Babakotia also is giving anthropologists a glimpse of a past where lemurs came in all sorts of shapes and sizes and exhibited a far greater range of bizarre behavior than they do today. "We still don't know the whole story of what took place," Simons says. But the wealth of lemur fossils in Madagascar should help fill in the gaps.

New Fossils Found In Ethiopia

For most of the 1980s, anthropologists were banned from doing field work in Ethiopia because the government was rewriting laws on antiquities, including fossils. But in 1990 foreigners were allowed to return, and now they're finding fossils that are sure to help flesh out a crucial period of prehuman evolution, according to anthropologist Berhane Asfaw, director of the National Museums of Ethiopia.

Asfaw announced in Las Vegas that researchers in the Middle Awash area, a lowland zone north of Addis Ababa that is part of the Awash River drainage, had found fossils of at least three species of hominids (the family containing humans and their extinct ancestors) that lived between 400,000 and 4 million years ago. Although the discoveries are so recent that the researchers haven't yet fully analyzed them, hominid fossils from these time periods are so rare that the specimens are sure to help produce a better picture of what early hominids were like—and how they evolved and were related to one another.

The most dazzling of the finds is a hominid mandible discovered by a team led by Asfaw and University of California anthropologists Desmond Clark and Tim White at a site known as Maka in the Middle Awash. Asfaw said this jawbone belonged to a hominid that lived 3 million to 4 million years ago, which would likely make it an example of *Australopithecus afarensis*, the species that includes the partial skeleton known as "Lucy," and is the oldest known ancestor of modern humans. The same researchers also did well at two other Middle Awash sites. At Bodo, they found a humerus from a hominid who lived about 400,000 to 500,000 years ago, probably an example of archaic *Homo sapiens*. And at Gameda, they found a mandible from a hominid who lived 2 million to 3 million years ago, although the dates on that site are preliminary.

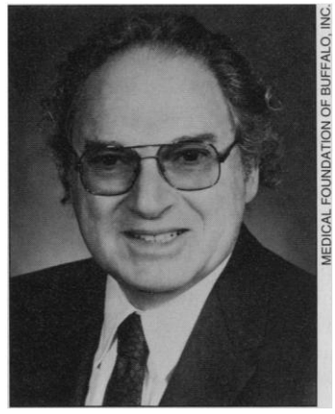
And the discoveries don't stop there. To the north in Hadar, anthropologists Donald Johanson and William Kimbel of the Institute of Human Origins in Berkeley found what Asfaw says are "beautiful hominids"—although Kimbel declined to discuss the find, saying he just returned from the field. All this prompted Asfaw to conclude that "despite other problems in the country, we keep moving: We have discovered more hominids."

–Ann Gibbons

CRYSTALLOGRAPHY

New Methods Make Mid-Sized Molecules Easier to Solve

In large families, the oldest child and the youngest often get most of their parents' attention, while the middle children seemingly get lost in the crowd. Until lately, such has been the fate of the in-between molecules—the ones having between 200 and 500 atoms—when x-ray crystallographers set about determining three-dimensional structures. The researchers have been able to devise techniques for solving the molecular structures of both the larger and smaller molecules, but the mid-sized molecules have proved tough to decode. Now, two research teams, each using an entirely different strategy—one chemical, the other mathematical—have come up with methods for solving mid-sized structures that should make the job far easier and faster than it used to be.



Advancing direct methods. Herbert Hauptman.

And that will come as welcome news to biochemists and drug designers. Many in-betweeners have important physiological roles. They include, for example, small proteins, such as the hormone insulin and the blood-pressure regulator angiotensin, as well as many antibiotics. Having detailed structural information about these molecules should not only give researchers a better understanding of how they work, but might also help in the design of more effective antibiotics or of drugs that either mimic or block a protein's effects. "The starting point of rational drug design," says crystallographer Bart deVoss of Genentech Inc. in south San Francisco, "is getting a protein structure to work with."

What the two groups have had to overcome for the mid-sized molecules is the "phase problem," a bugaboo for all crystallographic analyses, but especially intractable for structures of intermediate size. To determine a structure, a beam of x-rays is directed at a crystal of the material under investigation. The substance will scatter some of the x-rays, forming a diffraction pattern that can be detected on a photographic plate as an array of spots of varying intensities. Exactly how the crystal scatters the x-rays depends on how its atoms are arranged, and the goal is to work back from the diffraction pattern to calculate the structure.

