Understanding sterol biosynthesis pathways

Dedicated to understanding the highly complex evolution of sterol asymmetry and function, chemical biologist Dr W David Nes has spent over three decades studying the biochemical difference in phytosterol structure. He discusses his progress on his latest project.

How have advancements in technology enabled you to elucidate existing knowledge?

Advances in technology over the last 25 years have transformed how we study metabolism, significantly improving the amount and type of data that is generated on sterol biosynthesis and function. Indeed, we can now take a systems approach that allows for an entire genome-metabolome congruence in a cell to be studied concurrently. Breakthroughs in chemistry instrumentation have enabled sterol biochemists to use different chemical and molecular biology approaches to isolate and purify large amounts of sterol catalysts to study their properties and to visualise the active site of these catalysts bound with substrates and inhibitors.

The advent of RNAi technology has allowed sterol biosynthesis and functions to be studied in combination with sterol biosynthesis inhibitors. Isotopically labelling treatments using 13C and 2H-labelled intermediates have allowed for the accurate determination of enzymatic reaction sequences from precursor to final product in intact organisms. These global, world-changing categories of technological advancement have increased our understanding of the reaction mechanisms and orders of enzymes in sterol biosynthesis across kingdoms. The practical applications of these advances have led to the development of drugs that sustain life, or cure disease and have allowed researchers to engineer plants for value-added traits.

What have been some of the most surprising discoveries from experiments conducted so far?

Firstly, that the rate-limiting enzyme of cholesterol biosynthesis in animals is not similarly the rate-limiting enzyme in ergosterol biosynthesis in microorganisms or sitosterol in land plants. Indeed, the classic acetate-mevalonate pathway to sterols in animals does not operate in green algae where a mevalonate-independent methyl erythritol-4-phosphate (MEP) pathway is used to provide isoprenoid intermediates. This reveals interesting differences about sterol biosynthesis evolution. Alternatively, we were surprised to show that the 24-SMT is tetrameric and to be an allosteric enzyme sensitive to feedback modulation by the membrane insert and to be positively stimulated by ATP, the energy charge of the cell. Secondly, that the sterol biosynthesis pathway may have evolved by convergence in eukaryotes, rather than the pathway evolving step-wise from prokaryotes as often considered, by a patchwork organisation of promiscuous pre-existing enzymes that afforded substrate-binding segments for a sterol template. It is interesting to note that all the sterol genes from humans and yeast have been cloned and sequenced.

Bioinformation analysis of the primary structures of the corresponding proteins quite unexpectedly fail to show the existence of a set of amino acids that conform to a common binding motif for the sterol substrate. However, there is a common substrate feature recognised by all the sterol catalysts, that is a polar group in ring A of the sterol molecule usually a 3β-OH group; notably, the active site residues that contact this substrate anchor are not the same amongst sterol biosynthesising enzymes. Finally, that individual sterols play multiple functions differentiated by the structure and amount of compound affecting specific cell responses. Interestingly, the natural cholesterol with a 3β,20R-stereochemistry can be replaced by its enantiomer as the bulk membrane insert in yeast whereas the 24β-methyl group of ergosterol is essential for yeast to grow anaerobically.
PHYTOSTEROLS, COMMONLY REFERRED to as sterols that possess a 24-alkyl group, have become increasingly important to agriculture and medical research as their role in the physiology of eukaryotic organisms has been revealed. For example, some of the latest research into these natural products has indicated their ability to reduce serum cholesterol in humans. A group of researchers based at the Texas Tech University is now attempting to clarify the molecular and genetic basis of sterol biosynthetic reactions in an effort to improve the understanding of the role that sterol structure plays in biological activity and evolution. They are achieving this through a wide range of integrative research methods, such as mechanistic enzymology, organic chemistry and molecular biology. Leading this study is Dr W David Nes, the Paul Whitfield Horn Professor in the Department of Chemistry and Biochemistry and Director of the Center for Chemical Biology at the University. He explains that there is a need to better understand the genetic and molecular basis of sterol biosynthesis throughout nature: "The goal is to establish a full understanding of sterol diversity and function as a foundation for the genetic engineering of useful sterol traits and in the rational design of medicinal drugs to control their actions in normal and diseased systems".

The factors influencing how sterol biosynthesis operates in plants and microorganisms are still generally poorly understood or appreciated. A key effort for Nes is to investigate how phytosterol biosynthesis is controlled differently from those in cholesterol biosynthesis in humans. Nes believes that more research is needed to increase our knowledge of the mechanisms that influence phytosterol production and processing: "This is because engineered or inhibitor-treated modifications in 24-alkyl sterol compositions can lead to improved or harmful physiologies, depending on the organism affected". Their ongoing research project entitled 'Enzymatic C-Methylation Reactions of Phytosterol Biosynthesis', which aims to characterise the structure and function of the (S)-adenosyl-L-methionine-delta24-sterol methyltransferase (SMT), is known to be one of the important translating enzymes involved in phytosterol biosynthesis and homeostasis. This work has been funded for many years by the US National Science Foundation, who have recognised that these experiments hold much significance in improving understanding about design methods that may well control or even mimic the actions of sterols in animals and plants.

