Isolation and Characterization of a Humoral Factor That Stimulates Transcription of the Acyl-CoA-binding Protein in the Pheromone Gland of the Silkmoth, Bombyx mori*

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Atsushi Ohnishi, Hiroyuki Koshino, Shunya Takahashi, Yasuaki Esumi, and Shogo Matsumoto‡

From RIKEN (The Institute of Physical and Chemical Research), Hirosawa 2-1, Wako, Saitama 351-0198, Japan

Acyl-CoA-binding protein (ACBP) is a highly conserved 10-kDa intracellular lipid-binding protein that binds straight-chain (C14–C22) acyl-CoA esters with high affinity and is expressed in a wide variety of species ranging from yeast to mammals. Functionally, ACBP can act as an acyl-CoA carrier or as an acyl-CoA pool maker within the cell. Much work on the biochemical properties regarding the ACBP has been performed using various yeast and plant tissues, as well as different types of cells in culture, the regulatory mechanisms underlying ACBP gene expression have remained poorly understood. By exploiting the unique sex pheromone production system in the moth pheromone gland (PG), we report that transcription of a specific ACBP termed pheromone gland ACBP is triggered by a hemolymph-based humoral factor. Following purification and structure elucidation by means of high resolution electrospray ionization mass spectrometry and NMR analyses, in conjunction with biochemical analyses using acid hydrolysates, the humoral factor was identified to be β-o-glucosyl-O-L-tyrosine. Examination of the hemolymph titers during development revealed that the amount of β-o-glucosyl-O-L-tyrosine dramatically rose prior to eclosion and reached a maximum of 5 mg/ml (about 1 mg/pupa) on the day preceding eclosion, which was consistent with the effective dose of β-o-glucosyl-O-L-tyrosine in stimulating pheromone gland ACBP transcription in vivo. Furthermore, in vitro assays using trimmed PG indicated that β-o-glucosyl-O-L-tyrosine acts directly on the PG. These results provide the first evidence that transcription of some ACBPs can be triggered by specific humoral factors.

Acyl-CoA-binding protein (ACBP) is a highly conserved 10-kDa intracellular lipid-binding protein that binds straight-chain (C14–C22) acyl-CoA esters with high affinity and is structurally highly conserved from yeast to mammals as well as insects (1). Although the in vivo function of this protein has yet to be clarified in detail, in vitro investigations have revealed that ACBP protects acyl-CoA esters from hydrolysis (2–4); consequently, it can function as both an acceptor and a donor of acyl-CoA esters (3) by regulating their availability for various metabolic purposes such as mitochondrial β-oxidation (4–6), microsomal glycerolipid synthesis (4), and phospholipid synthesis (7, 8). ACBP can also generate an intracellular acyl-CoA pool that can serve a number of biochemical purposes (9, 10), because overexpression of ACBP in yeast cells results in a substantial increase in cellular acyl-CoA content (11).

In mammals, whereas the ACBP gene is a typical housekeeping gene (12), its expression differs markedly among different cell types; a high level of ACBP expression occurs in hepatocytes, steroidogenic cells, and adipocytes (reviewed in Ref. 13). The expression of ACBP is partly correlated with increased lipogenesis and occurs, for instance, in 3T3-L1 preadipocytes during in vitro differentiation, a process that is accompanied by a marked accumulation of triacylglycerol (TG) and de novo fatty acid synthesis (14, 15). Although the molecular mechanisms regulating ACBP expression have remained largely unknown, two functional regulatory elements, a sterol regulatory element-binding protein (SREBP)-binding site and a nuclear factor Y (NFY)-binding site, have been identified in the proximal promoter of the human ACBP gene (16). Recently, it has also been demonstrated that ACBP is a novel peroxisome proliferator-activated receptor (PPAR) target gene and that the PPAR-response element in intron 1 of the rat ACBP gene is a bona fide PPAR-response element (17).

