A Novel 43-kDa Protein as a Negative Regulatory Component of Phenoloxidase-induced Melanin Synthesis*

Received for publication, April 18, 2005, and in revised form, April 27, 2005
Published, JBC Papers in Press, April 27, 2005, DOI 10.1074/jbc.M504173200

Mingyi Zhao‡§, Irene Söderhäll†, Ji Won Park‡, Young Gerl Ma‡, Tsukusa Osakibe, Nam-Chul Ha‡, Chun Fu Wu§, Kenneth Söderhäll†, and Bok Luel Lee‡**

From the ‡National Research Laboratory of Defense Proteins, College of Pharmacy, Pusan National University, Jangjeon Dong, Kumjeong Ku, Busan 609-735, Korea, the †Department of Comparative Physiology, Evolutionary Biology Center, Uppsala University, Norbyvägen 18A, Uppsala SE-752 36, Sweden, and the §Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang 110016, China

The melanization reaction induced by activated phenoloxidase in arthropods is important in the multiple host defense innate immune reactions, leading to the sequestration and killing of invading microorganisms. This reaction ought to be tightly controlled because excessive formation of quinones and systemic hypermelanization are deleterious to the hosts, suggesting that a negative regulator(s) of melanin synthesis may exist in hemolymph. Here, we report the purification and cloning of a cDNA of a novel 43-kDa protein, from the mealworm Tenebrio molitor, which functions as a melanization-inhibiting protein (MIP). The deduced amino acid sequence of 352 residues has no homology to known sequences in protein data bases. When the concentration of the 43-kDa protein was examined by Western blot analysis in a melanin-induced hemolymph prepared by injection of Candida albicans into T. molitor larvae, the 43-kDa protein specifically decreased in the melanin-induced hemolymph compared with control hemolymph. Recombinant MIP expressed in a baculovirus system had an inhibitory effect on melanin synthesis in vitro. RNA interference using a synthetic 445-mer double-stranded RNA of MIP injected into Tenebrio larvae showed that melanin synthesis was markedly inhibited. These results suggest that this 43-kDa MIP inhibits the formation of melanin and thus is a modulator of the melanization reaction to prevent the insect from excessive melanin synthesis in places where it should be inappropriate.

The innate immune system is a host defense mechanism that is evolutionarily conserved from plants to humans and is mainly involved in the recognition and control of the early stage of infection in all animals (1, 2). It is activated by a group of germ line-encoded receptors and soluble proteins, conceptually termed pattern recognition receptors and proteins, which recognize microbial surface determinants that are conserved among microbes but absent in the host, such as lipopolysaccharide, peptidoglycan, 1,3-β-d-glucan, and mannan. Upon recognition, these receptors activate distinct signaling cascades that regulate specific immune-related proteins aimed at the aggressors. Recently, our knowledge of innate immunity in mammals and insects has increased dramatically (1–6). The recruitment of similar proteins and pathways in both insects and mammals in the fight against infection suggests that they have developed similar mechanisms and molecular pathways to recognize and eliminate pathogenic invaders (4, 6).

The prophenoloxidase (pro-PO) system, like the vertebrate complement system, is a proteolytic cascade comprising pattern recognition proteins, serine proteases, their inhibitors, and terminates with thezymogen, pro-PO (7–10). The pro-PO system is an important non-self-recognition system present in most invertebrates. Microbial polysaccharides lipopolysaccharide, peptidoglycan, and 1,3-β-d-glucan first react with pattern recognition proteins, which then induce activation of several serine proteases within the pro-PO system. Determining the molecular mechanism, by which pattern recognition molecules differentiate non-self from self and how to transduce signals that stimulate defense responses, is a key for understanding the ways in which innate immune systems are regulated. The pro-PO-activating enzymes or factors, which all are similar to Drosophila easter-type serine protease (clp domain-containing trypsin-like serine protease), cleave pro-PO to generate the active enzyme, phenoloxidase (PO) (11–14). This enzyme produces toxic compounds to microorganisms by oxidizing phenols to form the melanin pigment, and it also participates in the sclerization of the cuticle, which is vital for the survival of insects (8, 9).

