate, ribonuclease, which fits between the horseshoe's two ends. Another example of protein movement is the iron-binding protein lactoferrin which has two jaw-like "iron traps" that close around the metal atoms. There is probably little difference in energy between the open and closed states, so that the "trap" will readily shut around an iron atom. In the absence of iron, one of the two traps is open, while the other is closed. Researchers speculate that the reason the crystalline form of the iron-less molecule has one open and one closed trap is that the molecules can most readily pack together in this configuration. David Concar (1990) likens the "jaws" of lactoferrin to the sensitive leaves of a Venus flytrap. Such analogies are common in discussions of protein movement. The idea that molecules move and that this movement is functional is difficult for many of us to visualize, particularly when we are bombarded by static images of proteins. The easiest way for those who are exploring the dynamics of proteins to make their findings understandable is to compare protein movements to the movements of functional devices familiar to us. And since we all have highly functional jaws and we are all familiar with how jaws work, it makes sense to describe the movement of lactoferrin in just these terms.

This movement can also be described in terms of another common device: the hinge. My husband and I have a beautiful screen with four panels that was one of my aunt's most treasured possessions. The panels are connected to each other with hinges that allow easy movement of each panel. The hinges are very small relative to the screen as a whole, but they are essential to its function as an extendable partition. The two portions of each of lactoferrin's jaws are also held together by a hinge; in this case, a short, flexible amino acid sequence. So the rather large displacement of the jaw is accomplished by movement of a very small segment of the protein, much as the small hinges allow movement of the large panels. Any relatively discrete or independent segments of a protein are called domains. Thus, the large sections of the proteins that are moved by the hinges are domains; they are the regions of the protein involved in the movement but not responsible for generating it.

Movement around hinges is probably the most common form of motion found in proteins. Porphobilinogen deaminase (PBGD) is a key enzyme in the production of the tetrapyrroles found in hemoglobin and chlorophyll (Louie et al. 1992). It has a single catalytic site that has to accommodate an enlarging molecule as pyrroles are added. The two domains of the protein can hinge open, allowing for the increasing size of the substrate. The portion of the polypeptide chain that passes directly in front of the active site cleft appears to be highly mobile, and this may be a lid which transiently blocks solvent from the active site during synthesis.

Lids

A "lid" is one type of structure attached to a hinge. While with my screen, the two panels attached by hinges are of equal size and equally capable of movement, hinges are also useful in situations, such as the door of a cabinet or the lid of a chest, where only one of the attached elements can move. Such seems to be the case with the polypeptide chain covering PBGD's active site. "Lids" are common hinged elements in proteins. As with PBGD, the lids often cover or block the active sites of enzymes. Pancreatic lipase has a lid over its active site when it is in the inactive form (Huang & Coffin 1992). This enzyme is activated by contact with the lipid/water interface. The lid in this protein is a complicated device that pops open when the protein makes contact with a lipid surface (Riddihough 1993). Bourne (1993) compares the movable helical domain or region of the protein transducin to a "teapot lid." Transducin converts the light signal that activates rhodopsin, the photon-receptor in the eye, into a chemical signal, and in the process converts GTP to GDP. The region of transducin that

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catalyzes this reaction is where the teapot lid is found, and this lid opens to accept GTP when the protein is activated by rhodopsin.

Still another variation on the hinge theme is the "swinging gate" of the colicin Ia ion channel in cell membranes (Simon 1994). Researchers have found that not only is there a large conformational change when this channel opens, but a large region of the protein can reversibly flip across the membrane, very much like a swinging gate. While it has been assumed that there were conformational changes in ion channels as they opened and closed, such a large-scale and radical change hadn't been predicted. One reason for this may simply be that even those doing research on proteins have difficulty visualizing the extent of protein dynamism. With static images of protein structure firmly implanted in their minds, it's not surprising that the kinds of movements they visualize for proteins are rather minor. In the case of an ion channel, a slight shift in the subunits might open a pore in the center of a barrel-like structure. This view is quite different from an actively "swinging gate," a sequence of amino acids that is sometimes in contact with the inside of the cell and sometimes with the exterior. In the ion channel for potassium, a ball-and-chain model can explain its inactivation; with the gate or ball attached to a flexible amino acid sequence, the chain, which can move the ball onto or away from the pore and thus control access of ions to the pore.

In looking at the allosteric changes in hemoglobin and at hinge-like movement in enzymes, we've covered examples of the two major types of large-scale movement found in proteins: global movement involving the entire molecule as in hemoglobin and more local movement as with a swinging gate. Sometimes a protein can undergo both types of motion, as when the first oxygen molecule bonds to a subunit of a deoxygenated hemoglobin molecule. There is local change at the binding site and global modification as the entire molecule changes shape so that each subunit can more readily bind oxygen molecules.

Other Motions

Besides these two types of movement that are specific to particular proteins and determined by the protein's amino acid sequence and conformation, there is also more random movement called thermal motion due to the kinetic energy of the atoms at a particular temperature. Not surprisingly, atoms near the surface of a protein generally display greater thermal motion than those in the interior of the molecule (McCammon & Harvey 1987). In some enzymes, such as lysozyme, the substrate binding site is located in a cleft which fluctuates randomly in width as part of the normal thermal motion of the molecule. Entry of the substrate can only take place when the binding site has a certain width.