Unfortunately, there's a problem. To calculate the structure, crystallographers use a mathematical relation called a Fourier transform to derive a wave of electron density for each spot in the diffraction pattern. They then use the waves to reconstruct the electron density pattern—and thus the arrangement of atoms—in the crystal itself. But while crystallographers can easily get the amplitude of each wave from the intensities of the diffraction pattern spots, they have a much tougher time getting the phases of the waves, that is, the positions of the wave crests and troughs relative to one another. And without the phase information, the researchers can't solve the crystal structure.

For small compounds, with fewer than 200 atoms, crystallographers get around this phase problem with so-called direct mathematical methods that enable them to cull the phase information from the diffraction data. And for large compounds, such as proteins, they get around it by inserting atoms of a heavy metal, such as mercury or uranium, into specific sites in the protein molecule. The easily recognizable metal diffraction pattern then serves as a sort of landmark for determining the phases of the x-rays diffracted by the protein crystal.

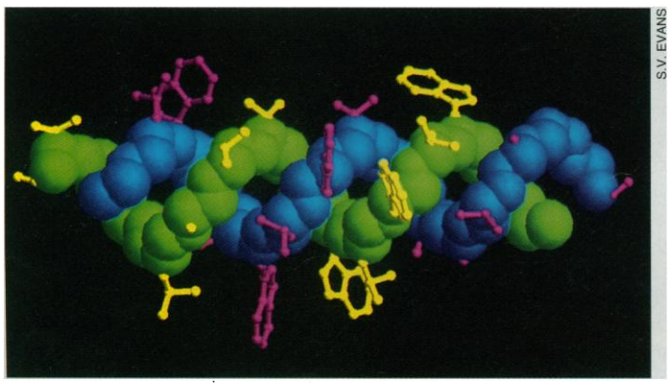
But neither method has worked well with the mid-range compounds. Their structures are so small that it's hard to insert heavy metals into them without causing unacceptable amounts of distortion, but they're nonetheless too complicated to be readily solvable by the standard mathematical approach. Take for example the antibiotic gramicidin A, which is a dimer consisting of two identical 15-amino acid peptides. David Langs of the Medical Foundation of Buffalo Inc. and his colleagues managed to solve the gramicidin A structure with traditional direct methods. But it was a struggle, requiring 10 years. And several other groups grappled unsuccessfully with the problem over the years. That's where the new work, done independently by biophysicist Jeremy Berg and his postdoc Laura Zawadzke of Johns Hopkins University, and by Herbert Hauptman of the Medical Foundation of Buffalo Inc. and his colleagues, should help.

The Johns Hopkins group was the one that took the chemical approach to solving the structure of mid-sized proteins. Proteins are, of course, made by hooking together

amino acids. And while each amino acid can come in two stereoisomeric forms, designated "D" and "L," that are mirror images of one another, all the amino acids in natural proteins are of the L type. Berg and Zawadzke reasoned that if they synthesized two versions of the protein they wanted to analyze, one containing the natural L amino acids and the other with D amino acids, and crystallized them together, the two protein molecules might arrange themselves in crystals as mirror images. And in that event, the x-rays scattered by corresponding atoms in the two molecules might interact with one another in such a way that the x-ray reflections in the final pattern would either be totally in phase or totally out of phase. Phase choice would thus be a simple matter of black or white, Berg says, whereas with ordinary methods the phases can be any shade of gray.

To see if their approach works, the Johns Hopkins workers decided to test it on a protein of known structure. They chose rubredoxin, which contains 45 amino acids and whose structure had previously been determined by Lyle Jensen and his colleagues at the University of Washington in Seattle, who used standard crystallographic methods. So far the results with the new method look quite promising, Berg says.