Melanin synthesis is essential for defense and development but must be tightly controlled because systemic hyperactivation of the pro-PO system, excessive formation of quinones, and inappropriate excessive melanin synthesis are also deleterious to the hosts, suggesting that the pro-PO activation system and melanin formation are tightly regulated by melanization-regulatory molecules. Only a few inhibitors of the pro-PO system have been identified from insects and crustaceans (15–18). Previously, it has been shown that Drosophila serpin-27A specifically inhibited the pro-PO-activating enzyme and prevented

* This work was supported by National Research Laboratory Grant M10400000028-04J0000-02 and International Collaboration Research Grant M6-0403-00-0048 from the Ministry of Science and Technology (to B. L. L.), a Pusan National University research grant, 2005, and postdoctoral fellow grant (to T. O.), a Pusan National University research grant, 2004 (to B. L. L.), and by grants from the Swedish Research Council (VR-NT) and Swedish Foundation for International Cooperation in Research and Higher Education (to K. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AB205184.

† Present address: National Cardiovascular Center, Osaka, Japan.

** To whom correspondence should be addressed. Tel.: 82-51-510-2809; Fax: 82-51-513-2801; E-mail: brlee@pusan.ac.kr.

---

1 The abbreviations used are: pro-PO, prophenoloxidase; DFP, diisopropyl fluorophosphate; dsRNA, double-stranded RNA; FPLC, fast protein liquid chromatography; GFP, green fluorescent protein; MIP, melanization-enhancing protein; MIP, melanization-inhibitory protein; nMIP, native MIP; PO, phenoloxidase; POI, PO inhibitor; PTU, phenylthiourea; rMIP, recombinant MIP; RNAi, RNA interference.
the melanin synthesis induced by activated PO (19) and that the proteinase inhibitor, pacifastin, efficiently inhibited the pro-PO-activating enzyme in a crayfish (18). However, the specific inhibitor protein for melanin formation induced by activated PO was not found. The identification of a specific melanization-inhibitory protein (MIP) will provide important information to clarify how an arthropod’s innate immune reaction such as the melanization response is regulated and controlled.

We reported previously that a 160-kDa vitellogenin-like protein was involved in melanin synthesis as a melanization-enhancing protein (MEP) in the beetle Tenebrio molitor (20). In our preliminary experiments we observed that a 43-kDa protein specifically disappeared from the hemolymph during melanin synthesis. Because the melanization-regulatory proteins usually disappear or are degraded during the melanization reaction, it is not easy to purify these proteins from crude hemolymph. Therefore, to purify a novel melanization-regulatory protein(s) of T. molitor larvae, we assumed that the components of pro-PO system should be in their nonactive form, and hence we prepared a nonactive pro-PO system by treating hemolymph with an irreversible serine protease inhibitor, diisopropyl fluorophosphate (DFP) to prevent activation of pro-PO-activating enzyme(s) (11). By using DFP-treated hemolymph, we isolated a novel 43-kDa protein to homogeneity, cloned its cDNA, and examined its function by using RNA anticoagulation buffer and eluted with the same buffer at a flow rate of 12 ml/h. Fractions showing specific PO activity in the presence of Ca²⁺ was applied to a Sephadex G-100 column (1 cm × 100 cm) and eluted with the same buffer. When these fractions were assayed for PO activity, we obtained a 160-kDa vitellogenin-like pro-PO-activating enzyme (11). By using DFP-treated hemolymph, we isolated a novel 43-kDa protein from the hemolymph, the head of each larva was pricked with a 25-gauge needle. The extruding crude hemolymph from 25 larvae (about 20 μl of hemolymph/larva) was collected in 1 ml of a modified anticoagulation buffer (136 mM trisodium citrate, 26 mM citric acid, 20 mM EDTA, and 15 mM NaCl, pH 5.5) and then centrifuged at 2000 × g for 10 min at 4 °C. The resulting supernatant was named hemolymph and stored at −80 °C until use. Hemocytes were collected from the extruding crude hemolymph by centrifugation at 200 × g for 10 min at 4 °C, washed with anticoagulation buffer, and stored at −80 °C. About 3 × 10⁶ packed cells were obtained from 500 μl of the crude hemolymph.

