N-terminal amino acid sequence of an insect neurohormone, melanization and reddish coloration hormone (MRCH): heterogeneity and sequence homology with human insulin-like growth factor II

Shogo Matsumoto, Akira Isogai, and Akinori Suzuki

Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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An insect neurohormone, melanization and reddish coloration hormone (MRCH), is responsible for cuticular melanization and epidermal red pigmentation in the armyworm. The three molecular forms of MRCH were isolated from adult heads of the silkworm, *Bombyx mori*, and their N-terminal amino acid sequences revealed a sequence homolgy with the C-terminal region of human insulin-like growth factor-II as well as N-terminal heterogeneity of MRCHs.

Amino acid sequence *Insect neurohormone* *Insulin-like growth factor-II* *Sequence homology* *Amino terminal heterogeneity* *Cuticular melanization*

1. INTRODUCTION

In insects that undergo color polymorphisms, various neurohormones are implicated in the control of pigment metabolism. Among them, only melanization and reddish coloration hormone (MRCH) has been isolated and chemically elucidated [1,2]. MRCH is concerned with formation of both melanin in the cuticle and ommochrome in the epidermis of armyworm species [3,4]. An attempt to purify and characterize MRCH was performed with both cephalic organs of the common armyworm, *Leucania sparata*, and whole heads of adult *B. mori*, and it has been demonstrated that MRCH is a peptide with an M_r of 6400–8000 [5,6]. In our previous paper, we reported the isolation and N-terminal amino acid sequences of two components, MRCH-I and -II, from the adult heads of *B. mori* [2]. We describe here isolation of the third component, MRCH-III, and determination of its N-terminal amino acid sequence which shows a homology with the C-terminal region of human insulin-like growth factor II (IGF-II) as well as N-terminal heterogeneity of MRCHs.

Abbreviations: HPLC, high performance liquid chromatography; IGF, human insulin-like growth factor; MRCH, melanization and reddish coloration hormone; TFA, trifluoroacetic acid; PTH, phenylthiohydantoin

2. MATERIALS AND METHODS

2.1. *Bioassay*

Bioassay of MRCH was performed by using the penultimate instar larvae of *L. separata* according to the method previously reported [6]. MRCH activity at each purification step was represented as MRCH units defined in [6].

2.2. *Materials*

The starting material for the isolation of MRCH-III was 'QAE-I' fraction derived from 211000 heads of adult *B. mori* (race, Showa) at the step 6 in the previous paper [2]. The procedures, in

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brief, were as follows. An acetone powder of *Bombyx* heads was extracted with 80% ethanol and then heated to 100°C for 10 min. The heated fraction was precipitated with 50–95% acetone to give 'crude MRCH'. Crude MRCH was applied to column chromatography with activated charcoal and then QAE-Sephadex affording two active fractions, QAE-I and 'QAE-II'. MRCH-I and -II were isolated from QAE-II as previously reported [2].

2.3. *Isolation of MRCH-III*

QAE-I was concentrated by ultrafiltration with Amicon UM-2 (Amicon) and then applied to a Sephadex G-50 (superfine) column (1.8 cm i.d. × 92 cm). The column was eluted with 0.05 M $AcONH_4$ containing 2% *n*-butanol at 14 ml/h at 5°C. The active fractions obtained from Sephadex G-50 were combined and subjected to the reversed-phase HPLC on a Hi-Pore RP-318 column (Bio-Rad, 4.6 mm i.d. × 250 mm). HPLC was repeated 3-times, successively, with a linear gradient of 5–30% acetonitrile in 3 different solvent systems; 10 mM phosphate buffer (pH 7.0), 5 mM sodium hexanesulfonate and 0.1% phosphoric acid, and 0.1% TFA.

2.4. *Amino acid sequence analysis*

N-terminal amino acid sequence of purified MRCH-III was analyzed by automated Edman degradation with a gas-phase protein sequencer, model 470A (Applied Biosystems) [7]. PTH amino acids were identified with reversed-phase HPLC as reported [2].

2.5. *Search for sequence homology with MRCHs*

Sequence homology between MRCHs and the other protein sequences were searched in the Dayhoff Atlas and the Newat sequence data base at the University of California, San Diego [8].

