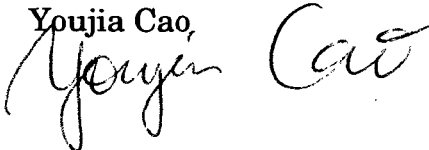


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**STRUCTURAL AND FUNCTIONAL STUDY
OF RABBIT MUSCLE GLYCOGENIN**

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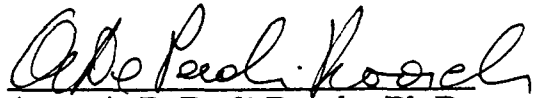
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ABSTRACT

The biosynthesis of glycogen involves a specific initiation event mediated by the initiator protein, glycogenin, which undergoes self-glucosylation to generate an oligosaccharide primer from which the glycogen molecule grows. Rabbit muscle glycogenin was expressed at a high level in *Escherichia coli* and purified close to homogeneity in a procedure that involved binding to a UDP-agarose affinity column. The resulting protein had subunit molecular weight of 38,000 as judged by polyacrylamide gel electrophoresis in the presence of SDS. Purified glycogenin was crystallized and X-ray diffraction data obtained. The preliminary data suggested a compact dimer of glycogenin. Analysis of peptide fragments by mass spectroscopy indicated that the recombinant glycogenin was already glucosylated at Tyr-194. The enzyme was active as a self-glucosylating enzyme and could incorporate up to ~8 glucose residues. The efficacy of the purified glycogenin as substrate for the elongation reaction catalyzed by glycogen synthase was significantly enhanced if glycogenin was first allowed to undergo self-glucosylation. The length of the priming oligosaccharide is thus critical for glycogen synthase action. Fully primed glycogenin was also a substrate for glycogen phosphorylase, which removed glucose from the oligosaccharide attached to glycogenin in a phosphorolysis reaction similar to that involved in glycogen degradation. Treatment of fully primed glycogenin with phosphorylase converted glycogenin to a form less effective as a substrate for glycogen synthase, and hence could affect the synthesis of glycogen. These results suggest a novel role for glycogen phosphorylase in

the control of the initiation of glycogen biosynthesis. Of several oligosaccharides of glucose surveyed, only maltose caused significant inhibition of the glycogenin self-glucosylation reaction. Mutation of Tyr-194 to either phenylalanine or threonine disabled self-glucosylation. However, both wild type and mutated glycogenin were catalytically active for the glucose transfer to a maltose acceptor, indicating that Tyr-194, though the site of carbohydrate attachment, is not necessary for catalytic activity.

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