**Assay of PO Activity and Ability of Melanin Synthesis—** An assay of PO and the preparation of G-100 solution were carried out according to our previously described method (20). Briefly, to obtain the solution showing PO activity in the presence of Ca²⁺ and 1,3-β-glucan, 50 μl of hemolymph was concentrated by ultrafiltration through a membrane filter (YM10, Amicon). Approximately 3 ml of the concentrated solution was applied to a Sephadex G-100 column (1 × 50 cm) equilibrated with anticoagulation buffer and eluted with the same buffer at a flow rate of 1 ml/min. The fractions showing specific PO activity in the presence of Ca²⁺ and 1,3-β-glucan were pooled and named G-100 solution. This solution was used to examine PO activity and melanin synthesis. To measure PO activity, 30 μl of G-100 solution (3.5 mg/ml proteins) was preincubated with 10 μl of 1,3-β-glucan (1 μg/ml) for 5 min at 30 °C, and then 460 μl of the substrate solution (1 mM 4-methylcoumaryl-7-amide (Boc-Phe-Ser-Arg-MCA)) was used. The resulting peptides were separated through high performance liquid chromatography on a reverse phase C₄ column (Waters) and applied to an amino acid sequencer (Applied Biosystem Procise Automated Gas Phase Sequencer). To determine the amino-terminal sequence, the purified protein (1 μg) transferred to a nitrocellulose membrane. For immunoblotting, the proteins separated by SDS-PAGE were transferred electrophoretically to a polyvinylidene difluoride membrane, which was blocked by immersion in 5% skimmed milk solution containing 1% horse serum for 1 h. The membrane was then transferred to rinse solution I (20 mM Tris-HCl buffer, pH 7.5, containing 150 mM NaCl, 0.1% Tween 20, and 2.5% skimmed milk) containing the affinity-purified protein (5 μg/ml) and incubated at 4 °C for 2 h. The amounts of the bound antibodies were determined using ECL Western blotting reagent kit (Amersham Biosciences).

**Detection of MIP-binding Protein in Hemolymph—** The proteins of control G-100 solution (20 μg of proteins) and the supernatant of melanin-induced G-100 solution (20 μg) separated on SDS-PAGE were transferred electrophoretically to a nitrocellulose membrane, and the filter was blocked by immersion in 5% skimmed milk solution containing 1% horse serum for 12 h. The membrane was then transferred to rinse solution I (20 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 0.1% Tween 20, and 2.5% skimmed milk) containing the affinity-purified 43-kDa protein antibody (50 ng/ml) and incubated at 4 °C for 2 h. The amounts of the bound antibodies were determined using ECL Western blotting reagent kit (Amersham Biosciences).
cDNA Cloning and Nucleotide Sequencing of 43-kDa MIP—A cDNA library from *T. molitor* larvae was constructed as described previously (24). Among chemically determined four partial amino acid sequences of the purified 43-kDa protein, DNA oligonucleotide corresponding to *T. molitor* cDNA was synthesized as follows: 5′-TA/TC/CA/AG/AG/GCIAAA/T/C/TA/TC/CA/TC/GA/ATGAT3′, and it was labeled with [γ-32P]-ATP by a previously described method (25). For the initial screening, ~50,000 recombinants of *T. molitor* larvae cDNA library were used and the membranes were prehybridized at 65 °C for 1 h in 4 × SSC (600 mM NaCl, 60 mM trisodium citrate, pH 7.0), 10 × Denhardt’s solution (50 × Denhardt's solution, 1% (v/v) bovine serum albumin, 1% (v/v) Ficoll, and 1% (w/v) polyvinylpyrrolidone) and 25 μg/ml salmon sperm DNA. The membranes were then hybridized at 51.5 °C for 12 h in the same solution during prehybridization. We obtained 10 hybridization-positive clones and analyzed two plasmids containing four chemically determined partial amino acid sequences and amino-termini sequence. The nucleotide and amino acid sequences of the 43-kDa protein cloned together with the protein sequence data base of the National Center for Biotechnology Information (NCBI) using the Genetyx system (Software Development Co., Ltd., Tokyo).

