Bacteriochlorophyll biosynthetic enzymes; molecular mechanistic studies on magnesium chelatase and *S*-adenosyl-L-methionine:magnesium protoporphyrin IX *O*-methyltransferase

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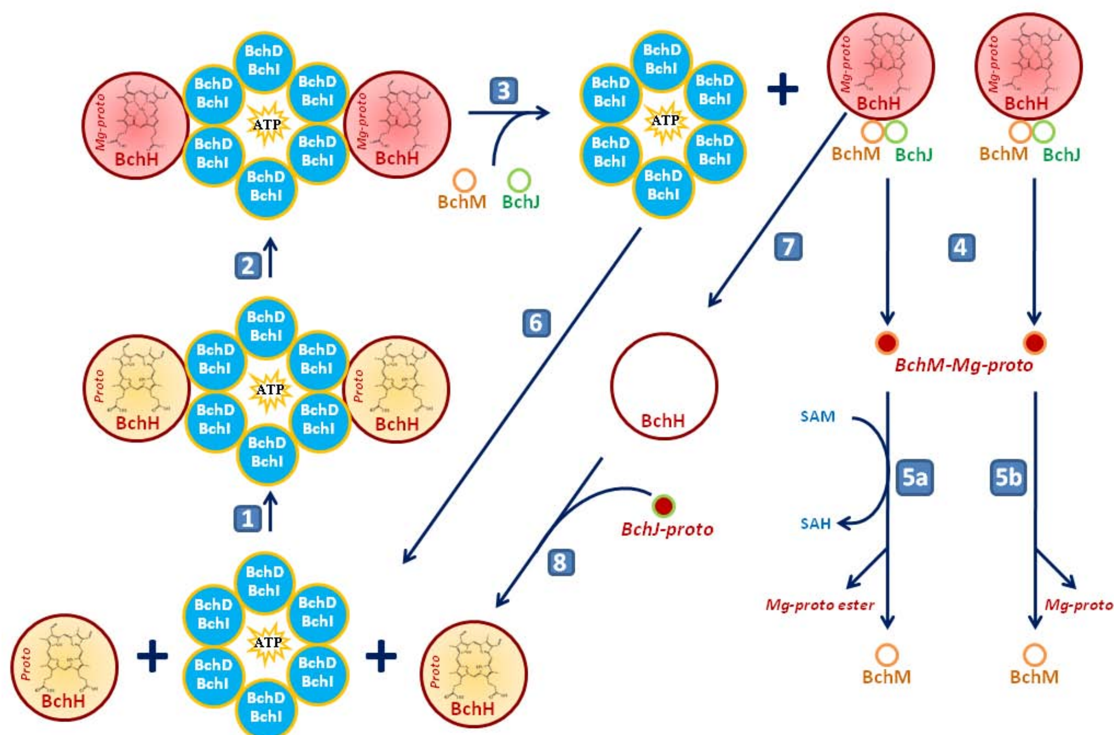

