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Physical organic chemistry

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Biography: Frank Westheimer was born (1912) in Baltimore, Md. and received his doctorate in Chemistry, with James B. Conant and E. P. Kohler, from Harvard in 1935. He served on the staff of the University of Chicago from 1936 to 1953, and at Harvard since then. His research concerns the mechanisms of both chemical and enzymatic reactions in solution, the invention of molecular mechanics and of photoaffinity labeling, and the application of pseudorotation to phosphate ester chemistry. In 1964-65 he chaired the National Academy of Sciences' survey of chemistry ("Chemistry: Opportunities and Needs", National Academy of Sciences Press, 1965). He has received a number of awards in chemistry here and abroad, including a National Medal of Science, and has been elected to membership in several honorary societies.

Physical organic chemistry was originally devoted to an understanding of the structures of compounds and the mechanisms of reactions in organic chemistry (1). It was marvelously successful, and not merely accompanied but fundamentally caused a revolution in synthesis. Today, the methods and ideas of physical organic chemistry can be applied more broadly, and in particular to enzymology, and other areas of bio-organic chemistry.

Prior to the elucidation of reaction mechanisms, successful synthesis depended on a vast knowledge of the chemical literature. Various past syntheses, mostly developed by intensive empirical experimentation, were reported, and anyone planning new work could and did draw extensively on the record of the past. So successful was this methodology that, fifty years ago, most practitioners refused to acknowledge the importance of the then fledgling science of physical organic chemistry, and paid little or no attention to mechanisms. But "nothing fails like success" (Note a); those trapped in memory without mechanism could not compete against those who, led by Robert Robinson and R. B. Woodward, based their syntheses, and in particular, based their stereochemical predictions on an understanding of reaction mechanisms.

Today virtually all successful syntheses are based on reaction mechanisms, and mechanisms are taught (usually *ex cathedra*) in elementary courses in organic chemistry. Is there much more to discover by the methods of physical organic chemistry? Or is the field "mature"?

The most important discoveries, almost by definition, are unexpected. But reasonable scientists nevertheless try to pick the areas in which to look for pearls. One of the areas that is active today, and which probably will remain active for quite a while is mechanistic enzymology. Fifty years ago no one knew how any enzyme catalyzed any reaction; today, we can explain the mechanisms of some enzymic processes with as much or more precision as we can specify mechanism for any reaction in solution (2).

The mechanisms of enzymic reactions relate to pharmacology, as well as to fundamental biology and chemistry. Although a great deal has already been accomplished in this field, much still remains to be done. In a practical world, the control and cure of disease profits from an understanding of the mechanisms of enzyme action; this aspect of physical organic chemistry is revolutionizing drug design just as the earlier understanding of reaction mechanisms in organic chemistry revolutionized chemical synthesis.

In early attempts to determine the mechanisms of enzyme action, the substrates for which an enzyme is active were determined, and where possible the amino acids that are responsible for its action were specified. Such studies have benefited from both amino acid sequencing and X-ray crystallography. For example, a generation ago a particular serine residue at the active site of chymotrypsin was marked by its specific reaction with diisopropyl fluorophosphate (one of the nerve gases) (3); an active histidine was marked by the tosyl amide of phenylalanyl chloromethyl ketone (4). Later the complete amino acid sequence of the enzyme was determined (5), and the detailed three-dimensional structure of the enzyme was worked out by X-ray crystallography (6). The kinetics of the process were essential to devising a useful mechanism (7).

[a] Note: Quotation from Phyllis McGinley, *The Province of the Heart; How to get along with men* (1959).

But today one can develop even more detailed mechanisms, and confirm what previously were hypotheses by the method of site-directed mutagenesis, borrowed from molecular biology. One can vary not only the substrates for enzymes, but the enzymes themselves, exchanging any amino acid in the protein for any other, and noting the effect.

For example, a tyrosine residue had been postulated as an essential catalytic residue in carboxypeptidase. This hypothesis was destroyed when the tyrosine was replaced by site-directed mutagenesis, by a phenylalanine residue, with only minor effect on enzyme activity (8). By contrast, a glutamate residue has been postulated as essential in triose phosphate isomerase; this hypothesis was supported by replacing the gluamate with aspartate, with a 1500-fold decrease in enzymatic activity (9).

An example where chemistry, X-ray crystallography, and site-directed mutagenesis were all applied to the elucidation of reaction mechanism is offered by the study of the action of mandelate racemase (10). Here the mechanism of action of an enzyme is known in as much detail as that of any other reaction in solution. George Kenyon, John Gerlt, and Gregory Petsko postulated that a histidine residue removed (and replaced) the *R*-asymmetric hydrogen atom of mandelic acid, whereas a lysine residue removed and replaced the *S*-hydrogen atom. They confirmed this hypothesis when they replaced the essential histidine, by site-directed mutagenesis, with an asparagine residue. The new enzyme did not catalyze the racemization of mandelic acid, but did catalyze the hydrogen-deuterium exchange of the *S*- but not of the *R*- mandelate, in conformity with prediction. Such detailed understanding of the action of an enzyme is now feasible, and offers exciting possibilities for the future.

The need for such understanding of mechanism for many, many enzymes arises from the increasing use of enzyme inhibitors in planning new pharmaceuticals. Examples of such drug development include, for example, the invention of the widely used blood-pressure regulators (11), Captopril and Enalopril. Silverman (12) lists over two dozen enzymes where knowledge, or partial knowledge, of the mechanism of enzyme action is stimulating and guiding drug development. In order to take advantage of these practical possibilities, modern physical organic chemistry must contribute to a determination of enzyme mechanisms.

But in addition to the predicted practical value to medicine of such mechanistic information, the detailed understanding of major fields in science adds to our intellectual grasp of biochemistry, and our mastery of science in general. This encomium applies of course, to the modern application of physical organic chemistry to other fields than enzymology such as, for example, metallo-organic chemistry.

Incidentally (or perhaps not so incidentally), we in the scientific community need to explain how mechanisms are determined, what are the experimental bases for our conclusions, and how firmly each statement can be made. A mechanism is a theory in science, not a fact, and offers all the possibilities of prediction and is subject to all the uncertainty that accompanies every scientific theory. The scientists who use the predictions of mechanism in synthesis or drug design should be aware of the way in which the mechanisms were determined, and so be conscious of the limitations as well as the power of the field.

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