The Structure of Rabbit Muscle Glycogenin: A Unique Self-Glucosylating Enzyme

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The structure of rabbit muscle glycogenin, a unique self-glucosylating enzyme, has been solved using MAD-phasing from selenomethionine-substituted enzyme crystals. Glycogenin initiates glycogen synthesis via self-glucosylation on Tyr-194. Once glycogenin has extended the glucose polymer to ~10 residues, glycogen synthesis is continued by glycogen synthase and the branching enzyme. Glycogenin-like self-glucosylating enzymes have been identified in many organisms from yeast to humans. Glycogenin is a 333 amino acid protein that forms ~80 kDa dimers in solution. The structure was solved using crystals that were tetragonal ($P4_32_12$, a=b=140 Å, c=417 Å) and diffracted x-rays weakly. A three-wavelength 3.8 Å Se-Met data set and a 3.4 Å native data set were collected at the Structural Biology Center, beamline 19-ID at the APS. The program Solve was used to find a 40-site solution to 4.5 Å. The program Resolve produced a density-modified map that showed clear evidence for an improper 10-fold noncrystallographic arrangement of the contents of the asymmetric unit. Phases to 4.5 Å from this initial solution were transferred to the native data set in CCP4. The program DM was used to apply 10-fold averaging, solvent flipping, and phase extension to the limit of the native data. The resulting 3.4 Å averaged map was of high quality and resulted in a model for residues 1-231 and 241-267. The remainder of the protein structure, residues 268-333, is disordered. The current model has been refined to 3.4 Å using strict noncrystallographic symmetry constraints to a R_{work} of 25.2% and a R_{free} of 28.6% (Fig. 1). Although sequence identity among glycosyltransferases is minimal (generally <10%), the overall fold of glycogenin is similar to other enzymes of this class. The core structure comprises a modified α/β domain containing 4 parallel β-strands and two antiparallel strands. A new orthorhombic crystal form with only a single subunit in the asymmetric unit that diffracts to 1.9 Å confirms the original 3.4 Å structure and has provided important information on substrate binding and catalysis. A manuscript describing the structure and mechanism of glycogenin is in preparation.

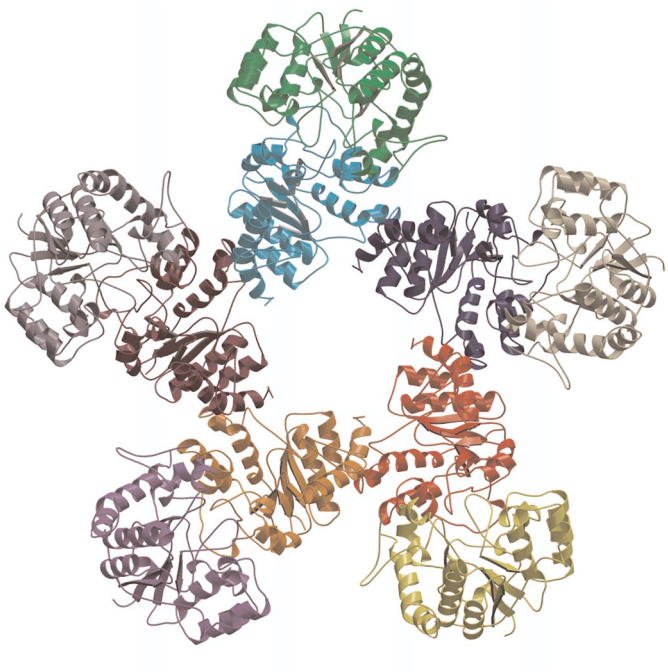

FIG. 1. Ribbon diagram of the glycogenin decamer showing the noncrystallographic five-fold axis. Each unit of the pentagonal arrangement shown here is composed of a dimeric assembly of glycogenin.

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