Transcience in enzymes

Protein-enzymes catalyze most biochemical processes through high-energy, transient intermediates. Structural characterization of intermediates can lead to stable analogs that inhibit cognate enzymes and serve as pharmaceutical agents. The enzyme pyruvate dehydrogenase catalyzes the oxidation of pyruvic acid to carbon dioxide and acetyl-coenzyme A, an important species in energy production. A central reaction involving the vitamin thiamine led to acetyl-coenzyme A through an unknown transient intermediate. Our group identified the kinetically invisible intermediate as 2-acetylthiamine diphosphate. This proved to be a key intermediate in other thiamine-dependent enzymes.

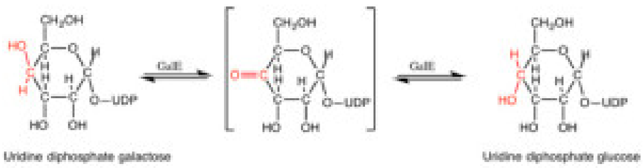

Fig. 1. Interconversion of uridine diphosphate derivatives of galactose and glucose by Gale.

In carbohydrate metabolism the uncommon sugar galactose can be used for energy as if it were the common sugar glucose. The two differ in stereochemical configuration at carbon-4 (Fig. 1.). Stereochemical differences are often described as “right and left handedness” of the order in which four substituents are attached to an atom. The two sugars differ in handedness at carbon-4. Enzymes generally display stereospecificity in acting on such molecules. However, the enzyme uridine diphosphate (UDP) galactose-4-epimerase (GalE) catalyzes the interconversion of UDP-galactose and UDP-glucose. Our group showed that GalE reversibly catalyzes transient dehydrogenation of the C_4–OH group in each molecule to C_4=O in Figure 1, that is, to the same structure.

Biochemical molecules such as UDP-glucose are organophosphorus compounds. The enzymatic production of these compounds requires transfers of phospho-groups from a phospho-donor D–P to a phospho-acceptor A. Transfers can occur either by initial transfer of the phospho-group from D–P to the enzyme E to form E–P, followed by phospho-transfer from E–P to A to form A–P, a double displacement. Alternatively, the phospho-group can be transferred directly from D–P to A at the enzyme site to form A–P, a single displacement. Our group used stereochemistry and chiral phospho-groups with sixteen enzymes to identify the two classes. We proposed the “principle of economy in the evolution of active sites” in enzymes..

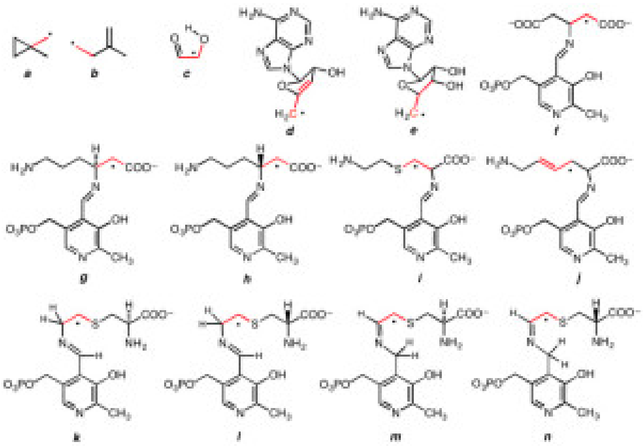

Fig. 2. Structures of enzymatic radicals identified in the Frey group. Adapted form Figure 4 in the reference.

Many chemically difficult biochemical reactions cannot be understood by conventional polar reaction chemistry. These reactions often involve radical intermediates. Radicals arise by homolytic cleavage of two-electron covalent bonds, a high energy process. Figure 2 shows the radicals identified in this laboratory. I first studied lysine 2,3-aminomutase (LAM), believing that radicals could be involved. LAM requires S-adenosylmethionine and the vitamin B6 coenzyme pyridoxal 5'-phosphate. We found radicals d, g, h, i, and j in LAM. The other radicals were identified in methane monooxygenase, dioldehydrase, glutamate 2,3-aminomutase, Class III ribonucleotide reductase, and lysine 5,6-aminomutase.

In certain enzymes, low-barrier (short-strong) hydrogen bonds (LBHBs) play a role. They are 5-10 times as strong as weak hydrogen bonds between water molecules. Based on published spectroscopy, our group assigned the first LBHB in a protein to a transient intermediate of chymotrypsin. We found and correlated chemical, structural, spectroscopic, and kinetic evidence in support of this assignment. The properties of the LBHB were in accord with the published properties of LBHBs in small molecules.

Perry Allen Frey
Department of Biochemistry
University of Wisconsin-Madison

Publication

Transient intermediates in enzymology, 1964-2008.

Frey PA.
J Biol Chem. 2015 Apr 24