**Purification of the Native 43-kDa MIP (nMIP) from Tenebrio Hemo**-

ymph.—To prevent the activation of Tenebrio pro-PO-activating enzyme(s), 600 μl of 0.5 mM DFP was added to 150 ml of hemolymph (2 mM DFP was used for purification). The supernatant was incubated for 2 h, and then the hemolymph was dialyzed at 4 °C for 12 h against 5 liters of buffer A (50 mM Tris-HCl, 3 mM EDTA, pH 8.0). After centrifugation at 3,000 rpm at 4 °C for 10 min to remove the precipitated proteins, the supernatant was loaded onto a Sepharose Cl-4B column (2 × 10 cm) pre-equilibrated with buffer B (50 mM Tris-HCl, 2 mM EDTA, 150 mM NaCl, pH 8.0) at a flow rate of 0.2 ml/min. Fractions containing the 43-kDa protein from the Cl-4B column were pooled and concentrated by ultrafiltration through a filter and Centricon (Amicon). The pooled fractions were then concentrated by ultrafiltration through a filter and Centricon to a final concentration of 1 mg/ml and then loaded to a Sephacryl S-200 column (2 × 120 cm). The column was preequilibrated with buffer C at a flow rate of 0.4 ml/min. After washing until the absorbance at 280 nm was 0, the column was eluted with a linear gradient of NaCl from 0 to 0.75 M over 120 min, and the fractions containing 43-kDa protein were pooled and concentrated with Centricon to a final concentration of 0.1 mg/ml. The amino-terminal amino acid sequence analysis was performed to verify the identity of rMIP.

**RNA Experiments**—The template (spanning from nucleotide 502 to 946) for synthesis of double-stranded RNA (dsRNA) for MIP was amplified by cDNA from PCR using the forward primer TAATACGACCTCAGATATAGGGCTGATTTAGGCACTAC and the reverse primer TAATACGACCTCAAAAGAAGGGGTTGACCAAGACT. The dsRNA was synthesized using the T7 MEGAscript kit (Ambion) according to the manufacturer’s instructions. The resulting RNA was extracted with phenol/chloroform and ethanol precipitated and finally dissolved in diethyl pyrocarbonate-treated water. Annealing of the complementary strands was performed by heating to 70 °C for 10 min and cooling overnight in water bath at room temperature. The template for synthesis of dsRNA for green fluorescent protein (gfp) was the PD2ZEGFP-1 plasmid (Clontech) was amplified by PCR using the forward primers TAATACGACTCATAGAGGCGCATAGAAACGCGACAGT and the reverse primer TAATACGACTCAAAAGAAGGGGTTGACCAAGACT, and dsRNA were then synthesized as described above. *Tenebrio* larvae were injected with 50 μl of dsRNA in 50 μg/ml phosphate-buffered saline using a 25-gauge needle. After the injection the larvae were kept at room temperature for 24 h.

