tween ferromagnetic MnO_2 bilayers through (La,Sr) blocking layers. There is also a significant out-of-plane magnetoresistance of 200% in low fields (<1 kOe).

These layered manganites are in many ways much more complicated than metallic multilayers, and much work needs to be done to develop a complete physical picture of their behavior. A full understanding will involve consideration of interplay between complicated electronic, magnetic, and structural features. As an example, neutron scattering studies of $La_{1.2}Sr_{1.8}Mn_2O_7$ by the Argonne group have revealed structural changes associated with the onset of ferromagnetism (*8*).

Certainly there is a long way to go before oxide manganites are technologically competitive with metallic magnetic multilayers. The synthesis of single crystals of a compound with strong anisotropy in the transport properties, with the out-of-plane transport being determined by spin-dependent tunneling between magnetic metallic layers, is an important advance. Technological application will require the compound in thin-film form and certainly an increase of the temperature at which low-field magnetoresistance is found. Another aspect of this work that should be recognized is the effective use of the floating-zone method for the growth of high-quality single crystals of complex compounds. This approach to crystal growth will impact many areas of materials research.

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PROTEIN STRUCTURE

Nanosecond Crystallographic Snapshots of Protein Structural Changes

William A. Eaton, Eric R. Henry, James Hofrichter

Šrajer *et al.* report in this issue on a dramatic development in protein science (*1*)—they have virtually "watched" a protein function. This group, led by K. Moffat and M. Wulff, has developed time-resolved x-ray crystallography to obtain nanosecond-long snapshots of the complete three-dimensional structure of a protein as it changes in response to ligand dissociation and rebinding. The new technology, made possible by the very intense x-ray pulses at the European Synchrotron Radiation Facility, opens the way to understanding the kinetics and dynamics of protein function in atomic detail. With this x-ray source it will be possible to determine the structure of intermediates with lifetimes as short as a few hundred picoseconds.

To take advantage of this enormous improvement in time-resolution, Šrajer *et al.* examined the photodissociation of the carbon monoxide (CO) complex of myoglobin, a protein reaction that can be rapidly triggered. Light dissociates CO from the heme of myoglobin in less than a picosecond (*2*), and the subsequent ligand rebinding and structural changes have been studied extensively by various time-resolved spectroscopies. The study of Šrajer *et al.* not only is a technological tour de force, but also addresses fundamental issues in understanding how proteins work.

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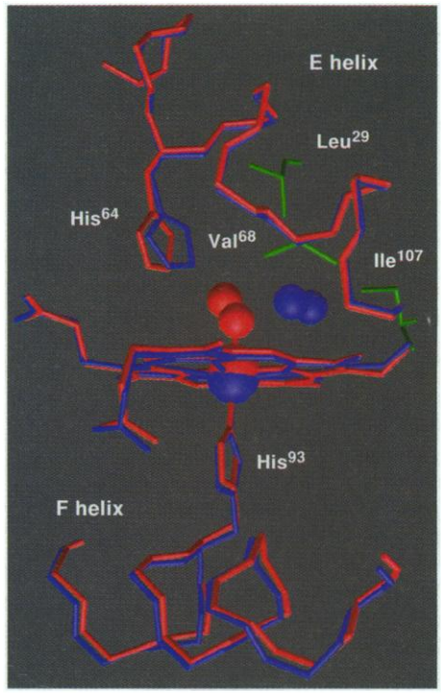
All proteins undergo some structural changes while carrying out their biological function (*3*). Enzymes alter their structure as part of the catalytic mechanism. Antibodies change conformation upon binding antigen. Ligand binding to cell surface receptors induces conformational changes that transmit the signal across the membrane. Viruses use conformational changes of their coat proteins to enter cells. Measuring and understanding the kinetics of conformational changes in proteins is, however, a largely unexplored area. Hemoglobin and myoglobin have been the paradigms for such studies. To facilitate oxygen unloading in the tissues, hemoglobin undergoes conformational changes that are coupled to the rearrangement of its four myoglobin-like subunits. The structure of myoglobin also changes upon oxygen dissociation. How fast does the protein conformation respond to oxygen dissociation? What is the sequence of structural changes? These and other questions have motivated a large number of time-resolved spectroscopic investigations since the initial observations of structural changes in hemoglobin and myoglobin on the microsecond (*4*), nanosecond (*5*, *6*), and picosecond (*7*) time scales.

The basic idea of the time-resolved x-ray crystallographic and spectroscopic experiments is very similar. A laser pulse breaks the covalent bond between the heme iron and CO. (CO is used instead of oxygen because of the higher quantum yield of photodissociation.) In the spectroscopic experiment, the probe is a second laser pulse that interrogates the photolyzed portion of a myoglobin solution at a series of time delays after the photolysis pulse. Theoretical methods and empirical correlations are then used to interpret the changes in the electronic and vibrational spectra in terms of structural changes. In many instances, however, interpretation is



Partial structures of myoglobin (blue) and the myoglobin–carbon monoxide complex (MbCO) (red) (*8*). Blue spheres, the iron and dissociated CO of photolyzed MbCO at 4 ns; red spheres, the iron and CO of MbCO. The two static x-ray structures have been superimposed by minimizing the differences in positions of backbone atoms in the A, B, D, G, and H helices. Residues in green (Leu^{29}, Val^{68}, Ile^{107}) contact the dissociated CO. The displacement of the F helix is only ~0.05 nm, but its motion is detected in the electron density difference maps.

difficult. In the crystallographic experiment of Šrajer *et al.*, the probe is a very intense x-ray pulse of 150-ps duration (or a 940-ns bunch of such pulses), which is used to collect diffraction patterns of a myoglobin crystal at various time delays after photolysis with an 8-ns laser pulse. (The time resolution of the experiment is therefore either 8 or 940 ns). The difference in the electron density between the photolyzed and unphotolyzed crystal is then computed from these diffraction patterns. The three-dimensional maps of the electron density differences directly show the changes in atomic positions that occur at each time delay after ligand dissociation.

