A Structured and Multi-cellular Model of Starch Biosynthesis in Potato

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ABSTRACT: Recently, systems biology has been increasingly applied to gain insights into the complexity of living organisms. Many inaccessible biological information and hidden evidences for example flux distribution of the metabolites are simply revealed by investigation of artificial cell behaviors. Most bio-models are models of single cell organisms that cannot handle the multi-cellular organisms like plants. Herein, a structured and multi-cellular model of potato was developed to comprehend the root starch biosynthesis. On the basis of simplest plant cell biology, a potato structured model on the platform of Berkley Madonna was divided into three parts: photosynthetic (leaf), non-photosynthetic (tuber) and transportation (phloem) cells. The model of starch biosynthesis begins with the fixation of CO_2 from atmosphere to the Calvin cycle. Passing through a series of reactions, triose phosphate from Calvin cycle is converted to sucrose which is transported to sink cells and is eventually formed the amylose and amylopectin (starch constituents). After validating the model with data from a number of literatures, the results show that the structured model is a good representative of the studied system. The result of triose phosphate (DHAP and GAP) elevation due to lessening the aldolase activity is an illustration of the validation. Furthermore, the representative model was used to gain more understanding of starch production process such as the effect of CO_2 uptake on qualitative and quantitative aspects of starch biosynthesis.

1 INTRODUCTION

Since ideas of constructing a model for living organisms were initiated to gain insights into complex cell behaviors, various kinds of modeling techniques have been increasingly developed. The development of techniques from simple mathematical modeling methods for small biological pathways such as glycolysis to highly complex calculation methods for huge systems such as modeling of the whole cell of *Saccharomyces cerevesiae* and *Escherichia coli*, shows the accomplishment of the modeling research to imitate the behaviors of living cells [1-3]. However, to develop a good model, the step of selecting techniques or calculation methods to construct model representatives of living organisms is also very important. Either a static or a dynamic mode of calculations should be appropriately chosen depending on each individual study. Some investigations require only the analysis of steady state quantity but some require the analysis on time scale. An example of the former case is the flux distribution study of high-branched pathways using metabolic flux analysis (MFA) [4]. For the latter case, observing behaviors of highly oscillated systems is essential to use a dynamic analysis with kinetic information. Besides the modes of calculations, defining a scope or size of studied pathways is considered to be equally important. Although, modelers normally expand/curtail the boundary of studied systems as they prefer, there are some limitations that prohibit modelers to extend the model boundary. Especially, the models of pathways involving in more than one cell do need a good strategy to model the processes that lay across the cells. This problem becomes more crucial as modeling techniques have been applied to understand more complicated biological cells like plants.

Plants are fantastic living organisms. They can produce different beneficial organic compounds from inorganic compounds and light energy. These compounds are, for examples, starch, alkaloids, lipids and other secondary metabolites. As a result, plants are recognized to be the biggest resources for food and energy of the world [5]. Starch, a secondary metabolite produced and stored in a specific compartment of plant cells, is not only very useful for all lives in term of food resource but it also has been utilized in a number of biotechnological and biochemical applications for instant pet food, cosmetic, and medical mixture compounds. Even though sufficient quantity of starch can be obtained from several sources of plants; each source does not give the identical properties of starch that enables to substitutionally use in particular applications. It results in insufficient amount of plant starch having the satisfied properties for a specific application. Typically, the applications of plant starch are dictated by the starch properties for example, granule size, shape, and viscosity [6]. To serve a starch based biotechnological industry, the plant starch with specific properties is highly required. Due to the high demand of knowledge about the starch production in plants, it has drawn many researchers to study the starch synthesizing process aiming to gain the better understanding of the starch biosynthesis. Starch biosynthesis is a process that operates continuously within three major types of cells involving in both photosynthetic and non-photosynthetic pathway. During the photosynthesis,

the fixed carbons are catalyzed through Calvin cycle and series of enzymes to produce transitory starch accumulated in leaf cells during a day. At night (non-photosynthetic pathway), the transitory starch is then broken-down into sucrose via the starch degradation process which locates in leaf cells before mobilizing through phloem tubes to storage organs. Sucrose being a substrate of the starch synthesis is subsequently converted into permanent starch in the storage cells for examples meristems, root cap cells, seeds, fruit, and tuber [7]. All the processes operate with highly related to each other. A perturbation at a specific enzyme in a single pathway may cause harsh alteration of the whole system. Moreover, the high interaction between each pathway inside plant cells leads to the unsteady operation of the system [8].