**Reverse Transcription-PCR and Western Blot Analysis of dsRNA-Treated Larvae**—To detect MIP transcript, total RNA was isolated from the fat body of dsRNA-injected *T. molitor* larvae using the TRIzol LS reagent (Invitrogen) according to the manufacturer’s instructions. Comminuted genomic DNA was removed by treatment with RNase-free DNase I (1 unit/μl), and the reaction was stopped by the addition of 20 mM EDTA, pH 8.0. After purification by phenol/chloroform extraction and ethanol precipitation the RNA (400 ng) was reverse-transcribed using the M-MLV reverse transcriptase (Invitrogen) and� according to the manufacturer's instructions. The PCR products were resolved on 1.2% agarose gel electrophoresis. The sequences of the reverse primers used were as follows: MIP (spanning from nucleotides 101 to 428) forward, CGTCGTC- ACTCTCTCTCCAAAAAG; reverse, TCAGTCATCTACGTCGTCGTCGTA. For controls (ribosomal protein L27a, accession number X99204) forward, GCAATGGCAAAACACGAAAAGCATC; and reverse, ATGACAGG-TGGTGTAGCAAGC. The PCR products were resolved on 1.2% agarose gel electrophoresis. The Western blotting analyses 40 μg of hemolymph proteins from MIP-dsRNA-treated larvae or GFP-dsRNA-treated larvae were transferred onto polyvinylidene difluoride membranes, and then immunoblotted with affinity-purified 43-kDa protein antibody as described above.

**Determination of MIP Localization**—To determine the localization of the purified 43-kDa protein, we prepared plasma, extracts of hemocyte, and fat bodies as described previously (25). The hemolymph and plasma (1 μl) was added to the sample wells as described above. The fat body (2×10^6 cells) were washed with 500 μl of buffer A, suspended with 500 μl of buffer A, sonicated for 15 s at 4 °C, and then centrifuged at 22,000 × g for 10 °C for 10 min. The supernatant was used as a source of hemocyte lysate. The soluble proteins of hemolymph lysate and fat bodies were precipitated with trichloroacetic acid, subjected to SDS-PAGE, and then immunoblotting with the affinity-purified 43-kDa protein antibody was carried out.

**RESULTS**

**Characterization of Proteins Associated with Melanization**—To identify melanin-regulatory proteins in insect hemolymph, we first prepared G-100 solution from *Tenebrio* hemolymph. This solution showed PO activity by incubation with 1,3-β-D-glucan and Ca^{2+}. Melanin pigments were induced when the G-100 solution was incubated with dopamine in the presence of 1,3-β-D-glucan and Ca^{2+}. After induction of melanization, the supernatants of the reaction mixtures were analyzed by SDS-PAGE under reducing conditions. As shown in Fig. 1A, six proteins clearly disappeared after a 30-min incubation (lane 8) compared with the G-100 solution (lane 4), indicating that these proteins are associated with melanin synthesis. It is known that melanins induced by arthropod PO are closely linked to protein matrices in the form of melanoproteins (7–9). We assumed that if some proteins in hemolymph were used in melanin synthesis, they would disappear as positive regulatory proteins such as MEPs during the melanization reaction. If some proteins functioned as negative regulatory proteins such as MIPs, they should be generated or
degraded from the hemolymph when the melanization reaction was started.

To characterize further these six disappearing bands, we determined their amino-terminal sequences. Bands 1 and 2 showed the same sequences as FNTLSPWDEKVIYNYW, which perfectly matched with the Tenebrio vitellogenin-like protein that was reported as a MEP (20). The amino-terminal sequence of band 3 was blocked. The sequence of band 4 was determined with trichloroacetic acid and analyzed in 12% SDS-PAGE under reducing conditions. Lane 1, G-100 solution only; lane 2, G-100 + Ca²⁺; lane 3, G-100 + β-1,3-glucan; lane 4, G-100 + Ca²⁺ + β-1,3-glucan; lane 5, G-100 + PTU; lane 6, G-100 + Ca²⁺ + PTU; lane 7, G-100 + β-1,3-glucan + PTU; lane 8, G-100 + Ca²⁺ + β-1,3-glucan + PTU. Arrows 1 and 2 indicate the 160-kDa vitellogenin-like protein (MEP). Arrows 3, 4, and 5 are Tenebrio pro-PO, 43-kDa protein, and Tenebrio desiccation protein, respectively. Gels were fixed and stained for proteins with Coomassie Brilliant Blue. All reactions were incubated for 1 h.

