Chimia 57 (2003) 74–76

ISSN 0009–4293

Prelog Lecture 2002

Eidgenössische Technische Hochschule Zürich
Laboratorium für Organische Chemie

Abstract: On Monday, November 11, 2002, the vice president, Prof. Dr. Gerhard Schmitt, presented the Prelog Medal 2002 to ***Prof. Dr. David E. Cane***, Brown University, Providence, Rhode Island, USA. The title of the lecture that followed was 'Mechanism and Structure of Biosynthetic Enzymes'.

Keywords: Cane D.E. · Prelog Lecture

Photo: R. Häfliger

David E. Cane Gerhard Schmitt

Prof. David E. Cane was born in New York, NY in 1944. He studied chemistry at Harvard University, where he received a B.A. in 1966 and a Ph.D. in 1971, studying under the direction of E.J. Corey. Following a two-year stay as a National Institutes of Health Postdoctoral Fellow in the laboratory of Duilio Arigoni at the Eidgenössische Technische Hochschule in Zürich, he joined the faculty of Brown University in Providence, Rhode Island, where he is Professor of Biochemistry as well as Vernon K. Krieble Professor of Chemistry.

In Zürich, Prof. Cane was first introduced to the subject that has fascinated him for over 30 years: the mechanism by which naturally occurring substances of diverse biological origin – including antibiotics, toxins, plant defense substances, essential oils, and vitamins – are formed. Using chemical, enzymological, and molecular genetic techniques, he and his coworkers have sought to unravel the biosynthetic pathways leading to many such natural products, most notably terpenes and polyketides.

In the area of terpenoid biosynthesis, he has carried out detailed investigations of enzymes that promote cyclizations of geranyl and farnesyl diphosphate, the linear precursors of monoterpenes and sesquiterpenes, respectively. These studies culminated in solving the first crystal structure of a terpene synthase, in collaboration with Prof. David Christianson of the University of Pennsylvania, and the engineering of families of novel cyclases by site-directed mutagenesis. They have helped illuminate how proteins achieve control over the course and stereochemistry of these complex transformations.

In the area of polyketide biosynthesis, Prof. Cane developed an influential stereochemical model that correlated the structure and stereochemistry of a large number of polyether antibiotics, such as important drugs like erythromycin, and provided evidence for a common biosynthetic origin. In collaboration with Prof. Chaitan Khosla at Stanford, Cane is now exploiting a combination of synthetic, enzymological, and genetic approaches to clarify how polyketide synthases orchestrate the intricate sequence of events involved in converting the simple building blocks acetate and propionate into these structurally complex natural products. Their work on these multifunctional, modular enzymes has gone far in showing how nature can carry out very complicated chemistry using remarkably simple tools.

Throughout his career, Prof. Cane has demonstrated a flair for combining innovative chemical and biological approaches to the elucidation of mechanistic and stereochemical details of biosynthetic transformations. As scientists attempt to characterize the plethora of new and fascinating proteins identified through genomic sequencing, such efforts will become increasingly important. In his own laboratory, for example, exciting progress has been made on the enzymology of bacterial vitamin B_6 biosynthesis, establishing the roles of two key gene products in the formation of the pyridoxine ring.

To mention only some of his many honors, Prof. Cane has been the recipient of the Kitasato Medal in Microbial Chemistry (1995), and the Arthur C. Cope Scholar Award (2000) and the Ernest Guenther Award in the Chemistry of Essential Oils and Related Products (1985) from the American Chemical Society. He has held fellowships from the John Simon Guggenheim Memorial Foundation (1990), the Alfred P. Sloan Foundation (1978–1982), Christ's College, Cambridge (1989–90), and the Japan Society for the Promotion of Science (1983). He was also a Visiting Professor at the Université Louis Pasteur in Strasbourg (1999), the University of California, San Francisco (1998–1999), the Technion in Haifa (1994–1995), and the University of Chicago (1980).