The starch biosynthesis is a large system which locates in several cell-types of multi-cellular organisms. Therefore, development of a model of starch biosynthesis requires a good strategy to deal with its complexity. A structured model of three types of cells: leaf, phloem and tuber, is designed for constructing the starch biosynthesis. In addition, kinetic mode calculation is selected to investigate fluxes and metabolite concentrations in the studied pathways since the operations in plants do not frequently establish in a steady state. In brief, this work attempts to apply the compartmentalized kinetic model concept to examine the starch biosynthesis aiming to gain more comprehension in behaviors of the studied pathway. Beginning with CO_2 fixation in photosynthesis, five pathways involving in the starch biosynthesis which are Calvin cycle, starch degradation, sucrose synthesis, sucrose transportation, and starch biosynthesis in tuber are modeled. Afterward, investigation the effects of change in the CO_2 concentration on starch yield and amylose to amylopectin ratio, an indicator of starch properties, is examined as an application of model once the model is successfully validated. For this short report, only one part of the model which is the kinetic model of the tuber cell will be shown. The separate model will be validated and used to analysis the effect of key enzyme activity on yield of starch and ration of amylose and amylopectin.

2 STRATEGY FOR DEVELOPING STRUCTURED MODEL OF STARCH BIOSYNTHESIS

A general outline for constructing the model of a given process starts with gathering data, developing a model, validating the developed model and then simulating it for prediction. This normal procedure is effectively applicable to uni-cell modeling. For multi-cell modeling, one important additional step is designing architecture of the model. This step enables modelers to deal with the complexity of multi-cellular system leading to obtain a good model layout of the studied organism. Model architecture designing is the beginning stage of the model development. The model layout of starch biosynthesis compartmentalized model defined in this work is shown in Figure 1. Each compartment is proposed to construct and validate separately before connecting altogether.

3 MODEL DEVELOPMENT

The tuber cell is the first part to be created. The starch biosynthesis in the tuber starts from the breakdown of transported sucrose from the phloem cells. Glucose-6-phopsphate which is a product of the sucrose breakdown in the cytosol is next transported to the amyloplast, a specific organelle for synthesizing and storing permanent starch. In the amyloplast, glucose-6-phosphate is an initial substrate for producing amylose and amylopectin which are main components of starch granule.

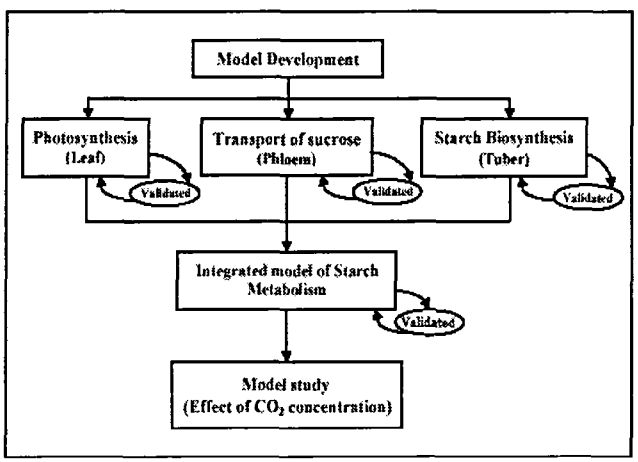

Figure 1 : The architecture of starch biosynthesis model

The pathway of starch production in the tuber cells is demonstrated in Figure 2. For the starch biosynthesis model in tuber cell, it is developed based on the following assumptions.

1. To avoid unnecessary complex system, only central pathways of the starch biosynthesis are modeled.
2. Since the mechanism of starch assimilation from amylose and amylopectin molecules is not clearly understood, the developed model ends at the production of amylose and amylopectin.
3. The yield of starch is assumed to be proportional to the amount of amylose and amylopectin.

