The Production and Analysis of Optically Pure Amino Acid Stereoisomers: the Work of Jesse P. Greenstein

Preparation of the Four Stereoisomers of Isoleucine

Optical Purity of Amino Acid Enantiomorphs

Jesse Philip Greenstein (1902–1959) was born in New York City. He received his B.S. in chemistry from the Polytechnic Institute of Brooklyn in 1926 and his Ph.D. from Brown University in 1930. His dissertation, which was done with Charles A. Kraus and Philip H. Mitchell, was on the dissociation constants of glycine and peptides containing glycine. After graduating, Greenstein joined *Journal of Biological Chemistry* (JBC) Classic author Edwin J. Cohn (1) at Harvard Medical School to study the dissociation constants of complex polypeptides. A year later, in 1931, he went to the Kaiser Wilhelm Institut at Dresden to work with Max Bergmann. There, he applied Bergmann’s newly discovered carbobenzoxy method of peptide synthesis to the synthesis of lysylglutamic acid and lysylhistidine. Upon returning to America, Greenstein spent a year in Carl L. A. Schmidt’s laboratory at the University of California, Berkeley where he continued to work on the relationship between structure and the dissociation of ionizable groups.

In 1933, Greenstein returned to Cohn’s laboratory at Harvard where he carried out a notable series of studies on peptides and proteins and published 35 papers in 7 years. This included studies on the synthesis, dissociation, dielectric constants, and electrostriction of amino acids and peptides and on the relationship of protein denaturation to the appearance of titratable sulphhydryl groups. He also served as a full-time tutor in the biochemical sciences and was a lecturer in biochemistry at Harvard College.

Greenstein began a new phase of his career when he accepted a position at the National Cancer Institute at the National Institutes of Health in 1939. There, he began studying the
components of tumors and eventually published the now classic monograph *Biochemistry of Cancer*. In 1946 he was appointed Chief of the newly created Laboratory of Biochemistry at the National Cancer Institute. Despite the administrative responsibilities that came with his new post, Greenstein continued to be actively involved in research. Realizing that there was a great need for a readily available supply of optically pure amino acids for research, Greenstein focused on remedying this situation. He figured out that he could use enzymes to aid in the preparation of pure amino acid isomers, and in 1949 he and Paul J. Fodor and Vincent E. Price published a paper on a new method for preparing large amounts of D- and L-alanine with a high degree of optical purity (2). The new method involved hydrolyzing acetyl-DL-alanine with hog kidney homogenate.

Using this enzymatic resolution procedure, Greenstein and his colleagues were able to prepare pure isomers of more than 60 amino acids, some of which had not previously been prepared. The preparation of the four stereoisomers of isoleucine is the subject of the first JBC Classic reprinted here. Greenstein, along with co-authors Leon Levintow, Carl G. Baker, and Julius White, resolved mixtures of DL-isoleucine and DL-alloisoleucine into D- and L-isoleucine and D- and L-alloisoleucine with an enzymatic hog kidney preparation.

This work also facilitated a number of studies by Greenstein and his colleagues on the stereochemistry of amino acids, which is the subject of the second JBC Classic paper reprinted here. In the paper Greenstein, along with JBC Classic author Alton Meister (3) and Leon Levintow and Rembert B. Kingsley, used the enzymatic resolution procedure to prepare several enantiomeric forms of amino acids. They then evaluated the purity of the resulting preparations with L- and D-amino acid oxidases and bacterial decarboxylases. They determined that their amino acid preparations contained less than 0.1% of the enantiomorph, thus proving that the enzymatic resolution procedure did indeed yield isomers of high optical purity. They also recommended “(a) that the readily available oxidase and decarboxylase preparations be routinely employed when possible to supplement optical rotation data in the characterization of amino acid isomers, and (b) that a standard of optical purity greater than 99.9% for each isomer be adopted to define an adequate resolution of both isomers from a racemic amino acid.”

Greenstein remained at the National Cancer Institute for the rest of his career. He spent the last few years of his life writing the three-volume treatise *The Chemistry of Amino Acids* with Milton Winitz.

In recognition of his scientific achievements, Greenstein was awarded the Carl Neuberg Medal in 1950, the Distinguished Service Award of the Department of Health, Education, and Welfare in 1952, and the Hillebrand Award in 1958. In 1954, he was elected Chairman of the Division of Biological Chemistry of the American Chemical Society.1

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REFERENCES


1 Biographical information on Jesse Greenstein was taken from Ref. 4.