氏　　名	織 田 康 則
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本　　籍	新潟県
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学位授与の題目	Hormonal and Biochemical Controls of Haemolymph Sugars in *Bombyx mori*(カイコ血糖の内分泌学的・生化学的調節機構)
論文審査委員(主査)	櫻井　勝(理学部・教授)
論文審査委員(副査)	岩見　雅史(研究科・助教授) 和田敬四郎(理学部・教授) 福森　義宏(理学部・教授) 板垣　栄治(理学部・教授)

学 位 論 文 要 旨

Abstract

(1) Haemolymph of *Bombyx* larvae contains two major sugars, trehalose and sorbitol-6-phosphate (S-6-P). Their concentrations changed throughout the postembryonic development but were relatively constant in the feeding period of 5th stadium. In the feeding period, haemolymph trehalose concentration was under the homeostatic control while haemolymph S-6-P concentration was not.

(2) Head factor(s) with hypertrehalosemic activity was involved in homeostasis of trehalose concentration. One candidate of such head factors was an adipokinetic hormone (AKH) since AKH exhibited hypertrehalosemic activity in *Bombyx* larvae.

(3) Haemolymph trehalose and S-6-P concentrations decreased at the wandering stage of 5th stadium. Such decreases were caused by an increased ecdysteroids in haemolymph at this stage.

(4) Ecdysteroid-inducible increase in membrane-bound S-6-Pase (m-S-6-Pase) activity in fat body was involved in the decrease in haemolymph S-6-P concentration at the wandering stage. m-S-6-Pase was purified to apparent homogeneity from fat bodies of wandering larvae by fractionation of membrane, solubilization with 1 % HTG and two chromatographic separations on Q-Sepharose and Heparin-Sepharose. Purified m-S-6-Pase was composed of a single protein with a molecular mass of 30 kDa. The optimum pH of purified enzyme was 6.0 and its Km value for S-6-P was 4.56 mM.

1. Difference and similarity of two haemolymph sugars

Haemolymph of *Bombyx* larvae contains two major sugars, trehalose and sorbitol-6-phosphate (S-6-P). Their concentrations changed throughout the postembryonic development but were relatively constant in the feeding period of 5th stadium (Figure 1). Haemolymph trehalose concentration was revealed to be under the homeostatic control since its concentration could recover the initial level after artificial increase or decrease of its concentration. Accordingly, trehalose is considered to be an insect blood sugar corresponding to glucose in mammals. In contrast, haemolymph S-6-P concentration was not homeostatically controlled. It accumulated in haemolymph during the feeding period and decreased at the larval-pupal transformation, indicating that it is a storage form of sugar. Along with the dynamics of storage protein in larval haemolymph, present findings suggest that insect utilizes haemolymph (haemocoel) as a storage site for energy source in addition to circulating system.

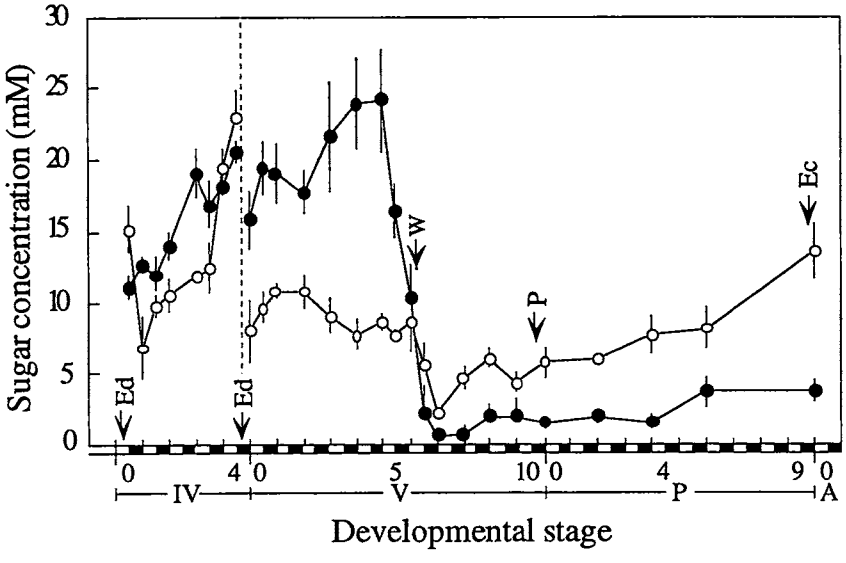

Figure 1 Developmental changes in haemolymph trehalose (open circles) and sorbitol-6-phophate (closed circles) concentrations in *Bombyx mori*. Ed, ecdysis; W, wandering; P, pupation; Ec, eclosion.

Modes of incorporation of trehalose and S-6-P from haemolymph into cells seem to be similar to each other (Figure 2). Both trehalose and S-6-P must be hydrolyzed prior to incorporation into cells. Trehalose is converted to two molecules of glucose by trehalase that locates on cell membrane, and glucose is incorporated into