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Contents

Abstract iv

Declaration v

Acknowledgements vi

Cover image description vii

List of Publications viii

Abbreviations ix

1. Introduction 1

1.1 Photosynthesis 1

1.2 (Bacterio)chlorophyll biosynthesis 2

1.2.1 Overview of the pathway 2

1.2.2 Historical background 6

1.2.3 Genetic map of the bacteriochlorophyll biosynthetic pathway 6

1.2.4 Differences between bacteriochlorophyll and chlorophyll biosynthesis 8

1.3 Regulation 11

1.3.1 Anaerobic/aerobic regulation of bacteriochlorophyll biosynthesis 11

1.3.2 Regulation of magnesium chelatase 12

1.3.2.1 Regulation of BchH 12

1.3.2.2 Light regulation of ChlI, ChlD, and ChlH 12

1.3.2.3 Additional regulation of ChlI, ChlD, and ChlH 13

1.3.2.4 Regulation of ChlI and ChlH by reducing disulphide bonds 15

1.3.3 Branchpoint of tetrapyrrole biosynthesis; magnesium or iron insertion 16

1.3.4 Regulation of *S*-adenosyl-L-methionine:magnesium protoporphyrin IX *O*-methyltransferase (BchM/ChlM) 17

1.3.4.1 Product inhibition 17

1.3.4.2 Other regulation 17

1.4 Cellular localization of protoporphyrinogen oxidase, magnesium chelatase, Gun4, and *O*-methyltransferase 18

1.4.1 Overview 18

1.4.2 Magnesium chelatase 19

1.4.3 Gun4 and ChlH 20

1.4.4 *O*-methyltransferase 20

1.5 Magnesium chelatase past and present 21

1.5.1 Overview 21

1.5.2 Early magnesium chelatase activity 21

1.5.3 *In vitro* assays 22

1.5.4 Reaction mechanism 23
1.5.5 Heterologous expression, reconstitution, and purification 25
1.5.6 Aerobic cobaltochelatase, a homologous enzyme to magnesium chelatase 27
1.5.7 BchI/ChlI subunit 27
1.5.8 BchD/ChlD subunit 30
1.5.9 BchI▪BchD/ChlI▪ChlD complex 31
1.5.10 BchH/ChlH subunit 33
1.5.11 ChlH and the role of Gun4 35
1.5.12 ChlH is a multi-functional protein 36
1.6 *S*-adenosyl-L-methionine:magnesium protoporphyrin IX *O*-methyltransferase (BchM/ChlM) 36
1.6.1 Overview 36
1.6.2 Early *O*-methyltransferase activity 37
1.6.3 Stopped and continuous assays 38
1.6.4 Purification and stability 39
1.6.5 Identification of BchM/ChlM as *O*-methyltransferase 40
1.6.6 Reaction mechanism 41
1.7 Evidence for magnesium chelatase and *O*-methyltransferase associations 44
1.7.1 Early studies 44
1.7.2 BchH-BchM/ChlH-ChlM interactions 44
1.8 BchJ 46
1.8.1 The role of BchJ in bacteriochlorophyll biosynthesis 46
2. *Paper I*: Recessiveness and Dominance in Barley Mutants Deficient in Mg-Chelatase Subunit D, an AAA Protein Involved in Chlorophyll Biosynthesis 47
2.1 Synopsis 47
2.2 Journal article 48
2.3 Supplementary data 59
3. *Paper II*: Kinetic Analyses of the Magnesium Chelatase Provide Insights into the Mechanism, Structure, and Formation of the Complex 64
3.1 Synopsis 64
3.2 Journal article 65
3.3 Supplementary data 74
3.4 Addendum 77
4. *Paper III*: *S*-Adenosyl-L-methionine:magnesium-protoporphyrin IX *O*-methyltransferase from *Rhodobacter capsulatus*: mechanistic insights and stimulation with phospholipids 80
4.1 Synopsis 80

4.2 Journal article 81
4.3 Supplementary data 91
5. *Paper IV*: BchJ functions like a magnesium-protoporphyrin IX carrier between magnesium chelatase and *S*-adenosyl-L-methionine:magnesium-protoporphyrin IX *O*-methyltransferase in *Rhodobacter capsulatus* 104
5.1 Synopsis 104
5.2 Manuscript 105
5.3 Supplementary data, schema and figures 123
6. Discussion 143
6.1 Overview 143
6.2 Magnesium chelatase 143
6.2.1 Is the BchD/ChlD subunit oligomeric? 143
6.2.2 Magnesium chelatase reaction mechanism 145
6.2.3 Stimulatory/inhibitory effect of detergents upon magnesium chelatase 148
6.3 *S*-adenosyl-L-methionine:magnesium protoporphyrin IX *O*-methyltransferase (BchM) 151
6.3.1 Purification of *O*-methyltransferase 151
6.3.2 Discovering that phospholipids stabilize and stimulate *O*-methyltransferase activity 152
6.3.3 Structural effects with phospholipids 155
6.3.4 Porphyrin substrates of *O*-methyltransferase 156
6.3.5 *O*-methyltransferase reaction mechanism 157
6.4 Interactions between magnesium chelatase and *O*-methyltransferase, BchJ, or detergent Tween 80 158
6.4.1 Effect of magnesium chelatase on *O*-methyltransferase activity 158
6.4.2 Aggregation of BchM and BchJ with magnesium 159
6.4.3 Binding of proto and Mg-proto to BchM or BchJ 160
6.4.4 Effect of *O*-methyltransferase, BchJ, and Tween 80 upon magnesium chelatase activity 160
7. Conclusions and future work 162
8. References 165

