Structure of the *E. coli* Branching Enzyme

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Introduction

I – Starch and Glycogen

The most important storage polysaccharides in nature are starch in plant cells and glycogen in animal and bacteria cells. Starch and glycogen are polymers of glucose residues. They are composed of two different molecules, amylose and amylopectin. Amylose is a linear glucose chain of 840 to 22,000 glucose residues.[1] Amylopectin is the branched polymer and major component of starch and glycogen. Glycogen structure differs from starch in that it is more extensively branched.

There are three enzymes involved in the biosynthetic pathway of starch and glycogen. The first enzyme, ADP-glucose pyrophosphorylase activates the glucose molecule by the formation of ADP-glucose. The elongation of the glucan chain is catalyzed by starch/glycogen synthase. The last enzyme in the pathway is the branching enzyme, which is responsible for the formation of the branching points.[2]

There has always be an interest in studying starch biosynthesis because of its relevance as an energy reservoir and as the main source of carbohydrate ingestion in humans. During the last decade starch's unique properties have awakened an interest for its use in industrial purposes. Understanding this biochemical pathway is not only of nutritional interest but is also of interest in the agriculture and material science fields.

II - Branching Enzyme

Branching enzyme has an important role in the determination of the structure of starch and glycogen. It catalyzes the formation of branching points by cleavage and transfer of the glucan chains.[3] The formation of these branching points are necessary to assure glycogen solubility in the cell and to increase the number of non-reducing ends, thus making the glycogen more reactive to both synthesis and digestion.[4] Previous studies performed on maize branching enzyme suggests that the C-terminal is responsible for substrate specificity, and the N-terminal and α/β barrel are responsible for chain transfer.[5]

III - Specific Aims

Starch synthesis has been a field studied since the 1940s. Although great progress has been achieved in the determination of its pathway, its chemistry is not fully understood. This is mainly due to the lack of structural models. There are no structures of any of the enzymes involved in starch/glycogen biosynthesis. The three dimensional structure of branching enzyme will reveal valuable information that will help in the understanding of the relationship between its structure and function.

The information that this structure will reveal may allow us to control the starch structure by predetermining the ratio of amylose to amylopectin and/or its degree of branching. This will not only be of great nutritional importance but will also be of great importance in the development of new biodegradable packing materials.

There are a number of inherited enzyme defects that result in impaired glycogen metabolism. One of these diseases is the Andersen glycogen storage disease, which is caused by a defective branching enzyme. This defective branching enzyme is unable to form the branching points in glycogen, and therefore the unbranched glycogen polymer is insoluble in the cell. This disease causes the death of many infants every year.

Methods and Results

Branching enzyme crystals belong to space group P21; a= 91, b=102.6, c=185.4 Å, β=91.4 and Z=4. Though these crystals diffract weakly on home sources (3.5 Å); we were able to collect a 2.3 Å native data set at the Structural Biology Center Collaborative Access Team (SBC-CAT)19-ID beamline at the Advanced Photon Source (APS) (March 2000). In order to find the phases, a one wavelength anomalous dispersion experiment at the selenium absortion peak was performed at the Industrial Macromolecular Crystallography Association Collaborative Access Team (IMCA-CAT) 17-ID beamline (August 2000). This information combined with a mercury heavy atom soak data set collected at our home source was used to search for the 64 selenium in the asymmetric unit. Using the programs Solve and Sharp, a total of 61 selenium and 4 mercury sites were found. The quality of the experimental electron density map was greatly improved by solvent flattening and four-fold averaging techniques.

Discussion

Branching enzyme belongs to the amylotic family of enzymes; these enzymes have a central α/β-barrel domain. We have been able to identify that domain in branching enzyme, as well as the seven residues involved in branching activity. We have already traced and assigned most of the first branching enzyme molecule and we are currently working on the other three. Once the whole asymmetric unit is traced, we will proceed to implement phase combination, which will improve the quality of the electron density map, and the structure will be refined to a resolution of 2.3 Å.

Acknowledgments

Use of the SBC-CAT beamlines at the APS was supported by the U. S. Department of Energy (DOE), Office of Biological and Environmental Research, under Contract No. W-31-109-ENG-38. Data were also collected at IMCA-CAT beamline 17-ID at the APS. These facilities are supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with the Illinois Institute of Technology (IIT), executed through IIT's Center for Synchrotron Radiation Research and Instrumentation. We would also like to thank Dr. Rongguang Zhang and Dr. Andrezej Joachimiak from the Structural Biology Center and Dr. Jorge L. Rios and Dr. Andrew Howard from the IMCA-CAT beamline. The APS is supporeted by the U. S. DOE, Office of Science, Office of Basic energy Sciences under Contract No. W-31-109-ENG-38.

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