In insects, many species of female moths produce species-specific sex pheromones to attract conspecific male moths. Their pheromone components are generally synthesized de novo through long-chain fatty acyl intermediates in the abdominal pheromone gland (PG), a functionally differentiated organ responsible for sex pheromone production. In the course of examining the molecular mechanisms underlying sex pheromone production in the silkmoth, Bombyx mori, we found that two distinct ACBPs termed pheromone gland ACBP (pgACBP) and midgut ACBP (mgACBP) are specifically expressed in the PG cells during pheromonogenesis (18). Northern blot analyses using various tissues also revealed that mgACBP, but not pgACBP, is highly expressed in the midgut during larval feeding stages, suggesting that there are at least two distinct ACBPs with different physiological functions in the moth and that both ACBPs simultaneously participate in sex pheromone production (18). In B. mori, the PG cells accumulate a large number of lipid droplets within the cytoplasm just before eclosion (19). The lipid droplets contain TGs composed of unsaturated C16 and C18 fatty acids in addition to the pheromone (bombykol, (E,Z)-10,12-hexadecadien-1-ol) precursor, Δ10,12-...
hexadecadecanoic acid, which is present as a major component. These droplets play a significant role in storing the bomyke precursor and eventually releasing it for pheromone production at eclosion in response to the neurohormone designated as pheromone biosynthesis-activating neuropeptide (PBAN) (19–21). Because the PG cells must retain large amounts of pheromone precursors during pheromogenesis, one of the functions of the ACBPs expressed in the PG seems to be to donate acyl-CoAs for the synthesis of the TGs in the lipid droplets.

Apart from these findings, we found that both of the ACBPs are expressed simultaneously in the PG and are up-regulated on the day preceding eclosion (18). Based on these observations, we hypothesized that there might be some kinds of physiological clues that trigger transcription and thus regulate the expression of the ACBPs in the PG. In this report, we describe the presence, isolation, and characterization of a humoral factor that appears in the hemolymph just before eclosion and that stimulates pgACBP transcription. Present results provide the first evidence that transcription of some ACBPs can be triggered by specific humoral factors.

**Experimental Procedures**

**Insects—** *B. mori* eggs (Shuko × Ryuhaku) were purchased from Katakura Kogyo (Matsumoto, Japan), and larvae were raised on an artificial diet as described (22). Bioassays were performed using 5–10 female pupae 3 days before eclosion (day −3). Pupal age was determined based on the morphological characteristics as described (21).

**Chemicals—** Para-amidinophenyl-methanesulfonyl fluoride hydrochloride (p-APMSF) was purchased from WAKO chemicals (Osaka). 2,3,4,6-Tetra-O-benzyl-d-glucopyranosyl fluoride (p-APMSF) in 0.2 mM phosphate buffer (pH 6.0) was added to the following method by incubating 0.1 mM phosphate buffer containing 150 mM KCl and 10 mM EDTA (pH 6.0) into the hemocoel (23). For purification, the hemolymph collected from female pupae (day −1) was immediately centrifuged at 4 °C for 10 min at 500 × g to remove hemocytes, and the resulting supernatant (plasma) was mixed with an equal volume of −20 °C acetone.

Following centrifugation at 4 °C for 10 min at 20,000 × g, the supernatant was passed through an Amicon Ultra PL-5 membrane (Millipore). The pass-through fraction was concentrated with the SpeedVac System (Thermo Savant) and designated as the plasma fraction.

**Bioassay in Conjunction with RT-PCR—** To examine the activity that stimulates transcription of pgACBP, test samples were diluted to 10 μl with phosphate-buffered saline (137 mM NaCl, 2.7 mM KC1, 8 mM Na2HPO4, 0.18 mM KH2PO4, pH 7.4, containing 0.05% phenylthiourea and 0.5 mM p-APMSF) in 0.2 mM phosphate buffer (pH 6.0) using the “flushing out” method by injecting 0.1 mM phosphate buffer containing 150 mM KCl and 10 mM EDTA (pH 6.0) into the hemocoel (23). For purification, the hemolymph collected from female pupae (day −1) was immediately centrifuged at 4 °C for 10 min at 500 × g to remove hemocytes, and the resulting supernatant (plasma) was mixed with an equal volume of −20 °C acetone.