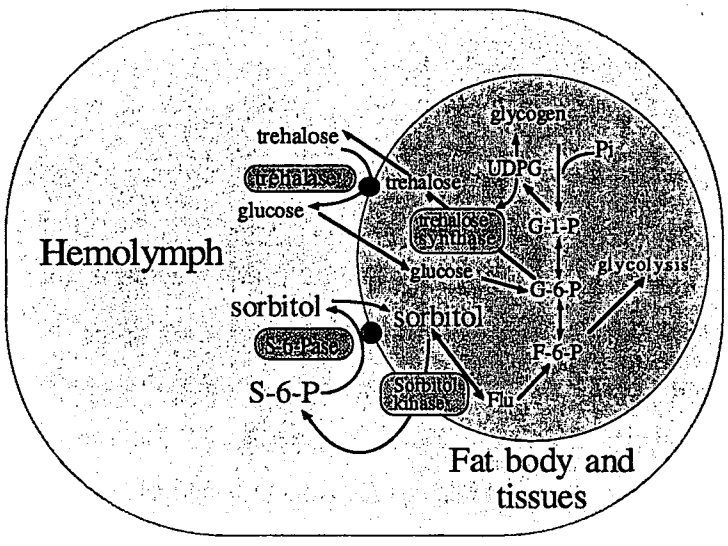

Figure 2 Putative metabolic pathway of trehalose and S-6-P.

cells with assistance of glucose transporter. Present study showed that S-6-P was converted to sorbitol by S-6-Pase that is probably located on cell membrane. Resulting sorbitol may be directly incorporated into cells through sorbitol transporter on cell membrane. These enzymes, trehalase and S-6-Pase, on cell membrane are keys to control haemolymph sugar metabolism.

2. Control of haemolymph trehalose

Haemolymph trehalose concentration is under two different controls, i. e. homeostatic and developmental controls. Trehalose concentration is maintained at constant level in a range of hours but developmentally changes in a range of days.

For homeostasis of trehalose concentration, head factor with hypertrehalosemic activity was prerequisite. One candidate of such head factors is adipokinetic hormone secreted from corpora cardiaca since present study suggested that AKH had hypertrehalosemic activity in *Bombyx* larvae. The mechanism for down-regulation of trehalose concentration is probably present in thoracic and/or abdominal portions.

In contrast to homeostatic control, developmental changes in haemolymph trehalose concentration are likely controlled by hormones that are involved in integrating all developmental events. We demonstrated that ecdysteroid, a moulting hormone secreted form prothoracic gland, was involved in the decrease in trehalose concentration at the wandering stage. The physiological meaning of the changes in

trehalose concentration is obscure. However, developmental changes in trehalose concentrations seem to reflect the changes in demand of tissues for trehalose throughout development.

3. Control of haemolymph S-6-P

S-6-P accumulates in haemolymph in the feeding period and the S-6-P concentration decreases to a low level at the wandering stage. Such decrease is caused by an increase in haemolymph ecdysteroids at this stage. Present study showed that an ecdysteroid-inducible increase in membrane-bound S-6-Pase (m-S-6-Pase) activity in fat body was involved in the decrease in haemolymph S-6-P concentration. Resulting sorbitol may be incorporated into tissues and stored or utilized for pupal metamorphosis, similar to the storage protein. m-S-6-Pase was purified to apparent homogeneity from fat bodies of wandering larvae by fractionation of membrane, solubilization with 1 % HTG and two chromatographic separations on Q-Sepharose and Heparin-Sepharose. Purified m-S-6-Pase was found to be composed of a single protein with a molecular mass of 30 kDa. The optimum pH of purified enzyme was 6.0 and its Km value was 4.56 mM. The Km value may be enough to cause a decrease in the haemolymph S-6-P concentration at the wandering stage. Accordingly, we propose that the purified S-6-Pase in present study is responsible for the decrease in haemolymph S-6-P *in vivo* and acts as a principle to cause metabolism-transformation at the pupal metamorphosis. The localization of m-S-6-Pase and the mechanisms of its activation by ecdysteroid must be elucidated by further studies. Such studies will provide new insights into the particular insect system for energy storage as a phosphorylated form of carbohydrate in haemolymph.

学位論文審査結果の要旨

本論文は，カイコ幼虫血糖の動態とそのホルモン支配を扱ったものである。昆虫の主要血糖はグルコース２分子からなる２糖類のトレハロースであるが，カイコ幼虫の血液はこの他にソルビトール-６-リン酸(S-6-P)を多量に含む。論文提出者はまず，トレハロースとソルビトールのガスクロマトグラフィーによる定量法を確立し，カイコ５齢幼虫期から成虫羽化にかけての血糖変動をそれぞれの糖について精査した。この結果，トレハロース濃度は幼虫期摂食期に一定であり，ガットパージ時に急激に低下すること。また，S-6-P濃度は摂食期に漸増し，ガットパージ前日に急減することを示した。これらの減少は体液中のエクジステロイド濃度の上昇によることを実証した上で，S-6-P濃度の減少がエクジステロイドによるS-6-Paseの活性化によることを示し，この酵素の活性動態とエクジステロイドとの関係，その細胞内分布などを調べた。その結果，S-6-Paseは脂肪体細胞膜体腔側に分布し，膜結合型フォスファターゼであり，この活性がエクジステロイドにより上昇することを示した。酵素精製を行った結果，分子量30Kの単量体で，その酵素特性は既知のフォスファターゼとは異るものであった。一方，トレハロース濃度はホメオスタシスの下にあり，そのホルモン支配について検討した結果，脂質動員ホルモン(AKH)が血糖上昇ホルモンとして幼虫期には作用することを発見した。幼虫期での血糖上昇にAKHが作用していること，またそれがホメオスタシスのup-regulatorとして機能していることは，初めての証明である。以上の結果は，昆虫の血糖の持つ意味，特にエネルギー保存形態としてのS-6-Pの昆虫発育における役割，及び血糖動態の内分泌支配を明らかにしたもので，昆虫生理学に新たな知見を加えたものである。よって，本論文は博士(理学)の学位を授与するに十分なものであると判定した。