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**INTRAMOLECULAR INTERSUBUNIT MECHANISM OF RABBIT
MUSCLE GLYCOGENIN SELF-GLUCOSYLATION**

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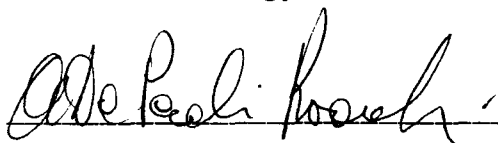
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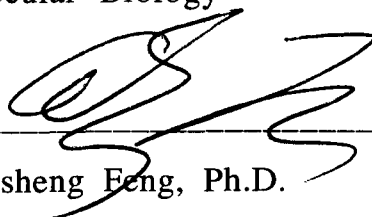
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ABSTRACT

Glycogenin, the self-glucosylating initiator for mammalian glycogen biosynthesis, transfers glucose residues from UDP-glucose to form an oligosaccharide chain attached to Tyr194 of the protein. Mutation of Tyr194 to Phe is known to result in the loss of self-glucosylation activity since the site of glucose attachment is lost. However, this mutant can still transfer glucose to exogenous acceptors, indicating that Tyr194 is not critical for catalytic activity. Kinetic studies had previously suggested that the reaction is intramolecular and gel filtration had indicated that glycogenin exists as an oligomer. However, the intramolecular reaction could be either intersubunit or intrasubunit. The objective of this work was to determine whether the intramolecular self-glucosylation reaction is intersubunit or intrasubunit.

Sequence alignment showed that Lys85 is highly conserved among glycogenins from different species. To test whether this lysine residue is required for activity, a K85Q mutant was constructed and expressed in *Escherichia coli* with an N-terminal hexahistidine tag. The protein was purified to homogeneity by Ni²⁺ chelate chromatography. A Y194F mutant was expressed as a GST-fusion protein and was also purified close to homogeneity. Neither mutated protein was active for self-glucosylation. However, intersubunit glucosylation was observed upon mixing the Y194F and the K85Q mutants.

Wild type and K85Q mutant glycogenin were also coexpressed in *E. coli* and copurified. The wild type polypeptide could glucosylate both itself and the

K85Q mutant. The ratio of the two proteins after a two step affinity chromatographic purification was not 1:1. A possible explanation is that there is an exchange of subunits between different oligomeric species.

Kinetic studies of separately purified Y194F and K85Q mutant proteins were conducted. Glucosylation of the K85Q polypeptide was observed, after an initial lag. The maximal rate reached was up to 20% of that observed for the wild type protein. The lag was abolished if the two proteins were preincubated. The results of this work provide strong evidence for the occurrence of inter-subunit glucosylation.

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