**Cloning and Characterization of the 43-kDa Band**

We determined four internal amino acid sequences of the 43-kDa protein as follows: EMFEPLADLGIPGSVTAEEFN, KGELETVIYLDKDELYG, VQPILLGTLSDTYEANDDMLSG, and WRVLVPNSSYYLV. To determine the whole amino acid sequence of the 43-kDa protein, we synthesized a degenerate oligonucleotide probe for YEANDDMI and screened a cDNA library of T. molitor larvae, and then obtained 15 positive clones. The nucleotide sequence of the longest insert and the deduced amino acid sequence encoded in the open reading frame of this clone are shown in Fig. 2. The open reading frame encodes a protein consisting of 352 amino acid residues. The five peptide sequences derived from the 43-kDa protein are all found within the complete sequence, indicating that the cDNA encodes for the 43-kDa protein. The deduced amino acid sequence of the 43-kDa protein has no significant homology to other proteins reported so far, but it contains an Asp-rich region, including 11 contiguous Asp residues, in the central part of the 43-kDa protein, which shows a high degree of homology to several unrelated proteins with different functions. As shown in Fig. 2B, the Asp-rich sequence of the 43-kDa protein shows high homology with the RNA polymerase II subunit 5 (RPB5)-mediating protein, which is an RPB5-interacting protein and which also counteracts transactivation.
This Asp-rich motif is also present in another protein such as *Xenopus laevis* RNA polymerase 1 transcript factor (Xl-RPTF, 30) or *Mus musculus* upstream binding factor (Mm-UBF), which is involved in the proliferation and differentiation of murine myeloid cells (31), and *Drosophila melanogaster* Gp150 protein (Dm-Gp150), which is related to early ommatidial development through modulation of Notch signaling (32). Also, the 11-Asp stretch is encoded by two different stretches of the triplets GAC and GAT, indicating that the Asp-rich region seems to avoid destabilizing the DNA structure by a long triplet repeat (33).

To ascertain the 43-kDa protein as a melanization-related protein, we examined the changes in concentration of the 43-kDa protein in vivo during melanin synthesis. As shown in Fig. 3C, *Tenebrio* larvae injected with *Candida albicans* cells (5 × 10^7^ cells) turned black after 24 h, whereas no change of color occurred in control larvae (Fig. 3A). When the concentration of the 43-kDa protein was examined by Western blot in the melanin-induced hemolymph by the injection of *C. albicans*, we found that the 43-kDa protein decreased but not in the control larvae (Fig. 3D), suggesting that the 43-kDa may be related with melanin synthesis. However, we could not determine whether the 43-kDa protein is a MEP or MIP.
system were strongly inhibited by DFP (10). As expected, the DFP-treated hemolymph showed very low PO activity compared with the crude hemolymph, indicating that Tenebrio pro-PO-activating factors were inactivated by DFP treatment (data not shown). To gain insights into the function of the 43-kDa protein in melanin synthesis induced by dopamine, we purified native and recombinant 43-kDa protein to homogeneity (lane 2 in Fig. 4A and lane 2 in Fig. 4B). To address whether the purified native or recombinant 43-kDa protein functions as MEP or MIP in vitro, we added the purified 43-kDa recombinant protein to the crude hemolymph solution and examined the effects on melanin synthesis using dopamine as substrate. As shown in Fig. 5A, the recombinant 43-kDa protein had an inhibitory effect on melanin synthesis in a dose-dependent manner. Under the same conditions, when we checked the effects of PO activity by addition of recombinant 43-kDa protein to the hemolymph solution, PO activity induced by synthetic substrates 4-methylcatechol and 4-hydroxyproline ethyl ester as PO substrates was not affected (Fig. 5B).

