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# Chromatographic Analysis of Pisum Sativum Porphobilinogen Synthase Morpheeins

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# CHROMATOGRAPHIC ANALYSIS OF PISUM SATIVUM PORPHOBILINOGEN SYNTHASE MORPHEEINS

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Porphobilinogen synthase (PBGS) is a metalloenzyme present in all organisms that synthesize tetrapyrrole compounds such as heme, chlorophyll, and vitamin B-12. PBGS catalyzes the condensation of two molecules of 5-aminolevulinic acid to form the tetrapyrrole precursor porphobilinogen. An artificial gene encoding PBGS of pea (Pisum sativum) has previously been designed, expressed, and purified (Kervinen, et al., Biochemistry 39:9018-9029). The specific activity is protein concentration dependent, which indicates that a maximally active enzyme can dissociate into less active smaller units. It has been hypothesized, and subsequently supported, that pea PBGS, essential for chlorophyll biosynthesis, exists as an equilibrium of an active octamer and an inactive hexamer. These different quaternary isoforms have been named "morpheeins." Using a gel filtration (sizing) column, attempts to separate and characterize the morpheeins of pea PBGS were made. Trials were carried out in various buffers, in which the pea protein ran at a characteristic octameric elution time. Further morpheein separation was achieved on an anion exchange column, where separation of putative octamer and hexamer peaks was observed and the ratio of octamer to hexamer was directly related to the concentration of PBGS. Peaks were analyzed multiple ways, leading to the conclusions that the presence of Mg2+ ions appears to stabilize the octameric form of the pea PBGS, and the absence of Mg2+ ions appears to pull the dynamic equilibrium toward the hexameric form. These are the first analyses of the equilibrium mixture of pea quaternary forms under assay conditions. Further study is needed to confirm any interpretations.