Beale

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APPENDIX III: PROGRESS REPORT FOR THE CURRENT FUNDING PERIOD

It should be noted that the focus of the research changed somewhat during the course of the current award. The initial focus is indicated by the title of the current grant, The Magnesium Branch of the Chlorophyll Biosynthetic Pathway. During the current grant period, Dr. Robert Willows, a postdoctoral associate, joined the faculty of McQuarie University in Australia. When he left my lab, we decided that he should independently pursue research on structure/function relationships in Mg chelatase and that our laboratories would collaborate on regulatory studies of this enzyme. Also, during the current award period, I began collaborating with Dr. Ariane Atteia and Mr. Robert van Lis, who were at the time located at the Autonomous University of Mexico. Dr. Atteia has since joined my laboratory and Mr. van Lis will also do so when he obtains his Ph.D. in the near future. These individuals bring to the laboratory their interests and expertise in the respiratory components of *Chlamydomonas* and their desire to become experts in tetrapyrrole metabolism. Recently, in a collaboration with Dr. David Bollivar, a former postdoctoral associate who is now at Illinois Wesleyan University, and Dr. Caroline Walker, who was at Clemson University but has since left this research area, we recently made a major breakthrough on the oxygen-independent cyclase reaction, which has now become an important component of the current proposal. Finally, our research on phycobilin biosynthesis in *Synechocystis* has revealed that this organism can grow at very low oxygen concentrations and its genome contains several genes that may encode for enzymes that catalyze alternative oxygen-independent reactions for tetrapyrrole biosynthesis, so characterizing the genes, their enzymes, and regulation of expression have also become parts of the current proposal.

Published papers. During the current award period, two research papers were published documenting work supported by the award. Copies of the papers in Appendix IV. These papers are listed below together with brief summaries.

Willows, R. D., and Beale, S. I. (1998) Heterologous expression of the *Rhodobacter capsulatus bchI*, *-D*, and *-H* genes that encode magnesium chelatase subunits and characterization of the reconstituted enzyme. *J. Biol. Chem.* 273, 34206-34213.

The *bchD*, *bchH*, and *bchI* genes from *Rhodobacter* were cloned separately into expression plasmids pET3a and pET15b. The pET15b constructs produced NH_2-terminally $(His)_6$-tagged proteins. All proteins were highly expressed and were purified to near homogeneity. The BchI and BchH proteins were soluble. BchD proteins were insoluble, inactive inclusion bodies that could be renatured by rapid dilution from 6 M urea. The presence of BchI in the solution into which the urea solution of BchD was diluted increased the yield of active BchD. A molar ratio of 1 BchI:1 BchD was sufficient for maximum renaturation of BchD. This result hinted at a possible protein remodeling role for BchI, which is consistent with the structure of BchI that was determined later. All of the proteins were active in the Mg chelatase assay except His-tagged BchI, which was inactive and inhibited in incubations containing non-His-tagged BchI. Expressed BchH proteins contained tightly bound protoporphyrin IX and they were susceptible to inactivation by light. This result was the foundation for later experiments that suggest a physiological role for this sensitivity to light in the presence of oxygen. Maximum Mg chelatase activity per mol of BchD occurred at a stoichiometry of 4 BchI:1 BchD. The optimum reaction pH was 8.0. The reaction exhibited Michaelis-Menton kinetics with respect to protoporphyrin IX and BchH.

Willows, R. D., Hansson, M., Beale, S. I., Laurberg, M., and Al-Karadaghi, S. (1999) Crystallization and preliminary X-ray analysis of the *Rhodobacter capsulatus* magnesium chelatase BchI subunit. *Acta Crystallographica* D55, 689-690.

These results were the first crystallization of any Mg chelatase component and provided the material for the later determination of the structure of BchI to 2 Å resolution.

Preliminary results. Unpublished results from the current award period that are directly related to this proposal are described briefly below.

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