Stereochemistry in Relation to Enzyme Mechanism

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Stereochemistry was a part of enzymology long before anything was known about the chemical nature of enzymes. Stereochemistry was born in 1874, enzymes were christened in 1877; and Emil Fischer, born in 1852, was of an age to take notice of both events. When Fischer studied the action of crude ferments from seeds on derivatives of the sugars, he did not even know the correct structures of his substrates, let alone of his enzymes; but he knew which of his substrates were stereoisomers, and he could formulate his lock-and-key model of enzyme-substrate interaction as a purely stereochemical hypothesis. It is important to recognize that this hypothesis was confined to the problem of substrate specificity in enzymes. At that time (1894) there was already a secure stereochemical theory of the structures of organic molecules, a theory to which X-ray crystallography brought confirmation rather than correction; but the stereochemistry of chemical reactions was largely unknown territory. Paul Walden was doing his work on stereochemical inversion, but for four decades it was considered a curiosity outside the main stream of chemistry. Alexander McKenzie had started his work on "asymmetric syntheses", but the stereochemical basis was supplied by Prelog more than half a century later.

The picture began to change in the 1930's. Then, recognition of the Walden inversion as the normal process of bimolecular nucleophilic displacement drew much attention to the stereochemistry of chemical reactions in general. It happened also about that time that the steroids became objects of intensive research because of the biological activities of some of them. Many transformations in this semi-rigid skeleton of carbon atoms were effected, and stereochemical control was often imperative. This work led eventually in Barton's hands to the theory of conformational analysis, which is just as much a theory of reactivity as of structure. The importance of geometrical arrangement in accelerating chemical reactions was emphasized.

On the biochemical side, the late 1920's and early 1930's saw the first crystallizations of enzymes and the demonstration that the catalytic activity resided in a protein molecule. In the same period, the separation of deuterium by the late Harold Urey began to liberate biochemists from the dilemma that to study a living system you must often begin by killing it.

Chemists were not slow to use isotopes to investigate reaction mechanisms, and indeed the most elegant demonstration of the Walden inversion used radioactive iodide in combination with polarimetry to show that every exchange of iodine between 2-iodo-octane and iodide ion was an act of stereochemical inversion. Biochemists at first tended to use isotopes incorporated in simple chemical compounds to trace biosyntheses in living organisms, and this technique is still much

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used today. But application of isotopic techniques to isolated organs, tissue slices and homogenates, cell-free systems and purified enzymes was not long delayed; and when this happened it soon produced an apparent paradox. The resolution of this paradox made stereochemistry of central importance to the study of enzyme mechanism.

The phenomenon now known as the Ogston effect could have been predicted from existing information long before 1948, and indeed before isotopes were discovered. For this omission the blame must be laid squarely on organic chemists, and it is instructive to ask why they not only failed to anticipate the phenomenon but were slow to explain it.

I think that the trouble lay, and still lies, in the ways chemists use to impart information about molecules. How liberated I should feel if I were able to show three-dimensional moving representations of my molecular subjects in such a way that the reader could take in their chemical and stereochemical nature and behaviour at the same time. But this would at present be prohibitively costly, and when this chapter is published I shall have to deal with my molecular representations as chemists have done for a century and more: that is, hammer them flat and show the result from one side only. This true on paper, on the blackboard, or on a projected slide. It is an expensive business to impart even the semblance of three dimensions to a two-dimensional projection, and it is a slow business to construct and transform three-dimensional models. A good visual imagination can help to remedy these defects, but the sad truth is that we still learn most of our chemistry in Flatland, to the detriment of our science. Adolf von Baeyer's strain theory, which predicted that rings became progressively less stable as they departed in either direction from a five-membered ideal, was a classic example of Flatland thinking.

So a 1948 organic chemist, making a two-dimensional representation of oxaloacetate and its enzymic reaction with "active acetate", as it was known before 1948, to form citrate, then dehydrated by a second enzyme to aconitate, would write something like this, without the asterisks:

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\begin{align*}
\text{O-C} & \text{CO}_2\text{H} + \text{CH}_3\text{COX} \rightarrow \text{HO}_2\text{C} \text{CH}_2\text{CO}_2\text{H} \\
& \text{HO}_2\text{C} \text{CH}_2\text{CO}_2\text{H} \rightarrow \text{HO}_2\text{C} \text{CH}_2\text{CO}_2\text{H} \\
\end{align*}
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And if a biochemist then told him that this reaction, when carried out with oxaloacetate isotopically labelled at *C, gives citrate in which the acetic acid chain carrying the label participates in the enzymic dehydration to the complete exclusion of the other chain, his first reaction would be disbelief.

The picture in three dimensions is quite different. Making no assumptions about the mechanism of citrate synthesis, we still know that a new C-C bond is formed between the carbonyl carbon of oxaloacetate and the methyl carbon of "active acetate". No matter what chemical mechanism is chosen for the condensation, there are two different ways of writing it, depending on the relative orientations of the two substrates. In Fig. 1a the new bond is formed on what is now called the re face of the oxaloacetate carbonyl: that is, the face showing oxygen, carboxyl and carboxymethyl groups in clockwise order around the carbon. In the alternative, (Fig. 1b), now known to be correct, the order is anticlockwise. The point is that these two arrangements are spatially not the same. In a statistically symmetrical medium like liquid water, there is no reason why reaction should occur more readily by