Obituary

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David Phillips, 1924–1999

Certain moments are deeply engraved in my memory. One is the Monday morning in March 1953 when Crick called me into his room to show me his and Jim Watson's double helical model of DNA which immediately revealed the molecular basis of heredity. Another is the moment when David Phillips, Louise Johnson and Charles Vernon made me first understand how an enzyme works.

In 1965, Phillips, Tony North, Colin Blake and others had solved the structure of lysozyme at 2 Å resolution, building on earlier work by Roberto Poljak (Blake *et al.*, 1962, 1965). Their atomic model was only the second of any protein and the first of any enzyme. That spectacular success made me decide to organize a Royal Society Discussion which took place on 3rd February of the following year. I was confident that it would prove very interesting, but I never expected it to become a turning point in the history of biochemistry.

Shortly before, Louise Johnson had solved the structures of lysozyme with two inhibitors, *N*-acetylglucosamine and tri-*N*-acetylglucosamine (Blake *et al.*, 1966). Both bound in a prominent cleft of the enzyme, but short of what clearly was its active site.

Phillips, using the observations on lysozyme substrates of John Rupley and Nathan Sharon and the experimental crystallographic results, built a hexasaccharide substrate into the cleft. The molecular model building, achieved by Phillips in one day, led to the identification of the catalytic site and explained in a remarkable way every feature of lysozyme's specificity and catalytic mechanism. In particular, it placed the C1 oxygen of the fourth sugar close to an acid residue, glutamic acid 35, and the C1 and ring O atoms of the fourth sugar close to another acid residue, aspartate 52. In order to comply with the stereochemical restrictions of the catalytic site cleft, the fourth sugar needs to be distorted to a 'sofa' conformation.

But how did they cleave that bond? Phillips asked Charles Vernon, a member of C. K. Ingold's laboratory at University College, to look at the models with him. Ingold was the author of the classic monograph 'Structure and Mechanisms in Organic Chemistry' and a pioneer in the elucidation of chemical reaction mechanisms. Vernon had worked for more than ten years on acid- and enzyme-catalysed mechanisms of carbohydrate hydrolysis. In 1961 his future wife, Barbara Banks, was the first author of a paper on the acid hydrolysis of methyl-α-D-glucopyranoside. This showed the reaction to proceed *via* the formation of a ring-closed carbonium ion-intermediate at C1, and the rate-determining step to be the subsequent heterolysis of the C1—O bond (Banks *et al.*, 1961).

By good fortune, this mechanism turned out to be the perfect model for the lysozyme-catalysed hydrolysis of oligosaccharides: the glutamate buried on one side of the glycosidic bond clearly had a high pK_a, so that its

uncharged carboxylic acid could donate a proton to the bridge O atom, while the negatively charged carboxylate of the external aspartate would then facilitate heterolysis of the C1—O bond and stabilize the positively charged carbonium ion so formed. In addition, this ion was stabilized by steric constraints in the cleft which distorted the hexose ring from its unstrained chair to a strained half-chair conformation, allowing the three bonds radiating from the carbonium ion to lie in one plane.

The mechanism stimulated a flood of papers on experiments designed to test and elaborate the crystallographic proposals and each one of these experiments provided further support. In particular, in 1991, on the occasion of a meeting in honour of David's retirement, Natalie Strynadka and Michael James published direct crystallographic observations on a cell wall tetrasaccharide complexed with lysozyme in which the fourth sugar was distorted into a sofa conformation, just as predicted.

At the time, transition-state chemistry was so new to me that I did not take in all the arguments, but the structure and mechanism that were now revealed made me ask why chemical reactions, which normally require powerful organic solvents or strong acids and bases, can be made to proceed in aqueous solution near neutral pH in the presence of enzyme catalysts. Organic solvents have the advantage over water of providing a medium of low dielectric constant, in which strong electrical interactions between the reactants can take place. The non-polar interior of enzymes seemed to me to provide the living cell with the equivalent of the organic solvents used by the chemists. The substrate is drawn into a medium of low dielectric constant in which strong electrical interactions between it and specific polar groups of the enzyme can occur.

I felt tempted to add, 'Once we understand the stereochemical basis of enzymic catalysis it may become possible to design and synthesize enzymes for specific catalytic functions, for both biological and industrial purposes. I look forward to a future Royal Society Discussion on that subject.'

When looking at a new structure, W. L. Bragg used to ask, 'What is it trying to tell you?' I doubt that Phillips, when he embarked on the X-ray analysis of lysozyme, ever expected that it would tell him so much. His fruitful collaboration with many biochemists, chemists and physicists may have convinced him that protein crystallography flourishes best in collaboration with other disciplines and may have led him later to found the highly successful Oxford Enzyme Group.

In conclusion, let me mention a more intimate episode which I shall always remember. In 1981 my son was a University Demonstrator at Oxford. One evening, when I was staying with him and my daughter-in-law, Phillips dropped in for a visit. He squatted down with my three-year-old grandson, normally shy with strangers, who instantly made friends with him. This taught me that Phillips had a way, not only with complex electronic equipment, but also with small children. His own grandson adored him.

References

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