After synthesizing the D and L versions of rubredoxin in mid-1991, Berg and Zawadzke began trying to make crystals containing the two stereoisomers last October. Their initial efforts were not successful; the crystals were too small for practical study. By early this year, however, they managed to grow crystals large enough to produce a diffraction pattern that's allowing them to solve the structure to a resolution of better than 2 Å, good enough to see the positions of all the atoms. They expect to finish computing the structure within the next month or so, Berg says. For comparison, it took the Jensen team about a year after they had crystals to determine the rubredoxin structure, and to a lesser resolution of 3 Å. In addition to speeding up structural determinations, Berg also expects that the new method should make them more accurate by eliminating the uncertainty in phase determinations, which is the major source of error in crystallographic studies.



S.V. EVANS

Coming in phase. New methods can solve the phase problem for mid-sized molecules such as gramicidin A.

Still, the method has its limitations, as even Berg concedes. It can be used only with proteins containing fewer than 100 amino acids, since larger proteins can't be chemically synthesized. And Berg points out that more than a little good luck is needed to get the D- and L-protein isomers to crystallize with the desired arrangement. Nevertheless, says protein chemist Fred Richards of Yale University: "The Berg method is very ingenious and will offer good structural information on those proteins small enough to be chemically synthesized."

"It is encouraging to see the benefits of this work creep up to larger compounds."

—Fred Richards

But Richards also hedges his bets, adding that the Hauptman group's new mathematical approach may prove to be more generally applicable in the long run. That approach is an extension of traditional direct methods, which were originally developed by Hauptman and Jerome Karle during the late 1940s and early 1950s when both researchers were at the Naval Research Laboratory in Washington, D.C.—an achievement that won them the 1985 Nobel Prize in chemistry. "The effect of the direct methods techniques on small molecules has been fantastic," Richards remarks, "and it is encouraging to see the benefits of this work creep up to larger compounds."

The traditional methods are based on Hauptman and Karle's recognition that there are always certain combinations of phases for particular x-ray reflections that are determined by the crystal structure alone. The researchers then went on to devise a probabilistic method for estimating the "structure invariants," as those phase combinations are called, from the diffraction intensities. With a reasonably large number of estimated structure invariants, individual phases could be calculated.

But as the number of atoms in a molecule goes up, so does the number of structure invariants. As a result, Hauptman says, the probabilistic method for estimating the invariants doesn't work well for molecules with more than 200 atoms and conventional direct methods for getting the phases become unreliable. In 1989, however, Hauptman discovered that a different kind of mathematical strategy could be used to get phase information from diffraction data for these larger molecules. He reformulated the problem so that the correct phases are those that are obtained by minimizing a new mathematical function that involves thousands of phases.

Advancing direct methods. The computation of those values is extremely complex, requiring the manipulation of several thousand variables. When Hauptman, Charles Weeks, who's also at the Medical Foundation of Buffalo, and their colleagues tested the method on gramicidin A, the computations took, Hauptman says, "perhaps a couple of months" on the CM5 supercomputer made by Thinking Machines Corp. in Cambridge, Massachusetts, which is currently the world's fastest parallel-processing supercomputer. That requirement for large amounts of supercomputer time could be a major limitation on the new method's application. "As the procedure becomes more computationally complex, it becomes more expensive. There is a question of how useful it may be," cautions crystallographer Wayne Hendrickson of Columbia University.

Still, it's likely that the new method is both faster and better than traditional direct methods for routine structure solving of mid-sized compounds. Whereas it took Langs 10 years to solve the gramicidin A structure, once Hauptman and his colleagues had crystals, they solved it in a matter of months. The next step, he says, is to improve the method and see if it can be applied to larger molecules, including small proteins such as rubredoxin and insulin.

Meanwhile, other groups, including those of George Sheldrick at the University of Göttingen, Germany, and Gerard Bricogne of the University of Cambridge in England, are also working on new mathematical strategies for solving the phase problem. So crystallographers may be at the beginning of a small revolution that will break down the barriers that have stalled their work for decades. And that in turn should help speed up new drug design and materials development, both of which depend heavily on accurate knowledge of molecular structures.

–Anne Simon Moffat

Additional Reading

L.E. Zawadzke and J.M. Berg, "A racemic protein," in press in the *Journal of the American Chemical Society.*

G. DeTitta *et al.*, "Solutions to the phase problem of x-ray crystallography: an update," in the *Proceedings of the Sixth Distributed Memory Conference,* IEEE Computer Society Press, 1991, pp. 587-594.