The Stereochemistry and Reaction Mechanism of Dehydrogenases and Their Coenzymes, DPN (NAD) and TPN (NADP): the Work of Birgit Vennesland

The Enzymatic Transfer of Hydrogen. I. The Reaction Catalyzed by Alcohol Dehydrogenase

The Enzymatic Transfer of Hydrogen. II. The Reaction Catalyzed by Lactic Dehydrogenase

Birgit Vennesland (1913–2001) and her twin sister, Kirsten, were born in Kristiansand, Norway. Their father had immigrated to the United States to study dentistry, and “tired of waiting for the end of World War I,” in 1917 their mother sailed for the United States with her two daughters to rejoin her husband in Chicago (1). After early schooling in Chicago, Vennesland entered the University of Chicago with a scholarship awarded on the basis of a competitive exam in physics. She settled on a biochemistry major and received her B.S. degree in 1934. After working for a year as a technician, she returned to the University of Chicago for graduate education in biochemistry and chose her own Ph.D. thesis project, the oxidation-reduction potential of a strict anaerobic bacteria, which was completed in 1938. During her thesis work, she discovered that bacteria require a small amount of CO2 for growth, a discovery that influenced her later work (1).

In 1939, Vennesland received a fellowship from the International Federation of University Women to work with Otto Myerhoff who was then in Paris after fleeing Germany and the increase of anti-Semitism. As war in Europe intensified, however, she decided instead to join A. Baird Hastings in the Department of Biochemistry at Harvard Medical School. With Hastings, Vennesland was among the first to use radioactive carbon, 11C, to investigate metabolism. Those were challenging studies because the 20.6-min half-life of 11C meant that experiments had to be completed very quickly. Among the most notable of their findings was the demonstration that starved rats incorporated the isotope from 11CO2 into liver glycogen. Vennesland’s work with Hastings was reported in an earlier JBC Classic (2).

Vennesland returned to the University of Chicago in 1941 as Instructor of Biochemistry intending to work on CO2 incorporation by non-photosynthetic plant tissues; however, she, like many scientists at the time, enlisted in the war effort and worked on a malaria research project until the end of the war. In 1946, she, along with an influx of post-war students and a newly available Beckman DU spectrophotometer, began to examine the enzymology of dehydrogenases that utilized DPN+ and TPN+, as NAD+ and NADP+ were then called. Upon reduction, these coenzyme molecules increase their absorbance at 340 nm thus providing a convenient spectrophotometric assay to quantify reaction progress. In 1950, after a great deal of experi-

1 The biographical information for Birgit Vennesland was taken from Ref. 1. We thank Eric E. Conn, Professor Emeritus of Biochemistry and Biophysics at the University of California at Davis for sharing his recollections of the work reported in these JBC Classics which was going on while during he was a postdoctoral fellow in Vennesland’s laboratory.
ence with these enzymes and their metabolic functions, Vennesland initiated a collaboration with Frank H. Westheimer, then in the chemistry department at the University of Chicago, that led to important mechanistic insight into the reactions of pyridine nucleotide-dependent dehydrogenases.

In the two JBC Classics reprinted here, Vennesland and co-workers describe experiments to show that the two hydrogen atoms at one of the carbons in the dihydropyridine ring of both DPNH and TPNH (NADH and NADPH) are enzymatically non-equivalent and that the dehydrogenases transfer hydrogen, as hydride ion, stereospecifically between substrate and coenzyme. Using alcohol dehydrogenase (ADH), they accomplished the first demonstration of the enzymatic discrimination between the two enantiotopic hydrogen atoms on the methylene carbon atom of ethanol. With dideuteroethanol as substrate, they established that the reaction products were monodeutero-reduced DPN and monodeuteroacetaldehyde.

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\text{ADH} \quad \text{CH}_3\text{CD}_2\text{OH} + (\text{oxidized}) \text{DPN}^+ \rightarrow \text{CH}_3\text{CDO} + (\text{reduced}) \text{monodeutero-DPN}
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Subsequent analysis also demonstrated that incubation of the enzymatically prepared reduced monodeutero-DPN with ADH and acetaldehyde resulted in complete transfer of deuterium from reduced DPN to acetaldehyde to form monodeuteroethanol. If, on the other hand, chemically reduced deuto-DPN was subjected to the same reaction, about one-half of the deuterium remained in the oxidized DPN. Vennesland and colleagues concluded that: 1) with enzymatic oxidation-reduction reactions, hydrogen is transferred directly from the alcohol to the coenzyme, and 2) the enzymatic reduction is stereospecific with respect to the position of the electrons in the dihydropyridine ring that are to be removed or added. The work described in the second JBC Classic is an extension of the initial work on alcohol dehydrogenase to lactate dehydrogenase, leading to the same conclusions. It is of interest that in these two papers the authors incorrectly designated the 6-position of the pyridine ring as the site of oxidation-reduction. They pointed out in an addendum to the first paper that M. E. Pullman had just reported (3) that the site of reduction was the 4-position, which is correct, but the validity of their work is independent of the position reduced.

Vennesland made several extended visits to work with Otto Warburg at the Max Planck Institute for Cell Physiology in Berlin, and Warburg offered her a position as both Director and his successor. She left the University of Chicago for Berlin in 1968. Soon after arriving, however, the circumstances, both personal and scientific, proved unsatisfactory and she moved to a nearby Max Planck Institute designated by the Max Planck Gesellschaft as Forschungstelle Vennesland or literally Vennesland research place. Her studies during this period focused on nitrate assimilation by photosynthetic organisms and included numerous noteworthy accomplishments before her retirement in 1984. Vennesland received many honors for her research including the Stephen Hales Prize from the Society of Plant Physiologists (1950), an honorary degree from Mount Holyoke College (1960), and the Garvin Medal of the American Chemical Society. She was revered by her students and post docs and was an excellent role model, particularly for women in science.

In 1953, shortly after the work for these JBC Classics was completed, Vennesland’s collaborator Frank H. Westheimer returned to Harvard where he had been a Ph.D. student with James B. Conant and E. P. Kohler. In his distinguished research career Westheimer was concerned with the mechanisms of both chemical and enzymatic reactions. He was an early pioneer in the field of molecular mechanics and invented photoaffinity labeling, as well as the application of pseudorotation to phosphate ester chemistry. As one of the most distinguished physical-organic chemists of his generation, he was the recipient of countless honors including election to the National Academy of Sciences (1954) and the National Medal of Science (1986).

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REFERENCES