Following centrifugation at 4 °C for 10 min at 20,000 × g, the supernatant was passed through an Amicon Ultra PL-5 membrane (Millipore). The pass-through fraction was concentrated with the SpeedVac System (Thermo Savant) and designated as the plasma fraction.

**Synthesis of β-p-Glucosyl-O-L-Tyrosine—** An authentic sample of β-p-glucosyl-O-L-tyrosine was synthesized from 2,3,4,6-tetra-O-benzyl-d-glucopyranose via stereoselective glycosidation of the corresponding O-glycosylpseudourea (27) with N-benzylcarboxyl-L-tyrosine benzyl ester followed by hydrogenation (H2, 10% Pd/C). N-benzylcarboxyl-L-tyrosine benzyl ester was prepared according to Tilak (28). For synthesis of β-p-glucosyl-O-L-Tyrosine, the plasma fraction was mixed with an equal volume of −20 °C acetone.

Following centrifugation at 4 °C for 10 min at 20,000 × g, the supernatant was passed through an Amicon Ultra PL-5 membrane (Millipore). The pass-through fraction was concentrated with the SpeedVac System (Thermo Savant) and designated as the plasma fraction.

**Experimentl Procedures—** The plasma fraction prepared from 500 female pupae (day −1) was loaded on to a TSK-GEL Amide-80 column (4.6 × 250 mm; Tosoh An). The column was equilibrated with 70% CH3CN (in a flow rate of 1.0 ml/min) and eluted with a linear gradient of 15% to 85% CH3CN in 30 min at 1.0 ml/min.

**Isolation Procedures—** The plasma fraction prepared from 500 female pupae (day −1) was loaded on to a TSK-GEL Amide-80 column (4.6 × 250 mm; Tosoh An). The column was equilibrated with 70% CH3CN (in a flow rate of 1.0 ml/min) and eluted with a linear gradient of 15% to 85% CH3CN in 30 min at 1.0 ml/min.

**Mass Spectrometry—** High resolution electrospray-ionization mass spectrometry (HR-ESIMS) was carried out in the positive ion mode using a JEOL JMS-T100LC spectrometer with methanol as the mobile phase at a flow rate of 0.2 ml/min (internal standard: sodium trifluoroacetate). The purified humoral factor and synthetic β-p-glucosyl-O-L-tyrosine were dissolved in water at a concentration of 1 μg/ml and 10-μl aliquots were subjected to analysis.

**NMRT—** The one-dimensional 1H at 600 MHz and 13C NMR spectra at 150 MHz, and two-dimensional DQF-COSY, HMOC, and HMB NMR spectra at 25 °C were recorded with a JEOL JNM-ECA600 spectrometer equipped with a Nalorac 3-mm gradient double resonance probe head. The purified humoral factor (100 μg) was also hydrolyzed with 1 N HCl at 110 °C for 20 h. The amino acid analysis of the resulting hydrolysates was carried out according to Hayashi and Sasagawa (26). The purified humoral factor (100 μg) was also hydrolyzed with 1 N HCl at 100 °C for 12 h. The resulting hydrolysates were loaded onto a TSK-GEL Amide-80 column and eluted with 70% CH3CN at a flow rate of 1.0 ml/min. The glucose fraction, which eluted at 7–8 min, was evaporated, redissolved in 1.5 ml of water, and then subjected to measurement of its optical rotation with a polarimeter DIP-370 (Asca, Japan) at a wavelength of 589 nm in a 1-cm cell with a path length of 0.1 mm.

**Results**

Presence of a Humoral Factor That Triggers pgACBP Transcription—The purified humoral factor (100 μg) was hydrolyzed in a vacuum-sealed tube with 6 N HCl at 110 °C for 20 h. The amino acid analysis of the resulting hydrolysates was carried out according to Hayashi and Sasagawa (26). The purified humoral factor (100 μg) was also hydrolyzed with 1 N HCl at 100 °C for 12 h. The resulting hydrolysates were loaded onto a TSK-GEL Amide-80 column and eluted with 70% CH3CN at a flow rate of 1.0 ml/min. The glucose fraction, which eluted at 7–8 min, was evaporated, redissolved in 1.5 ml of water, and then subjected to measurement of its optical rotation with a polarimeter DIP-370 (Asca, Japan) at a wavelength of 589 nm in a 1-cm cell with a path length of 0.1 mm.