Abstract

The majority of reactions in the bacteriochlorophyll biosynthetic pathway were first elucidated in the 1940-50's. It is only in recent times that molecular mechanisms of the intermediate steps have been determined. The work presented in this thesis is concerned with mechanistic studies of two successive steps of the pathway from *Rba. capsulatus*. The two enzymes involved are magnesium chelatase (consisting of BchI, BchD, and BchH subunits), and *S*-adenosyl-L-methionine:magnesium protoporphyrin IX *O*-methyltransferase (BchM). Their respective reaction mechanisms were analysed separately and shown how they operate in a coupled system. Also studied is the interaction between magnesium chelatase and an unclassified protein in bacteriochlorophyll biosynthesis, BchJ.

Dominant inhibition of magnesium chelatase activity *in vitro* with BchD mutants revealed this subunit was oligomeric. Kinetic data indicated that the molar ratio of BchI:BchD was 1:1, while there are ~2 BchH subunits that interacted with each BchI▪BchD complex. It was proposed that secondary catalysis of magnesium chelatase required ATPase activity of BchI for the structural reorganization of the BchI▪BchD complex and BchH subunit into catalytic-ready configurations.

O-methyltransferase required the phospholipid, phosphatidylglycerol for stability and optimal enzymatic activity. Enzyme kinetics showed the K_m of Mg-proto from *Rba. capsulatus O*-methyltransferase was approximately two orders of magnitude lower than the plant/algal enzyme, but similar to *O*-methyltransferase from another photosynthetic bacterium, *Chlorobaculum tepidum*. The reaction mechanism was random sequential which is comparable to previous studies with *O*-methyltransferase from *Synechocystis*.

Interactions between magnesium chelatase and BchM or BchJ were observed with magnesium chelatase assays. BchM or BchJ removed the product of the magnesium chelatase reaction, magnesium protoporphyrin IX from BchH. There was a 1:1 molar ratio of BchM or BchJ with BchH. BchH-BchM was the dominant interaction, so it is suggested that BchJ could play a role as a porphyrin binding protein.

Declaration

I certify that the work in this thesis entitled "Bacteriochlorophyll biosynthetic enzymes; molecular mechanistic studies on magnesium chelatase and *S*-adenosyl-L-methionine:magnesium protoporphyrin IX *O*-methyltransferase" has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research performed between March 2003 and January 2010 and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

Artur Sawicki (Student No. 40033910)

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Cover image description

From the information acquired in previous studies and the results presented in this thesis, a schematic was constructed showing the flux of porphyrin metabolites from magnesium chelatase to *O*-methyltransferase and BchJ. Starting at the bottom left of the image, the association of two BchH-proto subunits with the double-hexameric BchI▪BchD unit forms a complete magnesium chelatase complex. This triggers a large amount of ATP hydrolysis by the BchI subunit and causes conformational changes of the complex [1]. This energy is utilised to convert protoporphyrin IX (proto) to magnesium protoporphyrin IX (Mg-proto) which remains bound to BchH [2]. With the addition of *O*-methyltransferase (BchM) or BchJ, there is an association of each of these proteins with BchH-Mg-proto, and a dissociation of BchI▪BchD [3]. BchM-BchH-Mg-proto is the stronger interaction and BchM removes Mg-proto from BchH [4]. If BchJ does remove Mg-proto from BchH, the porphyrin is translocated to BchM. With *S*-adenosyl-L-methionine (SAM) present, BchM converts Mg-proto to Mg-proto ester. Either Mg-proto ester, or Mg-proto is released from BchM [5a and b respectively]. At the end of the first cycle of magnesium chelation, BchI▪BchD and BchH are structurally reorganized before carrying out additional catalysis [6, 7, and 8]. BchJ may be involved in delivering new proto substrate to BchH [8].

List of publications

Paper I: **Axelsson E, Lundqvist J, Sawicki A, Nilsson S, Schroder I, Al-Karadaghi S, Willows RD, Hansson M** (2006) "Recessiveness and Dominance in Barley Mutants Deficient in Mg-Chelatase Subunit D, an AAA Protein Involved in Chlorophyll Biosynthesis" *The Plant Cell* **18:** 3606-3616.