Former Prelog Lecturers

1986 Kurt Mislow
1987 Meier Lahav and Leslie Leiserowitz
1988 K. Barry Sharpless
1989 Jeremy R. Knowles
1990 Henri B. Kagan
1991 Clayton H. Heathcock
1992 J. Michael McBride
1993 Hisashi Yamamoto
1994 Jean-Pierre Sauvage
1995 Yoshito Kishi
1996 David M.J. Lilley
1997 Günter Helmchen
1998 Lia Addadi
1999 David Evans
2000 Helmut Schwarz
2001 Robert H. Grubbs

CHIMIA **2003**, *57*, No. 1/2

Mechanism and Structure of Biosynthetic Enzymes. The Biosynthesis of Vitamin B_6

David E. Cane*

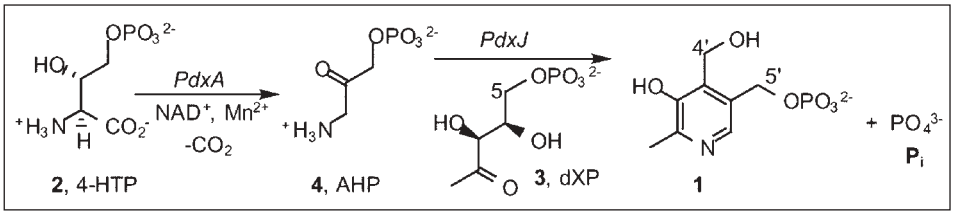
Keywords: Biosynthesis · Crystal structure · Oxidative decarboxylation · Pyridoxine synthase · Vitamin B_6

David E. Cane

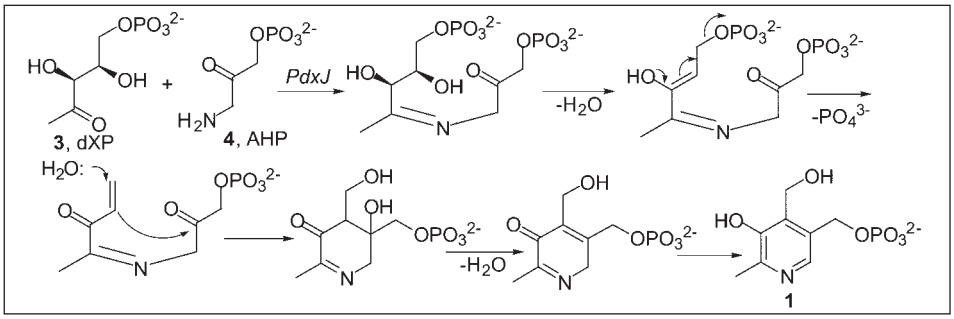
Pyridoxal phosphate and pyridoxamine phosphate are essential cofactors in numerous biochemical transformations of both amino acids and carbohydrates. Both pyridoxine derivatives are formed from the corresponding alcohol, pyridoxol phosphate (**1**), which is either biosynthesized *de novo*, as in microorganisms and plants, or acquired by animals in the diet, most commonly as pyridoxol. Two independent biosynthetic pathways to pyridoxol phosphate have been recognized. In *Escherichia coli* and other prokaryotes all of the relevant genes, enzymes, and biosynthetic intermediates have now been identified while the pathway in yeast and other eukaryotes, as well as certain eubacteria, is still very poorly understood. In *E. coli*, molecular genetic and biochemical investigations have established that pyridoxol phosphate is generated from the oxidative coupling of two building blocks, 4-hydroxy-L-threonine-4-phosphate (4-HTP, **2**) and 1-deoxy-D-xylulose-5-phosphate (dXP, **3**) in a series of reactions mediated by the gene products known as PdxA and PdxJ (Scheme 1).