To examine whether humoral factor(s) present in the hemolymph could stimulate pgACBP transcription, we injected various humoral factors into day 3 pupae and day 1 adults.

**Fluctuation of the β-p-Glucosyl-O-L-Tyrosine Content in the Hemolymph—** The fluctuation of the β-p-glucosyl-O-L-tyrosine titer in the hemolymph, plasma fractions were prepared directly from fifth instar larvae, and the amount of β-p-glucosyl-O-L-tyrosine was quantified by means of HPLC using a TSK-GEL Amide-80 column as described above. The pgACBP transcript levels following incubation were also measured by RT-PCR as described above.

**Measurement of the Total Volume of the Hemolymph in the Pupae—** Aliquots (10 μl) of Evans Blue (WAKO chemicals) solution (5 mg/ml) were injected into 10 female pupae. After maintaining for 15 min at 25 °C, the hemolymph (20 μl each) was collected into a chilled microcentrifuge tube and diluted 50-fold with H2O, and the absorbance at 610 nm was measured.
(lane 6) or immediately after eclosion (lane 7) caused a marked increase of the pgACBP transcript at 18 h postinjection. Although this activity in the plasma seemed to gradually increase during pupal development (lanes 4–6), no activity was detected in the plasma taken from either fifth instar larvae or female moths 1 day after eclosion (lanes 3 and 8). Since pgACBP transcripts were detectable as early as 12 h postinjection (Fig. 1B), we elected to monitor the progress of our purification of the plasma humoral factor by assaying pgACBP transcript levels 12 h after injection of the test samples.

Isolation of the Humoral Factor in the Female Pupal Hemolymph.—Preliminary experiments revealed that the humoral factor in the pupal plasma (day −1) could be recovered in the fraction that not only failed to be precipitated with 50% acetone but that also passed through an Amicon Ultra PL-5 membrane (Millipore), suggesting that the humoral factor was a low molecular weight substance. For purification, we prepared this plasma fraction, which was equivalent to 500 female pupae (day −1), and performed isocratic HPLC separation using a TSK-GEL Amide-80 column (Fig. 2A). The bioactive fraction that eluted from the column at 12–13 min was further purified using a C18 column with the activity recoverable in a single peak that eluted at 5.5 min (Fig. 2B).

Structure Elucidation of the Humoral Factor—HR-ESIMS spectral analysis of the purified factor resulted in molecular ions of m/z 566.10 ([M + Na]+), 382.08 ([M + K]+), and 388.09 ([M + 2Na]+), indicating a molecular formula of C15H21NO8. The UV spectrum in H2O showed absorption maxima at 271 and 276 nm. The one-dimensional 1H and 13C NMR spectra of the purified factor in the female pupal hemolymph. A, plasma fraction that was loaded onto a TSK-GEL Amide-80 column. B, the active fraction that eluted at 12–13 min in A was loaded onto a CAPCELL PAK C18 column. Horizontal bars, indicate active fractions.

To determine whether this concentration of β-D-glucosyl-O-L-tyrosine acts directly on the PG cells to stimulate pgACBP transcription, we synthesized β-D-glucosyl-O-L-tyrosine and examined its ability to stimulate transcription of the ACBPs in the PG. When we injected various concentrations of synthetic β-D-glucosyl-O-L-tyrosine into day −3 female pupae, the levels of pgACBP mRNA increased in a dose-dependent manner; the transcript was detected at doses of 0.5, 1.0, and 2.0 mg/pupa (Fig. 4A). These biological activities are consistent with those of the purified humoral factor (data not shown). At these concentrations, more than 1.0 mg/pupa seemed to be required to obtain the comparable level of pgACBP transcript of the untreated day −1 pupa (Fig. 1A, lane 2). Because injection of L-tyrosine and/or D-glucose alone failed to elicit a transcriptional response, even at concentrations of 2.0 mg/pupa, the covalent bond between L-tyrosine and D-glucose is indispensable for biological activity (data not shown). In contrast to the elevation of the pgACBP transcript levels, β-D-glucosyl-O-L-tyrosine essentially had no effect on the transcript of mgACBP (Fig. 4B).

