posed solely of RNA. The recognition of further catalytic versatility in RNA justifies consideration of its involvement in other early biochemistry. Recently, in vitro selection and amplification methods (*7*) have opened the way to the exploration of new RNA functions, without the constraints of biology. Over the coming years we may expect to see even further expansion of the catalytic capacity of RNA.

REFERENCES

1. H. F. Noller, V. Hoffarth, L. Zimniak, *Science*, **256**, 1416 (1992).
2. J. A. Piccirilli, T. S. McConnell, A. J. Zaug, H. F. Noller, T. R. Cech, *ibid.*, p. 1420.
3. H. F. Noller, *Annu. Rev. Biochem.* **60**, 191 (1991).
4. C. R. Woese, *Microbiol. Rev.* **51**, 221 (1987).
5. T. R. Cech, *Science* **236**, 1532 (1987).
6. C. R. Woese, *The Genetic Code: The Molecular Basis for Gene Expression* (Harper and Row, New York, 1967), p. 186.
7. J. Abelson, *Science* **249**, 488 (1990).

On the Other Hand . . .

Gregory A. Petsko

Almost 25 years ago my colleague, Prof. Dagmar Ringe, was asked during her Ph.D. thesis defense to speculate on what would happen if a proteolytic enzyme (one that cuts the peptide strand of a protein) could be made from all D-amino acids instead of the naturally occurring L-enantiomers. She replied that the protein would probably fold properly and have full catalytic activity, but only against peptide substrates of the same chirality. Her examiners did not believe her (she passed anyway), but now, a quarter of a century later, the experiment has been done and that is exactly what happens. Stephen Kent and his associates at the Scripps Research Institute have achieved the total chemical synthesis of an all-D-amino acid enzyme, the acid protease from the human immunodeficiency virus (HIV). As reported in this issue of *Science* (*1*), the protein displays a chiral specificity for both substrates and inhibitors that is the opposite of that shown by the naturally occurring all-L-amino acid enzyme.

"Chiral" comes from the Greek word for "hand," and the term "handedness" is often used interchangeably to describe the same property. An object is chiral if it cannot be superimposed on its mirror image by simple rotations and translations. The quintessential example of two objects of opposite chirality are the left and right human hands (see figure). Two objects of opposite handedness can always interact with the same achiral object: for example, both the left and right hands can hold a baseball in the same way. But their interactions with another chiral object must be different, and in some cases only one of them may be able to interact at all. You cannot use a left-handed baseball glove on your right hand. (Another example: Two right hands can shake with each other, as can two left hands. The left and right hands can also clasp, but one must turn upside down to do so.) Molecules are chiral if they lack a center, plane, or axis of symmetry. If they have any one of these, they will be superimposable on their mirror images.

Chirality is fundamental in biology. The building blocks of proteins, the naturally occurring amino acids, are chiral molecules. And in all proteins from all living organisms studied thus far, all amino acids are of one particular handedness: the so-called L-configuration (the nomenclature of chirality is changing at present, and for amino acids R- and S- are slowly replacing the older D- and L-designations). Some lower organisms use D-amino acids in certain specialized molecules, like cell walls and antibiotics, but proteins are always composed entirely of L-residues. Such chiral purity has advantages that have long been appreciated: as the baseball analogy indicates, a chiral protein has more specific recognition of, and interaction with, chiral substrates. That is why it was reasonable to expect that a proteolytic enzyme will only recognize one enantiomeric peptide substrate: its active site is chiral and the relative position of the peptide bond to be cleaved will be different in substrates of opposite handedness. Invert the hand of the enzyme and you must also invert the hand of the substrate in order to make the same interactions and obtain catalysis. A further reason for chiral proteins is that secondary structural elements such as alpha helices and twisted beta sheets have a handedness of their own, which is predicated on a consistently handed set of amino acid residues. Thus, all L-amino acid polymers make right-handed alpha helices whereas homopolymers of D-amino acid residues form left-handed ones. The reason is steric: there are unacceptable side chain clashes in a right-handed helix containing D-amino acids. Finally, chiral purity is necessary for the protein synthesizing machinery. It is essential to be able to assume that the side chain will always be in the same relative position with respect to the carboxylate and amino portions of every amino acid.