Previously it was reported that insect pro-PO system induced the activation of serine protease zymogen to active serine protease during 1,3-β-D-glucan-dependent pro-PO activation (11, 21). To explore the relationship between serine protease activity and PO activity in the presence of MIP, we compared the amidase activity in the presence and in the absence of MIP with 1,3-β-D-glucan and Ca2+ by using commercially available trypsin substrate, Boc-Phe-Ser-Arg-MCA. As shown in Fig. 5C, when Ca2+ ion and 1,3-β-D-glucan were added to G-100 solution (column 2), amidase activity increased compared with the G-100 solution alone (column 1). By addition of MIP to the G-100 solution in the presence of Ca2+ and 1,3-β-D-glucan, amidase activity was not changed even though increased amounts of MIP (columns 3, 4, and 5). These results suggest that MIP does not inhibit the amidase activity of any yet unidentified PO-activating serine protease(s). This indicates that MIP is not an inhibitor of the pro-PO-activating cascade itself, but rather that MIP inhibits the formation of melanin from PO-oxidized phenol substrates.

To characterize further the biochemical properties of MIP during melanin synthesis, we tried to find the MIP-binding protein(s) from G-100 solution and melanin-induced G-100 solution by Western blotting analysis. As shown in Fig. 5D, ~47-kDa protein of G-100 solution but not melanin-induced G-100 solution was specifically recognized by the MIP antibody, indicating that MIP might bind to this 47-kDa protein in G-100 solution. When melanin synthesis induced by the activated PO has been started, MIP might be dissociated from the 47-kDa protein and then degraded from hemolymph.
reactions in the melanin biosynthetic pathway (35). A search of inhibitors for tyrosinase is one of the major strategies in developing new whitening agents. Many tyrosinase inhibitors are polyphenol derivatives of flavonoids or of trans-stilbene (t-stilbene), such as resveratrol and its derivatives, which have been investigated intensively (36, 37). They are usually constructed from one of two distinct substructures, which dictate their mechanism of tyrosinase inhibition: containing either a 4-substituted resorcinol moiety or catechol. It was suggested that 4-substituted resorcinol-type inhibitors are potent in tyrosinase inhibition and bind to the enzyme binuclear active site (36). The catechol structure, with two OH groups at o-positions, may behave as a chelator to the copper ions in the tyrosinase (38). However, no report has shown that a protein, such as the...
43-kDa MIP described in this study, has an ability to inhibit the melanization reaction step.

From invertebrates, an endogenous PO inhibitor (POI) containing a dopa-containing 38-mer peptide from *Musca domestica* was purified and characterized (15). It was suggested that this inhibitor, POI, plays an important role in smoothing the way of emergence of the adult through hindering excessive melanization, as well as hardening, of cuticular proteins under the epicuticle. However, the amino acid sequence of 43-kDa MIP did not show any homology with that of POI. Another negative regulator of the pro-PO cascade in *Drosophila* is the blood serine protease inhibitor serpin-27A (17, 19). Serpin-27A has been shown to regulate the melanization cascade through the specific inhibition of the terminal protease of the pro-PO-activating cascade, which also was shown for the pacifastin proteinase inhibitor in a crustacean (18). Also, it was reported that the melanization reaction requires Toll pathway activator proteinase inhibitor in a crustacean (18). It was reported that several pattern recognition proteins for 1,3-

... continue reading...
A Novel 43-kDa Protein as a Negative Regulatory Component of Phenoloxidase-induced Melanin Synthesis
Mingyi Zhao, Irene Söderhäll, Ji Won Park, Young Gerl Ma, Tsukusa Osaki, Nam-Chul Ha, Chun Fu Wu, Kenneth Söderhäll and Bok Luel Lee

J. Biol. Chem. 2005, 280:24744-24751.
doi: 10.1074/jbc.M504173200 originally published online April 27, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M504173200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 39 references, 13 of which can be accessed free at http://www.jbc.org/content/280/26/24744.full.html#ref-list-1