Abstract of Thesis

Name (Widhi Dyah Sawitri)	
Title	Study on structure and function of sucrose phosphate synthase from sugarcane (サトウキビ由来sucrose phosphate synthaseの構造機能研究)

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Sucrose phosphate synthase (SPS; EC 2.4.14) catalyzes transfer of glycosyl group of UDP-glucose (UDP-G) to fructose 6-phosphate (F6P) to form sucrose 6-phosphate (S6P) and SPS activity is modulated by an allosteric activator glucose-6-phosphate (G6P). Plant SPS plays a key role in photosynthetic carbon metabolisms, whose function is regulated by environmental conditions such as diurnal cycles of light/dark and osmotic stress. Plant SPS consists of a single polypeptide with a molecular mass of 120 kDa. Bacterial SPS, which shows no allosteric regulation, consists of a polypeptide shorter by about 10 kDa than the plant enzyme by lacking two regions corresponding the N-terminal and C-terminal region of the plant SPS. My research aim was to study structure and function of the plant enzyme by focusing structural basis for the allosteric property.

I have tried expression and purification of recombinant sugarcane SPS (*So*SPS1) using *Escherichia coli* and Sf9 insect cells to investigate structure-function relationship of the regulation of the enzyme activity. When it was expressed in *E. coli*, two forms of SPS with different polypeptide sizes were accumulated in the bacterial cells. The larger form was comparable in size with the authentic enzyme from sugarcane leaves and the shorter one had an N-terminal region trimmed by about 20 kDa. However, only enzyme with the authentic size was produced in insect cells. It was shown that the purified trimmed SPS from *E. coli* and the full size enzyme from insect cells were active, but their enzymatic properties were markedly different: the full size enzyme showed changeable affinities with the two substrates with being converted to a high affinity form in the presence of G6P, while the trimmed enzyme showed the high substrate affinities without G6P.

It was further prepared a series of N-terminal truncated SPSs with variable lengths from 11 to 171 residues deleted. By kinetic analysis of the full length and truncated forms of *So*SPS1, three important results were obtained; 1) the full length enzyme showed a remarkable allosteric activation by G6P, while none of the truncated enzymes tested here had such characteristics, 2) specific activity of the full length and truncated SPSs showed a tendency that longer truncation has higher activity, and 3) the truncated forms showed a similar substrate affinity with F6P and UDP-G irrespective of the absence of G6P, while the full length enzyme showed such a high affinity only in the presence of G6P. These results indicated that the N-terminal region of *So*SPS1 is crucial for the allosteric regulation and may function like a suppressor domain for the enzyme activity.

It was previously reported that plant SPSs are tetramer or dimer depending on experimental conditions, whereas cyanobacterial and bacterial SPSs are monomeric enzymes. Our results determined that both native states of full-length and N-terminal truncation enzymes

were oligomeric. It could be hypothesized that the N-terminal domain of plant SPS plays a direct role in allostery by inducing a structural perturbation upon binding to G6P, which might include model states as a G6P-bound state with high activity and unbound state with intermediated or reduced activity. The 3D structural studies of plant SPS is necessary to draw more extensive story of the allostery of plant SPS and I am now intensively trying crystallization of the full length and truncated enzymes.

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論文審査の結果の要旨及び担当者

氏　名	(Widhi Dyah Sawitri)	
	(職)	氏　名
論文審査担当者	主査　教授	中川敦史
	副査　教授	長谷俊治
	副査　教授	栗栖源嗣

論文審査の結果の要旨

サトウキビによるショ糖の生産は、インドネシアなどの熱帯・亜熱帯諸国の重要な産業であり、生産の増強や効率化は重要な研究テーマである。本研究では、植物においてショ糖生合成の鍵となる fructose 6-P を sucrose 6-P に変換する酵素であるサトウキビ由来 sucrose phosphate synthase (SPS) の機能構造相関の解析を行った。高等植物では昼間と夜間でショ糖の合成が制御されているが、植物由来 SPS は、このような制御のないバクテリア由来 SPS と比べてN末端およびC末端領域付加的な領域を有しており、この領域が活性制御に何らかの関与をしてのではないかと考えられた。そこで、本酵素の大腸菌および昆虫細胞発現系を用いた大量発現系を構築し、全長、C 末端欠損変異体、及び数多くのN末端欠損変異体を発現させて、組換え体細胞内で容易に生じる限定分解産物を取り除く工夫を施しながら目的の変異酵素の部分精製標品を調製して、その酵素活性の速度論的特性を詳細に評価した。その結果、高等植物に特有のN末端領域が酵素活性発現に抑制的に働き、glucose 6-P が存在することでこの抑制効果が解除されることを見出した。この成果は、長年不明であった本酵素のアロステリック制御の分子基盤を蛋白質工学と生化学的手法を用いて明らかにしたものである。この研究は、植物のショ糖生合成の制御機構に関する重要な新知見を提供するとともに、今後の SPS の基礎的・応用的な研究発展に新たな糸口を与えるものである。

よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。