CHIMIA 1999, 53, No. 5

Chimia 53 (1999) 200–201

ISSN 0009–4293

Structure and Mechanism of Action of Hydroxymethylbilane Synthase

Alfons Hädener*

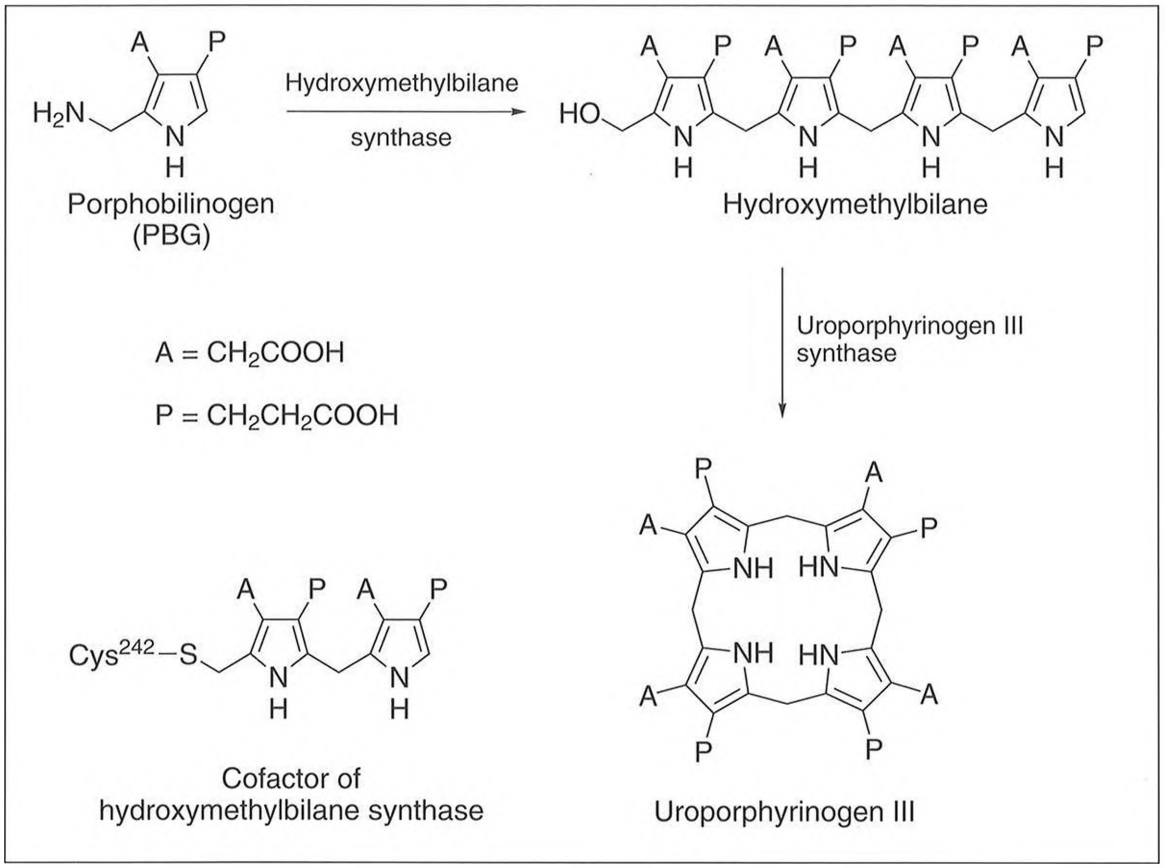
Abstract. The structure and mode of action of a vital enzyme involved in the biosynthesis of tetrapyrroles have been studied by using modern crystallographic and pre-steady-state kinetic methods.

Alfons Hädener was born in Aarau in 1951. He studied chemistry at the University of Basel and completed his Ph.D. in 1982 under the guidance of *C. Tamm*. He remained in Basel as a Research Associate until 1988 when he moved to Cambridge, UK, to study as a Postdoctoral Fellow in the group of *A.R. Battersby*. He returned to Basel in 1990 and completed his Habilitation in 1993. From 1995–1998, he was Head *a.i.* of the Institute of Medicinal Chemistry at the Department of Pharmacy.

A tetrapyrrolic ring system is a common feature of many vital pigments (chlorophylls, heme, vitamin B_{12}). It is biosynthesized *via* a common intermediate, uropor-

*Correspondence: Dr. A. Hädener
Departement of Pharmacy
Totengässlein 3
CH-4051 Basel
Tel.: +41 61 261 79 40
Fax: +41 61 261 79 07
E-Mail: haedener@ubaclu.unibas.ch

Scheme



phyrinogen III. Two enzymes, hydroxymethylbilane synthase (HMBS) and uroporphyrinogen III synthase, are needed to build this compound from porphobilinogen (PBG) (*Scheme*) [1].

To construct hydroxymethylbilane from PBG, HMBS uses an unusual dipyrromethane cofactor which is itself derived from two PBG building blocks and is covalently attached to the protein *via* the sulfur atom of a cysteine residue *(Scheme)*. The cofactor serves as an anchor to which further PBG units, one after the other, are covalently attached until a hexapyrrolic chain is formed. Cleavage of the chain releases the product and leaves the cofactor in place, ready for another reaction cycle [2].

In cooperation with British universities, we have elucidated the three-dimensional structure of enzymatically active, selenomethionine-labelled HMBS from *Escherichia coli* by using the multiwavelength anomalous diffraction (MAD) method followed by model refinement at 2.4 Å resolution [3][4].

To investigate the mechanism of the HMBS-catalysed reaction, the pre-steady-state kinetic parameters of wild-type HMBS and of a number of site-directed mutant variants were determined [5]. We showed that it is possible to trace the consequences of amino acid substitutions down to the individual steps of the five-step reaction sequence.

Building upon this work, time-resolved structures of crystalline [K59Q]HMBS as it is loaded with substrate in a flow cell were determined by *Laue* diffraction [6]. Detailed structural refinement on the data collected at 2 h into the reaction showed that extended electron density appears in the active-site region. This evidence may now serve as a basis for further crystallographic studies involving freeze-trapping techniques.

Received: February 26, 1999

[1] A.R. Battersby, F.J. Leeper, *Chem. Rev.* **1990**, *90*, 1261.
[2] G.J. Hart, A.D. Miller, U. Beifuss, F.J. Leeper, A.R. Battersby, *J. Chem. Soc., Perkin Trans. 1* **1990**, 1979.
[3] A. Hädener, P.K. Matzinger, V.N. Malashkevich, G.V. Louie, S.P. Wood, P. Oliver, P.R. Alefounder, A.R. Pitt, C. Abell, A.R. Battersby, *Eur. J. Biochem.* **1993**, *211*, 615.
[4] A. Hädener, P.K. Matzinger, A.R. Battersby, S. McSweeney, A.W. Thompson, A.P. Hammersley, S. Harrop, A. Cassetta, A. Deacon, W.N. Hunter, Y.-P. Nieh, J. Raftery, J.R. Helliwell, *Acta Cryst.* **1999**, *D55*, 631.
[5] A.C. Niemann, P.K. Matzinger, A. Hädener, *Helv. Chim. Acta* **1994**, *77*, 1791.
[6] J.R. Helliwell, Y.-P. Nieh, J. Raftery, A. Cassetta, J. Habash, P.D. Carr, T. Ursby, M. Wulff, A.W. Thompson, A.C. Niemann, A. Hädener, *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 2615.