We have established that PdxA catalyzes the NAD^+-dependent oxidative decarboxylation of 4-HTP to the unstable α-amino ketone, 1-amino-3-hydroxyacetone-3-phosphate (AHP, **4**) [1]. Although in the absence of dXP and PdxJ, AHP undergoes rapid dimerization, we have recently been able to detect and characterize this unstable intermediate by electrospray MS-MS. PdxJ catalyzes the coupling of AHP and dXP to give pyridoxol-5'-phosphate plus inorganic phosphate [2]. Labeling experiments with [^{18}O]-H_2O have shown that release of inorganic phosphate, derived from the C(5) phosphate of dXP, takes place with C–O bond cleavage [3]. Monitoring of the reaction by ^{31}P NMR showed simultaneous formation of both pyridoxol phosphate (**1**) and inorganic phosphate, with no detectable accumulation of any transient (enzyme-free) intermediates [2]. The precise timing of the loss of the phosphate group is not yet known. A proposed mechanism for the cascade of reactions that generates pyridoxol phosphate is illustrated in Scheme 2.

In order to better understand the mechanism of catalysis, in collaboration with our colleague, Prof. Joanne Yeh of Brown University, we undertook structural studies of the PdxJ protein with bound substrate dXP. The resulting 1.95 Å crystal structure [4], in combination with the independently described structures of PdxJ [5][6], both free and with bound products, has shed considerable light on the protein dynamics of the enzyme-catalyzed reaction, and has provided insights into the mechanism of the PdxJ reaction itself. The key features of the observed structure of PdxJ with bound dXP are: 1) PdxJ exists as an octamer in which each subunit is in the form of a classic $\alpha_8\beta_8$



Scheme 1



Scheme 2

*Correspondence: Prof. D.E. Cane
Department of Chemistry
Box H, Brown University
Providence, Rhode Island, 02912-9108 USA
Tel.: 401-863-3588
Fax: 401-863-3556
E-Mail: David_Cane@brown.edu

barrel. 2) Among the eight subunits of the octamer, three different binding states are observed – two that are unoccupied by substrate, four that have a bound dXP, and two that have both bound dXP and inorganic phosphate. 3) The occupancy of the active site is correlated with the conformational state of a mobile peptide loop consisting of amino acids 95–105. In the unoccupied state, this loop is folded back to provide an open active site; when only dXP is bound the loop is partially closed; when both dXP and inorganic phosphate are bound, the loop is closed down over the active site cavity, held in place by a series of cooperative interactions which properly align the substrates for catalysis. In the latter case, the bound inorganic phosphate is thought to be a surrogate for the phosphate ester of the actual co-substrate, AHP. 4) When only dXP is bound, the 5-phosphate moiety occupies the position ultimately occupied by the eventually formed inorganic phosphate product in the PdxJ-product complex, while the carbonyl oxygen of the 2-keto group of dXP occupies the site normally occupied by the phosphate group of AHP. Binding of inorganic phosphate causes the bound dXP to realign within the active site and to move into a conformation suitable for formation of a Schiff's base with the amino group of AHP. A movie simulating the proposed sequence of conformational changes, the binding of the substrates and the release of the products can be found online in the Supplemental Material for [4], or at *http://www.chem.brown.edu/brochure/people/dec/images/PdxJ_movie_8.gif*.

The crystal structure of PdxJ also suggests plausible roles for several of the active site residues (Fig.). For example, Glu72 is suitably positioned to act as the Lewis acid to promote Schiff's base formation by protonating the carbonyl oxygen of dXP. The nearby His193, in its imidazolium form, may assist in the subsequent dehydration reaction, while a variety of other residues, such as His45, His52, and Arg47, as well as Arg20 from the adjacent protein subunit, are likely to play a key role in the binding of the phosphate moiety of the dXP. Hydrogen bond interactions between the C(3) and C(4) hydroxyl groups of dXP and the corresponding hydroxyl groups of amino acid side chains of Thr102 and Thr103 in the mobile peptide loop serve to properly orient the substrate while acting as a latch to hold the peptide loop closed like a lid over the active site. These proposed interactions and others are currently under investigation in our laboratories by site-directed mutagenesis.