Symmetry's handiwork. The left and right human hands cannot be superimposed by simple rotations and translations; they are mirror images of each other. [Woodcut by Albrecht Dürer (1471–1528), from the Bettmann Archive]

So it is easy to rationalize why we have a chiral world, but it is not so easy to explain how we got the one we have. Why L-amino acids? Was the choice between L- and D- a random one, or does it offer some clues as to the early biochemistry of life? Prebiotic experiments in which, for example, electrical discharges are passed through mixtures designed to mimic the chemical composition of the primordial atmosphere, produce racemic products, that is, equal amounts of both D- and L-amino acids. It has been suggested that the choice of L- reflects an asymmetry in the environment in which the first proteins were formed: mineral deposits, or clays perhaps. Yet most such substances are themselves racemic, so the chance of encountering something that would favor L-amino acids is presumably random. It has also been suggested that the earliest life forms were imported onto Earth from other planets, so that the chiral preference we see is simply a reflection of what existed elsewhere. This panspermia hypothesis, of course, merely transfers the problem of choice of hand—as it does the problem of the origin of life—outside of our purview and so is really no help at all. Another, more productive, rationale is that beta decay is intrinsically asymmetric and will preferentially destroy D-amino acids (*2*). The discrimination, however, is only a few percent and probably not a sufficient explanation for the observed preference. We are left then with the view that the choice was probably random. For that to be possible, it would be helpful to

The author is at the Rosenstiel Basic Medical Sciences Research Center, Departments of Biochemistry and Chemistry, Brandeis University, Waltham, MA 02254.

show that the other choice would have been just as good: that an all-D-amino acid enzyme would fold to a stable mirror image of its natural counterpart and would show absolute, but opposite, chiral specificity toward substrate. That is precisely what Kent and his associates have demonstrated.

All-D-HIV protease is not the only opposite-handed protein to be synthesized. Laura Zawadzke and Jeremy Berg at Johns Hopkins have made all-D-rubredoxin, a small iron-sulfur protein that is involved in microbial electron transport processes (3). The "unnatural" protein binds metal ions (which are achiral like baseballs) with the same affinity as that of the natural protein. (Similarly, D-HIV protease is inhibited by an achiral inhibitor with the same affinity as is L-HIV.) D-Rubredoxin is not a substrate for hydrolysis by the digestive enzyme chymotrypsin, which of course has evolved to recognize only L-peptide substrates. Presumably, it could be digested by the Scripps team's D-HIV protease. Perhaps they ought to get together. (Or perhaps, given the expense and difficulty in making a functional protein by chemical synthesis, they ought to stay away from each other.) But the objective of Zawadzke and Berg was not to produce a molecule of opposite activity—rubredoxin is not an enzyme—but rather to make a racemic mixture because racemic mixtures, which are bad for biology, are very good for x-ray crystallography.

To solve the crystal structure of a molecule one needs to measure the amplitudes of the scattered x-ray waves and also determine their phases, that is, the relative time of arrival of each wave at the detector. It is impossible to measure all of the phases directly, so elaborate methods have been developed to deduce them from the amplitudes (4). For a crystal made up of chirally pure molecules, most phases can have any value from 0 to 360 degrees. But racemic mixtures can crystallize in packing arrangements (called space-groups) such that all phase angles are restricted to one of two possible values. It is much simpler to solve the phase problem in these so-called centrosymmetric space-groups, and the structure produced should be of higher accuracy as well. Of course, proteins could never crystallize in centrosymmetric space groups, that is, until now. Zawadzke and Berg have obtained high-quality crystals of the racemic mixture of their all-D-rubredoxin with its all-L mirror image, which they also synthesized. The crystals indeed have the symmetry of a centrosymmetric space group, and the Hopkins group has been able to solve the structure by using the known three-dimensional structure of the protein as a model. The uses of racemic proteins in crystallography are limited, but they may allow the solution of a set of reference structures with very low phase error, and they may be of considerable help in the solution of the structures of small proteins that prove refractory to other methods of phase determination. In this context, it is encouraging that Kent reports that his group has now been able, using the chemoselective ligation approach (5), to make more than 30 mg of high-purity D-HIV protease and an even larger amount of L-HIV protease. He indicates that targets up to 250 amino acids should be possible to synthesize in reasonable amounts.