**THE IMPORTANT FUNCTION OF STEROLS AND SMT**

Sterols are involved in a panoply of functions, but their primary function in eukaryotes is as an architectural component of cell membranes. In phytophagous insects, phytosterols are ingested by eating plants which they then use to convert to cholesterol. The ingested sterols also impact on a number of developmental functions, such as larval growth through the ecdysteroids. Nes observes that there is a
homeostasis in cells makes it an important target for control of insect damage of crop plants and for the parasite-specific inhibition of sterol biosynthesis that thereby provide opportunities to develop chemotherapeutic leads to cure neglected diseases,” observes Nes. Because phytoesters can be traced back in the fossil record to around 2.7 billion years ago, the time of the first eukaryotes has sparked his interest in terms of their evolution. It is these subjects, along with the function that 24-SMT plays in sterol patterning, which the project collaborators are focused upon.

THE VALUE OF STUDYING STEROL EVOLUTION

Complex and stereochemically varied sterol metabolomes have been observed in nature, and understanding how these sterol mixtures originate and how the structure of sterols changes over time is essential. To establish the exact chemistry and phylogenetic distribution of sterols, the researchers are examining the sterol metabolomes from a varied collection of organisms. They will then isolate these from their natural sources, or clone the relevant genes and functionally express the corresponding recombinant protein in bacteria. Through this approach, they are consequently able to calculate the exact kinetic properties of each of these enzymes. Nes believes that by assuming there is a relationship between sterol structure and the enzyme that catalyses its formation, organisms and sterols produced by primitive organisms should synthesise enzymes with higher energetic costs to product formation: “By studying the activation energies of catalysis of 24-SMTs from plants, fungi and protozoa, we have confirmed this new idea about sterol evolution”.

The project’s efforts have already yielded rewards, as through their investigations into the reaction mechanism that is catalysed by 24-SMTs the researchers are gaining new insights into the evolution of enzymes. “Notably, we have argued a novel view that ‘channel switching’ in the 24-methylation reaction mechanism resulting from mutations in the catalyst’s active site structure is responsible for directing change in product diversity; yet, how these changes in enzyme structure are retained is a matter of functional adaptation to the resulting product in the organism’s evolution,” elucidates Nes.

EMPLOYING MULTIPLE LABORATORY TECHNIQUES

The team is using a number of different tools and methods to determine enzyme structure and catalytic function in their laboratory work. To analyse the 3D structure of 24-SMT they have chosen to use the Circular Dichroism spectroscopy technique, which measures the differences in the absorption of left-handed polarised light against the right-handed polarised light. This approach helps to confirm the changes to the solution conformation in both wild-type and mutant enzymes. X-ray techniques are then being used to characterise solid state properties and active site topography. Each of the 24-SMTs must be sequenced from the whole cells, or cloned, and then these can be expressed functionally in bacteria.

Fast protein liquid chromatography and gel permeation and SDS-polyacrylamide electrophoresis, which is one of the most commonly employed methods for determining the size and amount of protein chains, is used to define the properties of each of the isolated enzymes. In order to confirm the primary structure, Nes and his collaborators have chosen to use Edman degradation and bioinformatics tools to compare the amino acid sequences of the different 24-SMTs. Classic enzymology approaches are also used to study 24-SMT properties and function. Finally 13C- and 2H-labelled substrates and mechanism-based inhibitors and site-directed mutagenesis are employed to investigate the catalytic mechanism. These are then used to map out the location and role of active site amino acids. The multiple approaches that have been utilised to study 24-SMT protein structure and action by this group are now being used globally for studying sterol biosynthesis enzymes.

Nes and his colleagues have been using suicide substrates against the 24-SMT they have prepared for the first time to map out the location of residues in the active site responsible for catalysis, to quantify the amount of active sterol SMT in the cell, and to selectively inhibit ergosterol biosynthesis in parasites and opportunistic pathogens without effects on human cholesterol biosynthesis.

In addition, this research partnership is keen to determine the role of sterols as a catalyst for methylation. In order to do so they need to isolate the C-methylated sterol. To achieve this, the researchers remove sterols from cells by using a number of extraction methods followed by various forms of chromatography. This allows them to determine the products of 24-SMT catalysis under physiological conditions, or the intact organism. “We use high-performance
ENZYMATIC C-METHYLATION REACTIONS OF PHYTOSTEROL BIOSYNTHESIS

OBJECTIVES

• To understand the relationship between sterol biosynthesis and function in plants and microorganisms using wild-type and genetically modified organisms
• To create a combination of rational design and functional analyses to investigate novel S-adenosyl-L-methionine-Δ24-sterol methyltransferase (24-SMT) properties from different phyla across kingdoms
• To probe conserved active site amino acid residues in cloned SMTs by directed mutagenesis and suicide substrate labelling to understand roles of these residues in the evolution of sterol methylation activities affecting the pattern of sterol diversity

KEY COLLABORATORS

Drs Michael R Waterman and Galina I Lepesheva, Vanderbilt Medical School, USA
Drs Fernando Villalta and Minu Chaudhuri, Meharry Medical College, USA
Dr William J Snell, University of Texas Southwestern Medical School, USA
Dr Henry T Nguyen, University of Missouri, USA
Dr Steven L Kelly, Swansea University, UK

FUNDING

National Science Foundation – Award No. 0920212

CONTACT

Dr W David Nes
Paul W Horn Professor and Chair of Biochemistry Division
Director, Center for Chemical Biology
Department of Chemistry and Biochemistry
Texas Tech University
Lubbock, Texas 79409 USA
T +1 806 742 1673
E w david.nes@ttu.edu
http://webpages.acs.ttu.edu/wnes/

W DAVID NES is the Paul Whitfield Horn Professor in the Department of Chemistry and Biochemistry, and Director of the Center for Chemical Biology at Texas Tech University.