Bio-Organic Chemistry in Retrospect

Frank H. Westheimer

Department of Chemistry, Harvard University, Cambridge, MA 02138 (USA)

Forty years ago, no-one knew the mechanism of any enzymic process, or could explain the action of any coenzyme. Today we understand the mechanisms of many enzymes and of most of the coenzymes. This extraordinary advance has been the work of numerous chemists and biochemists, who applied the methodology of physical-organic chemistry to enzymatic systems. My own part in this achievement includes two investigations that relate to stereochemistry, and are therefore appropriate to this Symposium: the direct and stereospecific transfer of hydrogen in the reductions that require NADH, and the pseudorotation that accompanies the hydrolysis of certain esters of phosphoric acid.

In 1951, *Birgit Vennesland, Harvey Fisher* and I demonstrated the direct and stereospecific transfer of hydrogen between nicotinamide adenine dinucleotide (NADH) and the substrates for alcohol dehydrogenase and lactic dehydrogenase. When deuterated NADH (i.e., NADD) was prepared enzymatically, only one deuterium atom was incorporated into the 4-position of the dihydronicotinamide ring, and when acetaldehyde was reduced enzymatically with this NADD, only the deuterium, and not the hydrogen atom from the 4-position was transfered. The CH_3CHDOH that was produced was chiral; we also prepared its enantiomer, and converted one to the other by a standard chemical inversion process.

Subsequently, these methods have been widely used in biochemistry, including applications by *Cornforth* and *Arigoni* and *Battersby* among others. The details of the transfer process have been elucidated in large part by *T. C. Bruice*, and *Steven Benner* has offered a generalization and explanation for the precise stereochemistry of the processes.

The phenomenon of pseudorotation about a phosphorus atom was first invoked by *Stephen Berry* to explain the nuclear magnetic resonance spectrum of PF_5. That compound has a trigonal bipyramidal structure, with three equatorial and two apical fluorine atoms. Nevertheless, the ^{19}F-NMR spectrum shows only one kind of fluorine atom. The simplest mechanism by which the apical and equatorial fluorine atoms can become equivalent on the NMR time scale is a «pseudorotation», a vibration where two of the equatorial bonds and the two apical bonds are simultaneously bent through modest angles to produce a new trigonal bipyramid where the old equatorial bonds are now apical, and vice versa.

The hydrolysis of cyclic phosphates present some unusual features that can be explained by this pseudorotation process. We found that methyl ethylene phosphate is a strained molecule, and that it hydrolyzes a million times as fast as does trimethyl phosphate. But on hydrolysis in acid, it yields only 50% of a ring-opened product, and 50% of methanol and ethylene phosphate. In other words, half of it hydrolyzes extraordinarily rapidly without breaking the strained ring. This surprising finding can be explained on the assumption that the attacking nucleophile produces a trigonal bipyramidal intermediate according to a set of reasonable rules, and that this trigonal bipyramidal intermediate can undergo a pseudorotation about the central phosphorus atom. The pseudorotation mechanism has been confirmed in many subsequent investigations, including investigations of the NMR spectrum of numerous oxyphosphoranes.

The pseudorotation mechanism has quite specific stereochemical consequences; in particular, a single pseudorotation about phosphorus leads to retention of configuration about the phosphorus atom. Although no enzymatic hydrolysis of a phosphate ester has yet been found to occur with pseudorotation, the process has been essential in developing the stereochemistry of many of the chiral phosphates needed for the investigations of enzymic mechanisms.