Elucidation of the mechanism of formation of vitamin B_6 has required a combination of chemical, biochemical, molecular biological, spectroscopic and protein structural approaches. Further progress in this area is anticipated to reveal fascinating details about the reactions that Nature has evolved to generate this ubiquitous and essential cofactor.

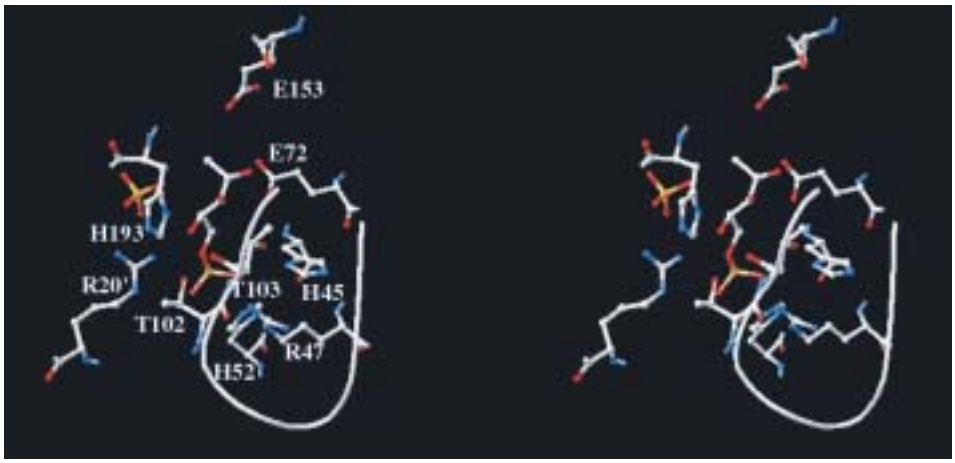


Fig. Crossed-eye stereo view of the active site of PdxJ occupied by both dXP and P_I, and showing key active residues. The white loop corresponding to amino acids 95–105 closes down over the active site and is held in place by interactions of both T102 andT103 with dXP.

Acknowledgments

In carrying out many of the studies described here, I have had the great pleasure of collaborating with two senior colleagues, Prof. Joanne I. Yeh of Brown University, who carried out the crystallographic studies, and Prof. Ian D. Spenser of McMaster University. In fact, Prof. Spenser has been almost single-handedly responsible for elucidating the biosynthetic building blocks for vitamin B_6 in *E. coli* over the last 25 years and he has recently begun to unravel the still mysterious pathway of pyridoxine biosynthesis in yeast. Finally I would like to acknowledge the assistance of my very able coworkers, Dr. Yuju Hsiung, Dr. J. Kenneth Robinson, and Dr. Shoucheng Du, as well as the more recent efforts of Dr. Valerie Frydrychowski and Mr. Jerel Banks who are continuing the investigations of vitamin B_6 biosynthesis in my laboratory.

[1] D.E. Cane, Y. Hsiung, J.A. Cornish, J.K. Robinson, I.D. Spenser, *J. Am. Chem. Soc.* **1998**, *120*, 1936.
[2] D.E. Cane, S. Du, J.K. Robinson, Y. Hsiung, I.D. Spenser, *J. Am. Chem. Soc.* **1999**, *121*, 7722.
[3] D.E. Cane, S. Du, I.D. Spenser, *J. Am. Chem. Soc.* **2000**, *122*, 4213.
[4] J.I. Yeh, S. Du, E. Pohl, D.E. Cane, *Biochemistry* **2002**, *41*, 11649.
[5] M.G. Franco, B. Laber, R. Huber, T. Clausen, *Structure* **2001**, *9*, 245.
[6] M. Garrido-Franco, B. Laber, R. Huber, T. Clausen, *J. Mol. Biol.* **2002**, *321*, 601.