To examine whether β-D-glucosyl-O-L-tyrosine acts directly on the PG cells to stimulate pgACBP transcription, we prepared trimmed PGs from female pupae (day −3) and incubated each PG for 12 h in the presence of β-D-glucosyl-O-L-tyrosine at concentrations ranging from 0–5 mg/ml. Similar to the in vivo assays described above, RT-PCR revealed that the levels of pgACBP mRNA in the trimmed PG increased in a dose-dependent manner (Fig. 5A). These results demonstrate that the humoral factor β-D-glucosyl-O-L-tyrosine acts directly on the PG to stimulate pgACBP transcription. In addition, the titer of β-D-glucosyl-O-L-tyrosine within the cytosolic fraction of PGs increased proportionately with the concentration of β-D-glucosyl-O-L-tyrosine used in the incubations (Fig. 5B). At a concentration of 5.0 mg/ml, the titer in the cytosolic fraction reached a maximum (0.6 μg/PG) at 6 h (Fig. 5B), whereas the pgACBP transcript appeared at 12 h (Fig. 5C).

Fluctuation of Hemolymph β-D-Glucosyl-O-L-Tyrosine Titers During Development—The in vivo and in vitro assays described above revealed that β-D-glucosyl-O-L-tyrosine in the hemolymph could act on the PG to stimulate pgACBP transcription; however, it required at least 0.5 mg/pupa to exert this activity. To determine whether this concentration of β-D-glucosyl-O-L-tyrosine reflects the physiological state and correlates with the hemolymph titer during pheromonesis, we examined the fluctuation of β-D-glucosyl-O-L-tyrosine titers in the hemo-
lymph during development (i.e. from the larval fifth instar to the adult stage) (Fig. 6). Although there was no significant difference in the patterns between female and male, two prominent peaks in the $\beta\-\text{D-glucosyl-}O\-\text{L-tyrosine}$ titer appeared during the molting stages; the smaller of the two surges occurred at the larval-pupal molt and reached a maximum level (1 mg/ml) on the day of pupation, whereas the larger pulse occurred during the pupal-adult molt. This second surge began 3–4 days prior to eclosion, reached a maximum of as much as 5 mg/ml at 1 day before eclosion, and then rapidly declined to the lowest level (0.2 mg/ml) within 2 days of emergence. When we measured the total volume of the hemolymph in female pupae (Table I), it was estimated that a single female pupa (day 2) contains, on average, ~190 µl of hemolymph. This estimation implies that the effective dose of $\beta\-\text{D-glucosyl-}O\-\text{L-tyrosine}$ (more than 0.5 mg/pupa) is comparable with the $\beta\-\text{D-glucosyl-}O\-\text{L-tyrosine}$ titers around 1 day before eclosion (0.95 mg/pupa). These results therefore indicate that the upsurge of $\beta\-\text{D-glucosyl-}O\-\text{L-tyrosine}$ in the hemolymph just before eclosion is indeed responsible for the up-regulation in pgACBP transcription during pheromonogenesis.

DISCUSSION
ACBP is a highly conserved 10-kDa $N$-acylated polypeptide that is expressed in a wide variety of species ranging from yeast to mammals (29). Because ACBP binds straight-chain
specifically expressed in the PG during pheromonogenesis and undergo up-regulation on the day prior to eclosion in the female *B. mori* moth (18). In the PGs of many moth species, various long chain fatty acyl-CoAs participate as precursors or intermediates in the biosynthesis of species-specific sex pheromones (30), suggesting that ACBPs expressed in the PG could possibly function as carriers or cellular deposits for the acyl-CoAs utilized in pheromone biosynthesis.