What all this means is that the movements of most proteins are rather complex and are made up of several components, several different kinds of motion. Individual atoms have vibrational motions that are superimposed on the slower and larger amplitude motions of groups of atoms. All these vibrations occur more rapidly than do the more concerted movements related to the binding of substrate or of regulatory molecules. All this movement indicates that proteins are more like Calder mobiles than Rodin statues. While we have been concentrating on movement in enzymes [Jacques Monod calls hemoglobin an "honorary" enzyme (Judson 1980)], other kinds of proteins are also in motion. Large-scale intramolecular motions are important in the aggregation of antibodies, in the formation of the protein coat of viruses, and in muscle contraction.

The actin-myosin interaction of muscle contraction is an example of a protein machine. Thomas Pollard (1992) writes that there are 13 different categories of these machines, which he defines as "driving units that produce macromolecular movement in living organisms" (p. 17). While the movements of protein molecules themselves seem rather unusual to many of us, the movements in the cell produced by proteins are more familiar, such as actin and myosin producing movement in muscles and within the cytoskeleton as well. Molecular machines can act like engines pulling intracellular cargo along protein polymer tracks, or like processors on an assembly line to copy the base sequence of DNA into RNA, or like selective valves or pumps to transfer macromolecules across lipid membranes.

Protein movement is also important in the process of protein folding—of getting a polypeptide chain or chains into the proper conformation. Though some small proteins may fold spontaneously as they are synthesized, more and more evidence is accumulating that protein folding is directed by other proteins including ones called chaperonins that, as their name implies, guide the folding process and prevent unwanted interactions between the protein being folded and others in the vicinity (Gething & Sambrook 1992). GroEL is a chaperonin that interacts with a cofactor called GroES. The binding of GroES to GroEL causes massive changes in GroEL so that a space opens in the middle of the GroEL molecule where the protein to be folded can fit in. Further conformational changes in GroEL take place during the folding and release process (Hartl 1994). While chaperonins are thought to facilitate the folding of polypeptides into the "correct" shape, it must be kept in mind that the correct shape is really a collection of shapes, since all proteins undergo at least thermal motion.

Moving Metaphors

As I noted earlier, in order for researchers to appreciate just how dynamic proteins are and in order for them to communicate this dynamism to others, they have had to rely on metaphors, on comparing the dynamism of proteins with moving objects familiar to us. Swinging gates and teapot lids and hinges are all commonplace and readily visible objects. These objects are mentioned in research articles because comparing protein movement to the motion of familiar objects is the easiest way for researchers to get the idea of protein dynamism across to readers who find it difficult enough to visualize proteins as three-dimensional objects, when most of their experience with these molecules involves looking at two-dimensional images on the printed page.

Metaphors are used by scientists not just to communicate their ideas to others, but also to clarify their own thinking. Picturing the two domains of lactoferrin closing around an iron atom as "jaws" makes the movement of this protein seem much more real. We can "see" what is going on more clearly when we have an image to hold on to and mentally manipulate. And if biologists need metaphors to make protein movement more understandable, how much more must these figures of speech be needed by students? Protein motion is important for students to understand if they are to develop an accurate picture of how macromolecules work, and thus of how cells function. Of course, we use metaphors

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all the time in trying to get concepts across to our students. Many linguists argue that most of our speech is metaphorical; that we can only get new ideas across by comparing them to what is already familiar. But in our discussions of the "kicking and screaming" of proteins, I think we should be more explicit in our use of comparison; we should tell our students why we speak of jaws and hinges when discussing proteins. By examining them more fully, we will make these metaphors more meaningful and also give students an insight into how science is communicated and how scientists formulate new ideas.

Another example of this is the set of metaphors I mentioned earlier, namely those involving protein "machines." When I think of the word machine, what comes to mind is a rather low-tech image of a lathe shaping an object or of a die press spewing out identical forms. In other words, I see a machine with moving parts producing a product or movement. And that's the way protein chemists visualize protein machines. Protein machines can "pull cargo" or work on the DNA "assembly line" or act as an ion "pump." In any case, they get some useful work done like the machines we have around the house, such as the drill and the electric mixer, do. As with the metaphors mentioned above, these terms are used by researchers to help them understand what's going on as well as to help them explain their work to others. While a criticism can be made of mechanistic metaphors in biology because they de-emphasize the organic aspect of living things, such metaphors are useful because machines are such familiar parts of our lives. Of course, we should point out the dangers of mechanistic metaphors to our students, but I think it would be difficult to avoid using them and also counterproductive because we would be denying ourselves a rich stock of metaphors from which to draw images in our teaching.

It is trite to say that life involves ceaseless change. We are all developing and aging at the same time. Ecosystems are never static; cellular chemistry must constantly adapt to the needs of the organism. Now we must add the movement of proteins to our picture of organic dynamism. That proteins are in constant motion should not amaze us. It is not surprising that the dynamism of life extends to the molecular level. What is more surprising is that this idea remains novel, that the popping lids and swinging gates of proteins are not more familiar to us. That they are not says something about the limitations of the images found in textbooks and about the way those images influence the way we teach. We have to attempt to overcome these limitations by stressing the dynamic nature of proteins in our discussions of these molecules. We may be helped in this task by the computer. David Goodsell (1992) argues that the greatest virtue of molecular computer graphics lies in its potential to improve scientific communication, and sees computers as important in imaging movement, something which is difficult to communicate in standard texts. Using imaging software, the extremely rapid internal motions of an antibody can be depicted as a multiple image in which the moving parts appear as closely packed parallel lines, or as one in a series of "snapshots" of moments during the movement. So as more is learned about protein movement and better ways are found to portray that movement, we have a great deal to look forward to, and so do our students.

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