

CITY UNIVERSITY OF HONG KONG
香港城市大學

**Studies of Key Enzymes Involved in
the Assembly of the Tetrapyrroles**
參與生成四吡咯化合物的酶的研究

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Li Nan
李楠

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Abstract

The structurally related tetrapyrrolic pigments are a group of natural products that include the haems, the chlorophylls, and the corrinoids. These compounds participate in many of the fundamental biosynthetic and catabolic processes of living organisms. They are all intensely colored and almost every living organism has an absolute requirement for one or more of them. It is for this reason that they are called the “pigments of life.” All the natural tetrapyrroles have their four pyrrolic rings and are derived from a single common tetrapyrrolic macrocycle, uroporphyrinogen III, abbreviated to Urogen III. Urogen III is biosynthesized from eight molecules of 5-aminolaevulinic acid (ALA) in only three enzymatic reactions, which are catalyzed by porphobilinogen (PBG) synthase, hydroxymethylbilane (HMB) synthase, and Urogen III synthase.

PBG synthase catalyzes the dimerization of two molecules of ALA to give the monopyrrole intermediate, PBG. The gene of rat PBG synthase was cloned into the bacterial expression vector pLM1 and pET28a+ with six continuous histidine codons attached to the 5' of the gene. The two recombinant plasmids were overexpressed in *E. coli*, and the enzymes were purified with a nickel metal affinity column to apparent homogeneity. A series of substrate analogues of PBG synthase were synthesized and characterized. Their interactions with the enzyme were studied and some inhibitors were found. Analogues with removal of amino group were found to be good inhibitors. But if the amino group remained and carboxyl group was replaced by different other functional groups, the analogues became

weak or moderate inhibitors. This result strongly suggested that the carboxyl group played an important role for the substrate binding. If the amino groups of these compounds were replaced by bromine, all of them became the irreversible inhibitors. Interestingly, the compound that had both the carboxyl group and the bromine was found to be the weakest irreversible inhibitor, indicating that the tight binding to the fixed position in the active site is unfavorable to the alkylation of the protein. Two analogues were designed to have a long carbon chain connecting two carboxyl groups, which could bind to both P-site and A-site of the enzyme. One of them was found to be a competitive inhibitor, and the other one with an α , β -unsaturated double bond was found to be an irreversible inhibitor.

HMB synthase catalyzes the polymerization of four molecules of PBG to furnish a highly unstable HMB intermediate. The gene of rat HMB synthase was cloned and overexpressed, and the purified protein was found to be a mixture of enzyme complexes by FPLC system. The enzymatic reaction rate of the holoenzyme was found to be lower than those of the enzyme complexes. Several mutant expression plasmids were constructed by site-directed mutagenesis, and the variant proteins were purified to apparent homogeneity. The mutation of the residue D44 stopped the stepwise enzymatic reaction in an enzyme-substrate intermediate step, indicating that this residue played an important role in the enzymatic reaction. Four substrate analogues of HMB synthase were synthesized and characterized. If the carboxyl group in the acetic acid chain on the pyrrole ring of the PBG was removed or replaced, the analogues were found to be very weak substrates. The result indicated that the acetic acid chain was important for substrate binding.

Urogen III synthase catalyzes the conversion of linear tetrapyrrole HMB to the macro cyclic Urogen III. We cloned, purified, and characterized His-tagged rat Urogen III synthase. The mechanism of enzymatic reaction was studied through site-directed mutagenesis of eight highly conserved residues around the active site. The activity assays of these mutant proteins confirmed that Tyr168 was an essential residue for enzymatic reaction catalyzed by rat Urogen III synthase.

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LIST OF ABBREVIATIONS

AIP	Acute intermittent porphyria
ALA	5-Aminolevulinic acid
BSA	Bovine serum albumin
CEP	Congenital erythropoietic porphyria
CTAOH	Cetyltrimethylammonium hydroxide
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DHP	1,2-Dihydropyran
DMAP	4-Dimethylaminopyridine
DNA	Deoxyribonucleic acid
<i>E. coli</i>	<i>Escherichia coli</i>
FPLC	Fast protein liquid chromatography
HMB	Hydroxymethylbilane
HMBS	Hydroxymethylbilane synthase
IPTG	Isopropyl-beta-D-thiogalactopyranoside
kb	kilobase(s)
kDa	kilodalton
Native-PAGE	Native polyacrylamide gel electrophoresis
N-terminus	Amino terminus
PCC	Pyridinium chlorochromate
PCR	Polymerase chain reaction
PBG	Porphobilinogen
PBGS	Porphobilinogen synthase

PDB	Protein data bank (http://www.rcsb.org/pdb/)
PPTS	Pyridinium <i>p</i> -toluenesulfonate
PTSA	<i>p</i> -Toluenesulfonic acid
RNA	Ribonucleic acid
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
THF	Tetrahydrofuran
THP	Tetrahydropyranoxy group
TLC	Thin layer chromatography
Urogen	Uroporphyrinogen
UrogenIIS	uroporphyrinogen III synthase

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