Whereas numerous studies regarding the biochemical properties of the ACBP have been performed using various vertebrate and plant tissues as well as different types of cells in culture, the regulatory mechanisms underlying ACBP gene expression have remained poorly understood; only the involvement of transcription factors such as SREBP and PPAR expression have remained poorly understood; only the involvement of transcription factors such as SREBP and PPAR have provided the first evidence that transcription of a specific ACBP is triggered by a hemolymph-based humoral factor. To initiate the present experiments, we hypothesized that there would be a specific physiological cue that triggered ACBP transcription within the PG cells 1 day prior to eclosion. Because insects contain an open circulatory system, the PG is continuously exposed to the hemolymph; consequently, we examined whether the hemolymph 1 day prior to eclosion could stimulate ACBP transcription and found that a transcriptional activator was indeed present (Fig. 1A). Following purification and structure elucidation by means of HR-ESIMS and NMR analyses, in conjunction with stereochromical analyses using hydrolysates, the active factor in the hemolymph was identified as a specific humoral factor with synthetic \( \beta \)-D-glucosyl-O-L-tyrosine injection.

**TABLE I**

| Hemolymph volume* (µl) | Day −3 | Day −2 | Day −1 |
|-------------------------|-------|-------|-------|
| Day −3                  | 194.6 ± 16.97 | 194.2 ± 18.38 | 193.4 ± 11.31 |

*Pupae of 1.45–1.55 g were used for measurement.*

(Fig. 6) Fluctuation of hemolymph \( \beta \)-D-glucosyl-O-L-tyrosine during development (○, male; ●, female). Data are given as means ± S.D. Measurements were performed six times using separately prepared plasma samples.

**TABLE I**

The total volume of *B. mori* pupal hemolymph (mean ± S.D.) Measurements were performed with 10 female pupae taken from the same developmental stage.

| Hemolymph volume* | Day −3 | Day −2 | Day −1 |
|-------------------|-------|-------|-------|
| Day −3            | 194.6 ± 16.97 | 194.2 ± 18.38 | 193.4 ± 11.31 |

*Pupae of 1.45–1.55 g were used for measurement.*

(Fig. 4) Biological activity of synthetic \( \beta \)-D-glucosyl-O-L-tyrosine. Stimulation of pgACBP transcription (A) or mgACBP transcription (B) in the PG of day −3 female pupae. RT-PCR was performed 18 h after \( \beta \)-D-glucosyl-O-L-tyrosine injection.

(Fig. 5) Effects of \( \beta \)-D-glucosyl-O-L-tyrosine in vitro. A, dose-dependent effects of \( \beta \)-D-glucosyl-O-L-tyrosine in vitro. Trimmed PGs were incubated with \( \beta \)-D-glucosyl-O-L-tyrosine and RT-PCR was performed after a 12-h incubation. B, incorporation of \( \beta \)-D-glucosyl-O-L-tyrosine into PG cells. Trimmed PGs were incubated with \( \beta \)-D-glucosyl-O-L-tyrosine (0 mg/ml (A), 1.0 mg/ml (C), and 5.0 mg/ml (●)). Trimmed PGs were homogenized at each time point, and the amount of \( \beta \)-D-glucosyl-O-L-tyrosine in the cytosolic fraction was measured. Bars, means ± S.D. C, stimulation of pgACBP transcription in the PG in vitro (time response). RT-PCR was performed at the indicated times after incubation with \( \beta \)-D-glucosyl-O-L-tyrosine (5.0 mg). As a control (−), PGs were incubated without \( \beta \)-D-glucosyl-O-L-tyrosine.