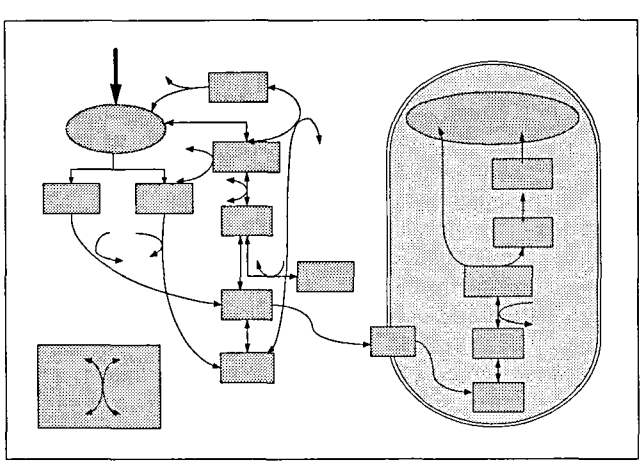

Figure 2 : The modeled pathway of starch biosynthesis in tuber cell

Based on the metabolic pathway of starch biosynthesis along with the assumption of the model, the kinetic model constructing from a set of ordinary differential equations (ODEs) is developed based on the material balance around each metabolite and the rate law. The kinetic parameters of each enzyme in the pathway are collected from available published data and databases [9]. More details of model information can be seen in the website of Systems Biology and Bioinformatics Research Group (KMUTT): http://www.kmutt.ac.th/sbi/. After the model is completely developed, a further important step is the model validation. This step indicates the ability of models to represent behaviors of master cells and also implies the predictability of constructed models.

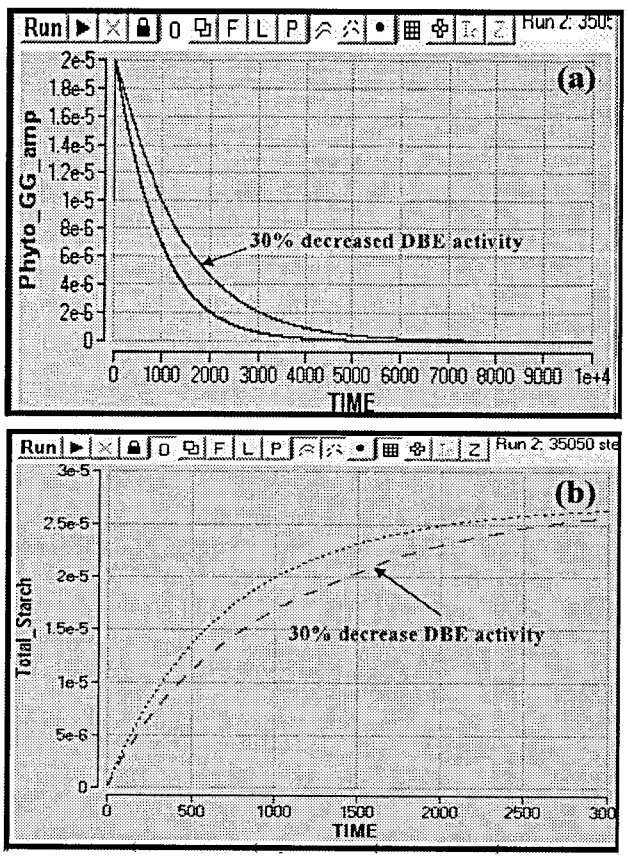

Figure 3 : (a) The alteration of phytoglycogen concentration and (b) the amount of total starch due to 30% decreasing of DBE activity