By static x-ray crystallography, it has been shown that dissociation of CO displaces the iron from the heme plane, domes the porphyrin ring, and allows the distal histidine (His^{64}) to relax into the space vacated by the CO (*8*) (see figure). These changes are already present in the first structure at 4 ns, as expected from time-resolved optical spectroscopy (*7*) and molecular dynamics simulations of photodissociation (*9*). The dissociated CO appears in a pocket in the protein a few angstroms from the heme iron at 4 ns (see figure) and disappears by 1 μs. This site, however, appears to be only partially occupied by CO at 4 ns, suggesting multiple paths from the heme to the solvent (*10*).

The difference electron density maps and optical spectra of the same crystal show much more geminate rebinding than occurs in solution (*11*). "Geminate" signifies rebinding of the dissociated CO to the heme before it escapes into the solvent, and it occurs in the crystal on both a subnanosecond and nanosecond time scale. The clue to understanding the increased geminate rebinding in the crystal is the structural response of the protein to ligand photodissociation. Static x-ray crystallography had shown that among the conformational differences between liganded and unliganded myoglobin there is a very small displacement of the F helix (*8*) (see figure). The F helix contains the proximal histidine (His^{93}), and its position is believed to be critical for protein control of the geminate rebinding rate (see figure). To reach the transition state in forming the iron-CO bond, the histidine and iron must move toward the heme plane, requiring motion of the F helix and presumably an overall adjustment in protein conformation in the direction of the liganded conformation (*6*, *12*). This explains why geminate rebinding is several thousand times faster if the protein is already in the liganded conformation, as it is immediately after photodissociation and before relaxation to the structure of unliganded myoglobin (*13*).

The most exciting result from the x-ray work is the direct visualization of the conformational relaxation. The difference electron density map suggests that the F helix has already moved by 4 ns, in agreement with the interpretation of solution kinetics that this conformational relaxation is mostly a subnanosecond process (*14*). However, in contrast to the solution studies, there is also an indication in the difference electron density maps that the F helix continues to move until at least 1 μs. The slowing of the conformational relaxation in the crystal would immediately explain the increased geminate rebinding. If this motion is confirmed by more detailed analysis of the x-ray data, it would provide the most direct evidence so far for the mechanism of protein conformational control of geminate rebinding (*13*). Šrajer *et al.* attribute the slowing of the conformational relaxation in the crystal to the constraints of the lattice contacts, a phenomenon previously observed in other protein crystals, most notably for the quaternary conformational change of hemoglobin (*15*).

Direct observation of the complete three-dimensional structure of a protein during conformational changes raises interesting issues on exactly how they occur. Although there are as yet no x-ray data in the interval between 4 ns and 1 μs, the present results suggest that the conformational relaxation is complex. It is not a process with a single exponential time course, but is extended in time. Nonexponential spectral kinetics are also observed in the solution experiments (*14*). This behavior could result from a sequence of a few discrete structural events or from a single continuous process similar to diffusion (*16*). With innovative refinement procedures the x-ray results may eventually distinguish between these two mechanisms. Conformational kinetics in response to geminate ligand rebinding have also been observed in solution by infrared spectroscopy (*17*). Describing these changes in the crystal is yet another challenge for time-resolved x-ray crystallography.

What can we expect in the future from this powerful new technique? Once the problem of timing the x-ray pulse with a picosecond laser pulse is solved, we can look forward to 150-ps snapshots. This will permit direct comparison of the time dependence of atomic positions determined by x-ray crystallography and molecular dynamics calculations. Time-resolved crystallography should have a major impact on time-resolved spectroscopic studies, not only by aiding in structural interpretations, but also by pinpointing regions of the protein for detailed kinetic investigations. On the other hand, in cases where protein conformational changes are small and complex, as they are in myoglobin, time-resolved spectroscopy in crystal and solution will be necessary for completely interpreting the x-ray results (*13*, *15*, *18*, *19*). Finally, it is clear that ultrafast time-resolved crystallography can be applied to many more photoactive systems than the well-known heme protein complexes, bacteriorhodopsin, and photosynthetic reaction centers. The rate-limiting step for extending this technology to other proteins will be the development of rapid triggers. One could, for example, use a laser pulse to photochemically trigger catalytic events in the active site of an enzyme or the release of ligands from receptors. For reactions with large enthalpy changes, triggering could also be carried out by jumping the temperature with a laser pulse. There are numerous possibilities for discovery with time-resolved crystallography, and we expect this new technology to produce major advances in our understanding of proteins.

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