There is some hope that racemic protein mixtures might crystallize more readily than their chirally pure components do. Brock, Schweizer, and Dunitz have argued that there may be enthalpic effects favoring racemate crystallization (6), and a wider variety of ordered packing arrangements should be available when centrosymmetric crystal forms are possible. Even a slight improvement in the ease of crystallization would be worth considerable synthetic effort, at least in the view of the average protein crystallographer.

What other uses are there for these "gegen-eins," as one might call them? One could use them to synthesize chiral products of the opposite hand than those normally obtainable. Sugars are particularly attractive targets for that approach. Another use may be as protein pharmaceuticals, for they are not likely to have the same pharmacokinetics as the natural enantiomers. Of course, this utility probably only extends to enzymes that operate on achiral substrates because only these will be able to function within the context of biochemistry that has been selected to be recognized by L-amino acid enzymes. All-D-hormones or cytokines or growth factors, which must interact with chiral receptors, are inactive in vivo for the same reason (7). But even in this limited context, gegen-eins could be useful. They may be much less immunogenic, for example: the immune system must process protein antigens into peptides and present them on the surface of immune cells; it is unlikely that the processing system will be able to degrade D-proteins or that the presentation system, which includes peptide transport machinery, will handle long D-peptides properly even if they could be produced. But complete freedom from the immune response is not likely. The binding repertoire of the immune system is so diverse that some accidental recognition of gegen-eins by preexisting antibodies could happen anyway. However, as the work by Milton, Milton, and Kent illustrates (1), D-proteins should be resistant to attack by most proteases and should have long half-lives in vivo.

These considerations become important with peptide antibiotics because degradation often limits their usefulness and many of their functions do not involve recognition of or by chiral molecules. Channel-forming antibiotics fall into this category, and a number of recent studies have shown that the mirror images of naturally occurring antibiotics are able to form channels in lipid bilayers. Gramicidin A, a pentadecapeptide that dimerizes to form cation-selective channels, has a sequence containing both L- and D-amino acids. Synthesis of the true gegen-ein by Koeppe and associates, with the L-residues being replaced by their D-counterparts and vice versa, produced a molecule that formed channels of the opposite handedness, but indistinguishable in terms of ion selectivity (ions are like baseballs, of course) and conductance, from the natural antibiotic (8). Merrifield and co-workers showed that the D-enantiomers of the natural channel-forming peptides cecropin A, magainin 2 amide, and mellitin were resistant to enzymatic degradation and were potent antibacterial agents (9). All three gegen-eins formed functional channels in vitro, with conductances identical to those produced by the "correct" enantiomers. Merrifield points out that some of these molecules may be effective orally. Finally, James Tam of Rockefeller University has recently succeeded in synthesizing gegen-eins of the defensins, peptide antibiotics containing disulfide bridges that form beta sheets. These D-peptides are also fully active (10).

It seems likely that useful products will come out of this burgeoning interest in molecules as seen through the looking glass, but even if they are few, these studies have a philosophical impact. Science fiction writers have long speculated about parallel universes; we now must consider the possibility that their molecules and ours may be incompatible. And the same considerations may hold for life elsewhere in our own universe; if the choice was indeed random, then the opposite choice may have been made on other planets. In any case, studies of gegen-eins may help us understand what framed our fearful asymmetry.

REFERENCES AND NOTES

1. R. C. deL. Milton, S. C. F. Milton, S. B. H. Kent, *Science* **256**, 1445 (1992).
2. W. A. Bonner, M. A. Van Dort, M. R. Yearian, *Nature* **258**, 419 (1975).
3. L. E. Zawadzke and J. M. Berg, *J. Am. Chem. Soc.*, in press.
4. See the report by A S. Moffat, *Science* **256**, 309 (1992).
5. M. Schnölzer and S. H. B. Kent, *ibid.*, p. 221.
6. C. P. Brock, W. B. Schweizer, J. D. Dunitz, *J. Am. Chem. Soc.* **113**, 9811 (1991).
7. G. Flouret and V. du Vigneaud, *ibid.* **87**, 3775 (1965).
8. R. E. Koeppe, II, *et al.*, *Proteins: Struct. Funct. Genet.* **12**, 49 (1992).
9. D. Wade *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4761 (1990).
10. I am grateful for comments both frivolous and serious to D. Ringe, D. S. Kemp, S. H. B. Kent, J. Tam, and J. M. Berg.