To validate the kinetic model of starch biosynthesis in the tuber cell, the kinetics of enzymes in the studied metabolic pathway is perturbed and its effects on certain metabolite concentrations are investigated. The following corresponding behaviors of simulated results to numerous evidences from many literatures indicate a good represent ability of the model to the real cell. Primarily, the operation of debranching enzyme is used to check the representativeness of the model. Debranching enzyme (DBE) is a key enzyme of starch synthesis responding in modifying highly branched-carbohydrate molecule (phytoglycogen) to amylopectin. The significance of DBE on starch biosynthesis is to produce amylopectin whose structure compatible for forming the starch granule. Naturally, starch granules are formed after amylose and amylopectin are produced. Several hypotheses and disagreements of the starch granule formation mechanism from amylose and amylopectin molecule have been discussing [10-13]. The most admired postulation believes that amylopectin molecules firstly form the structure of granule and amylose molecule afterward fill up in between the space of the amylopectin structure [11, 12]. The structural characteristic of amylopectin is then very critical to perfectly form the starch granule. Therefore, deficient in DBE will directly affect both yield and properties of starch. Dinges and his colleagues [14] found the accumulation of phytoglycogen concentration or sometimes called water-soluble polysaccharide (WSP) when gene encoding DBE is defected. Figure 3a shows coincident event as found in Ding's work. As can be observed, when 30% of DBE activity is repressed the concentration of phytoglycogen significantly increases comparing to the control model experiment. Moreover, Figure 3b shows a significant decrease in the starch in the particular condition which agrees with the previous study [15].

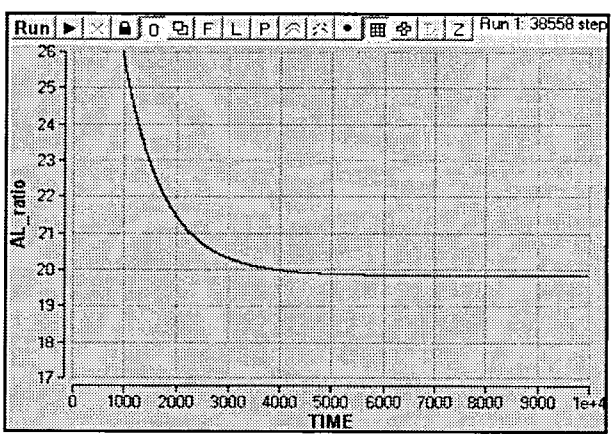

Figure 4 : Percent of amylose content of potato starch biosynthesis model.

The further evidence used for testing the model is the content of amylose in the produced starch. Amylose content of plant starch is generally specific to the sources of starch. Cassava starch holds the amylose content about 17 percents [16] whilst corn starch approximately contains 28 percents of the amylose [16]. Normally, potato starch composes of the amylose about 18-20 percents [16, 17]. The percent of the amylose content from the simulated results in Figure 4, 19-20 percents, shows the agreeable value as indicated in literatures.

4 MODEL APPLICATION

The validated kinetic model of starch biosynthesis is further simulated to comprehend the behaviors inside the starch biosynthesis pathway once the system is perturbed. Effect of the change of the granule bound starch synthase (GBSS) activity on yield and properties of produced starch is

investigated using the built model. GBSS is an important enzyme that is believed to have a significant role on the amylose synthesis. As waxy starch which contains very small amount of the amylose becomes more interesting, the action of GBSS in the starch biosynthesis pathway is studied further. Herein, approximately 50 percents of the GBSS activity in the model is repressed and every metabolite concentration and flux through enzyme is monitored. The simulation shows the dramatic decrease in the amount of the amylose, Figure 5a, leading to vividly lessen the amylose content in the total starch, Figure 5b. These results may not surprise plant physiologists because similar behaviors are exhibited in several literatures [10, 18]. Nevertheless, the results of GBSS activity repression on the amylopectin concentration would attract all researchers. Figure 5b demonstrates that the amount of the amylopectin increases according to the reduction of the GBSS activity. Interestingly, there is no report that indicates the influence of the GBSS activity on the amylopectin production. This result might be a new behavior revealed by model simulation. The elevation of amylopectin concentration is believable and is able to describe with the alteration of ADP-glucose partition at the branch point. To illustrate, a part of ADP-glucose that ever belongs to the GBSS enzyme is suddenly occupied by the soluble starch synthase (SS) for producing the amylopectin. The increment of the flux through the SS enzyme shown in Figure 6 supports the explanation.

