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Heterologous Expression, Purification, and Characterization of Porphobilinogen Synthase from Rhodobacter Sphaeroides

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Poster Presentation P19

HETEROLOGOUS EXPRESSION, PURIFICATION, AND CHARACTERIZATION OF PORPHOBILINOGEN SYNTHASE FROM RHODOBACTER SPHAEROIDES

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The enzyme porphobilinogen synthase (PBGS, EC 4.2.1.24) catalyzes the first common step in the biosynthesis of tetrapyrrole pigments-- such as heme, chlorophyll, and vitamin B12 (cobalamin)-- by converting two molecules of d-aminolevulinic acid (ALA) into porphobilinogen (PBG) 1. PBGS is categorized by presence or absence of catalytic and allosteric metal ions. All known PBGS sequences contain either a catalytic zinc ion or an allosteric magnesium ion except for those sequences expressed by Rhodobacter capsluatus and Rhodobacter sphaeroids 2. This study presents initial efforts to characterize PBGS in R. sphaeroides in order to better-understand the enzymeís unique characteristics. Evaluating ion dependence for R. sphaeroides PBGS is especially important due to an observed dependence upon divalent cations in the majority of know PBGS enzymes. Protein assays were carried out to determine the effect of various ions including monovalent cations (Na+, NH4+, K+), d! ivalent cations (Mg2+), and divalent anions (SO42-). Additionally, substrate concentration was altered for use in Km and Vmax determinations at varying pH values. The observation that specific activity shows protein concentration dependence suggests that PBGS can dissociate into smaller and less active subunits.