\((C_{14}-C_{22})\) acyl-CoA esters with high affinity and thereby protect them from hydrolysis (2–4), functionally, ACBP can act as an acyl-CoA carrier or an acyl-CoA pool maker within the cell to increase total cellular acyl-CoA content (9–11). In previous studies, we have demonstrated that two distinct ACBPs are
levels comparable with that observed in the PGs of untreated day 1 pupa (Fig. 4). Quite interestingly, we found that the \( \beta-d\)-glucosyl-O-t-tyrosine titer in the hemolymph dramatically rises prior to eclosion and reaches a maximum of 5 mg/ml (about 1 mg/pupa) on the day preceding eclosion (Fig. 6). This relationship between the effective dose of \( \beta-d\)-glucosyl-O-t-tyrosine and its upsurge in the hemolymph just before eclosion is consistent with its proposed role in the up-regulation of pgACBP during pheromogenesis. Furthermore, the \textit{in vitro} assays using trimmed PG (Fig. 5) indicate that \( \beta-d\)-glucosyl-O-t-tyrosine acts directly on the PG to stimulate pgACBP transcription, although the molecular mechanism behind how the \( \beta-d\)-glucosyl-O-t-tyrosine signal stimulates the transcription of pgACBP within the PG cells remains to be clarified. In mammals, two functional regulatory elements, a SREBP-binding site and an NF-Y-binding site, have been identified in the proximal promoter of the human ACBP gene (16). In addition, it has been reported that the ACBP gene is a novel PPAR\(\gamma\)-retinoid X receptor target gene and that PPAR\(\gamma\)-retinoid X receptor activates transcription through an intrinsic PPAR-response element in both human and rodent ACBP (17). In the present experiments, we found that there is a time lag of several hours between the incorporation of \( \beta-d\)-glucosyl-O-t-tyrosine into the PG cells and the appearance of the pgACBP transcript (Fig. 5, B and C). This finding suggests that transcription of pgACBP does not result from a direct interaction with \( \beta-d\)-glucosyl-O-t-tyrosine itself. We also found that the titer of \( \beta-d\)-glucosyl-O-t-tyrosine in the hemolymph rapidly declines following adult emergence (Fig. 6), whereas we had previously reported that the pgACBP transcript level remained high for several days after eclosion (18). These results suggest that \( \beta-d\)-glucosyl-O-t-tyrosine stimulates pgACBP transcription through a signal transduction cascade that probably includes some as yet unidentified transcription factor similar to SREBP, NFY, and/or PPAR\(\gamma\).

The presence of \( \beta-d\)-glucosyl-O-t-tyrosine in the hemolymph has been reported in several insects (31–34). In addition, it has been demonstrated in the tobacco hornworm, Manduca sexta, that \( \beta-d\)-glucosyl-O-t-tyrosine is synthesized in the fat body by the action of a specific \( \beta\)-glucosyltransferase present there (35). In M. sexta, the regulation of \( \beta-d\)-glucosyl-O-t-tyrosine synthesis and hydrolysis is under the control of hormones that regulate molting and metamorphosis; the decline in juvenile hormone titer after the last larval ecdysis initiates \( \beta-d\)-glucosyl-O-t-tyrosine synthesis (36), whereas the major pulse of 20-hydroxyecdysone triggers hydrolysis of \( \beta-d\)-glucosyl-O-t-tyrosine by a \( \beta\)-glucosidase in the fat body (33). In insects, \( \alpha\)-tyrosine is an important precursor for the diphenols and quinones that cross-link cuticular proteins during tanning or sclerotization, the process of which results in the hardening and pigmentation of the new cuticle following the molt (33). Consequently, the function of \( \beta-d\)-glucosyl-O-t-tyrosine has long been suggested to serve as a reservoir of \( \alpha\)-tyrosine for the eventual incorporation into the newly formed cuticle following the molt and/or metamorphosis. This idea seems to be consistent with the present finding that an increase in the \( \beta-d\)-glucosyl-O-t-tyrosine titer occurs, regardless of sex, at the time of pupation and eclosion (Fig. 6). Alternatively, since we have unequivocally demonstrated that \( \beta-d\)-glucosyl-O-t-tyrosine triggers pgACBP transcription during pupal-adult metamorphosis in the female moth, our present results also suggest a multifunctional role for \( \beta-d\)-glucosyl-O-t-tyrosine.

We found that \( \beta-d\)-glucosyl-O-t-tyrosine essentially failed to stimulate mgACBP transcription (Fig. 4B). Since we have detected mgACBP transcriptional activation capabilities in a hemolymph fraction other than the \( \beta-d\)-glucosyl-O-t-tyrosine fraction, it is likely that up-regulation of this protein in the PG is under the control of another humoral factor.

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Isolation and Characterization of a Humoral Factor That Stimulates Transcription of the Acyl-CoA-binding Protein in the Pheromone Gland of the Silkmoth, *Bombyx mori*

Atsushi Ohnishi, Hiroyuki Koshino, Shunya Takahashi, Yasuaki Esumi and Shogo Matsumoto

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