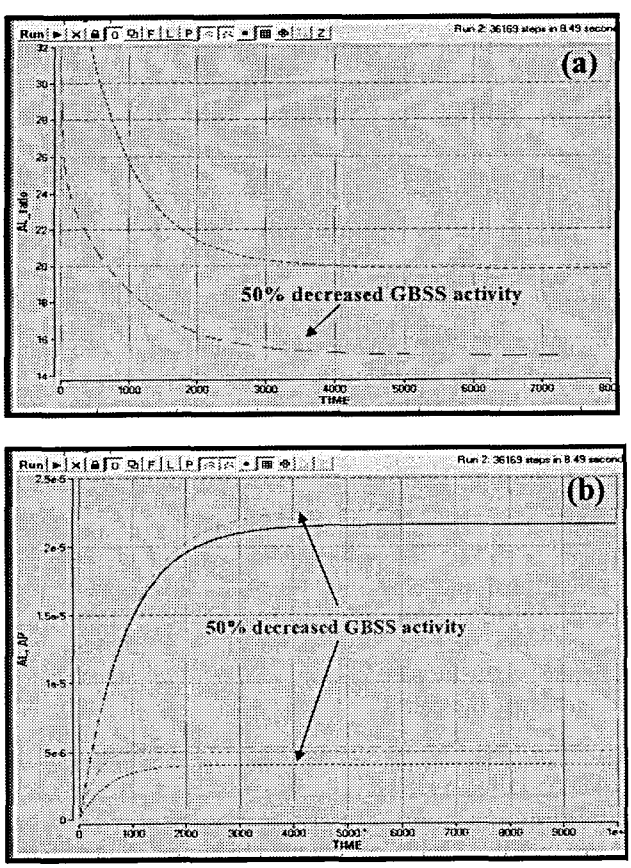

Figure 5 : (a) The alteration of amylose content and (b) amylose and amylopectin concentration due to 50% decreasing of GBSS activity

As seen in the previous part that the mathematical model extents us to observe and understand beyond the frontier of experimental world. Insight the complex system as living organism, high capability tools and techniques for example mathematical modeling and high-throughput technology are extremely required. Although the model prediction contributes the goodness in various aspects, it has to be careful that it works very well within its assumptions.

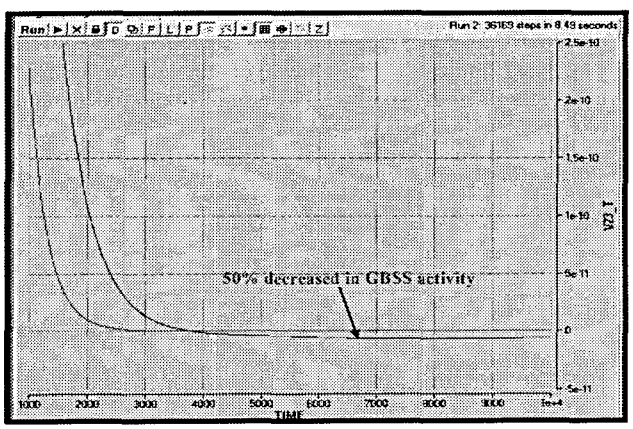

Figure 6 : The alteration of flux through enzyme soluble starch synthase (SS) due to 50% decreasing of GBSS activity

5 CONCLUSION

The tuber model gives us a reasonable hypothesis for further study. However, the complete model of the three-cell model will gain more understanding and useful hypotheses. The structured model will allow us to model the huge pathway that operates across the cells. The starch biosynthesis in this case is a good example of the structured model representing the real behaviors of pathway operation. In addition, the model enables us to examine the biological processes and the cell behaviors through the level of metabolites under metabolic regulation. The predictive results lead to a number of applications: (i) investigating the metabolite flux/concentrations at operation condition, (ii) generating the hypothesis on the basis of predicted data, and (iii) predicting altered behaviors of cell under one/many factors perturbed condition. These are the contribution of modeling whose benefit will be synergized when collaborating with the strong biological background.

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