Paper II: **Sawicki A, Willows RD** (2008) "Kinetic Analyses of the Magnesium Chelatase Provide Insights into the Mechanism, Structure, and Formation of the Complex" *Journal of Biological Chemistry* **283:** 31294-31302.

Paper III: **Sawicki A, Willows RD** (2007) "*S*-Adenosyl-L-methionine:magnesium-protoporphyrin IX *O*-methyltransferase from *Rhodobacter capsulatus*: mechanistic insights and stimulation with phospholipids" *Biochemical Journal* **406:** 469-478.

Paper IV: **Sawicki A, Willows RD** "BchJ functions like a magnesium-protoporphyrin IX carrier between magnesium chelatase and *S*-adenosyl-L-methionine:magnesium-protoporphyrin IX *O*-methyltransferase in *Rhodobacter capsulatus*" Submitted, *FEBS Journal*, December 24, 2009.

The work in this thesis centres on the findings of four papers presented in chapters 2-5 inclusive. Chapter 6 is devoted to unifying these findings into a discussion section. As a co-author in *Paper I*, I was involved in optimizing and conducting all magnesium chelatase assays. In *Papers II-IV* I was involved in conducting all experiments, data analysis, and preparation of manuscripts. The role of the supervisor was in contributed to the planning of experiments, assistance in data interpretation, and general support and guidance.

Abbreviations

A. rubrum	*Acidiphilium rubrum*
A. thaliana	*Arabidopsis thaliana*
ABA	Abscisic acid
AcsF	Aerobic oxidative cyclase
ADP	Adenosine diphosphate
ALA	δ-aminolevulinic acid
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BchD/ChlD	Magnesium chelatase D subunit
BchE	Anaerobic oxidative cyclase
BchH/ChlH	Magnesium chelatase H subunit
BchI/ChlI	Magnesium chelatase I subunit
BchJ	Protein with unknown role in bacteriochlorophyll biosynthesis
BchM/ChlM	*S*-adenosyl-L-methionine:magnesium protoporphyrin IX *O*-methyltransferase
C. reinhardtii	*Chlamydomonas reinhardtii*
C. tepidum	*Chlorobaculum tepidum*
C. vibrioforme	*Chlorobium vibrioforme*
CD	Circular dichroism
CMC	Critical micelle concentration
Da	Dalton
DOPG	Dioleoyl phosphatidylglycerol
DPOR	Dark-operative protochlorophyllide oxidoreductase
DTT	Dithiothreitol
E. coli	*Escherichia coli*
E. gracilis	*Euglena gracilis*
EM	Electron microscopy
GC	Gas chromatography
Gun	Genomes uncoupled
His	Histidine
IPTG	Isopropyl-β-D-thiogalactopyranoside
IR	Infra-red
kDa	Kilodalton
K_d	Dissociation constant
K_m	Substrate concentration at half maximal velocity, Michaelis-Menten constant
ELIP	Early light induced protein

ICM	Intracytoplasmic membrane
LDAO	Lauryl dimethylamine oxide
LC-MS	Liquid chromatography-mass spectrometry
Mg-proto	Magnesium protoporphyrin IX
Mg-proto ester	Magnesium protoporphyrin IX monomethyl ester
NEM	N-ethyl maleimide
P-20	Polysorbate-20 (highly purified Tween 20)
PCMB	*p*-chloromercuribenzoic acid
PCMBS	*p*-chloromercuribenzene sulphonate
PCR	Polymerase chain reaction
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
pK_a	Acid dissociation constant
POPG	Palmitoyl-oleoyl phosphatidylglycerol
POR	Protochlorophyllide oxidoreductase
PS	Phosphatidylserine
Proto	Protoporphyrin IX
Rba	Rhodobacter
RP-HPLC	Reversed phase-high performance liquid chromatography
SAH	*S*-adenosyl homocysteine
SAM	*S*-adenosyl-L-methionine
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TLC	Thin layer chromatography
V_{max}	Maximal velocity, Michaelis-Menten constant