3. RESULTS AND DISCUSSION

3.1. *Isolation procedure*

QAE-I derived from 211000 heads of adult *B. mori* was first subjected to a gel filtration on Sephadex G-50. MRCH activity was recovered at the position corresponding to an M_r of 7000. The fractions with MRCH activity were combined and subjected to further purification with reversed-phase HPLC on Hi-Pore RP-318 in the same manner as that used for the isolation of MRCH-II [2]. Fig.1 shows elution profiles of HPLC and table 1 summarizes the purification of MRCH-III. After a 430000-fold purification from crude MRCH, 0.3 μg of pure MRCH-III was isolated. The specific activity was 6 ng/unit that was coincident with that of MRCH-I or -II [2].

3.2. *N-terminal amino acid sequence of MRCH-III*

Since minute amounts of purified MRCH-III were obtained (40 pmol), it was subjected to

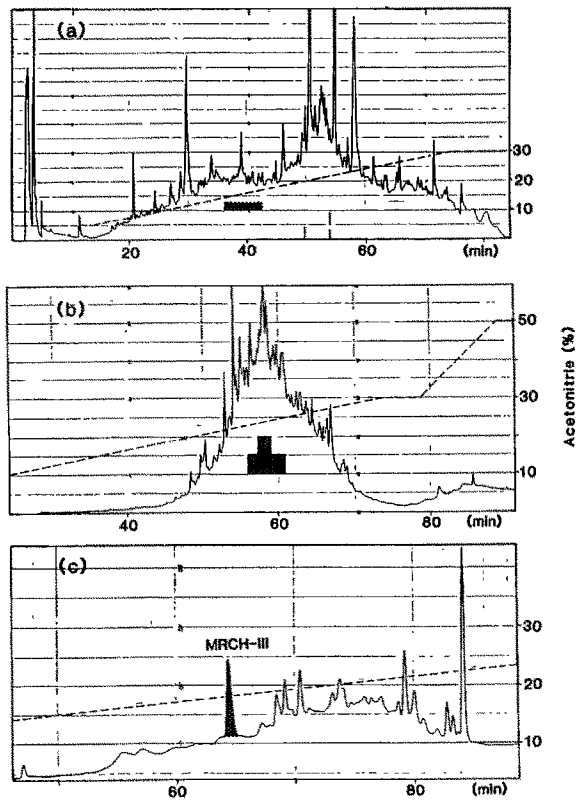

Fig.1. Reversed-phase HPLC on Hi-Pore RP-318. Absorbance at 220 nm was detected. Shaded fractions showed MRCH activity [6]. (a) Sephadex G-50 active fraction derived from QAE-I was chromatographed with acetonitrile in 10 mM phosphate buffer (pH 7.0). 1.0 AUFS. (b) The active fraction in (a) was chromatographed with acetonitrile in 5 mM sodium hexanesulfonate and 0.1% phosphoric acid. 0.19 AUFS. (c) The center two active fractions in (b) were chromatographed with acetonitrile in 0.1% TFA. 0.034 AUFS.

Table 1

Purification of MRCH-III from 211000 *Bombyx* heads

Purification (step)	Weight[a] (mg)	Total activity (units)	Specific activity (ng/unit)
Crude MRCH (4th)	26000	10000	2600000
QAE-Sephadex (6th)			
QAE-I	150	2500	60000
QAE-II	150	5000	30000
Sephadex G-50 (7th)	3.0	1250	2400
Hi-Pore RP-318 (10th)			
MRCH-III	0.0003	50	6

[a] Estimated from the absorbance at 220 and/or 280 nm except crude MRCH

analysis of N-terminal amino acid sequence with a gas-phase protein sequencer, model 470 A. Fig.2a shows the N-terminal sequence of MRCH-III along with MRCH-I and -II [2]. These results indicate that MRCHs are single chain peptides and reveal a heterogeneity in the amino terminal portion. MRCH-III possesses an addition proline residue to the N-terminal leucine of MRCH-I. It may be that N-terminal portion does not participate in the biological activity of MRCHs, since several aminopeptidases failed to inactivate MRCH as previously reported [6].

We have previously called attention to the molecular heterogeneity of MRCH [6], and we now conclude that MRCH in *B. mori* consist of more than 3 molecular species. The multimolecular forms of other insect neurohormones have been described for adipokinetic hormone (AKH) of the locust [9], diapause hormone of the silkworm [10], diuretic hormone of the locust [11], prothoracicotropic hormone of the silkworm [12], and periplanetin of the cockroach [13].

3.3. *Sequence homology between MRCHs and IGF-II*

A comparison of the MRCH N-terminal sequences with the IGF-II C-terminal sequence [14] revealed a similarity between these peptide hor-

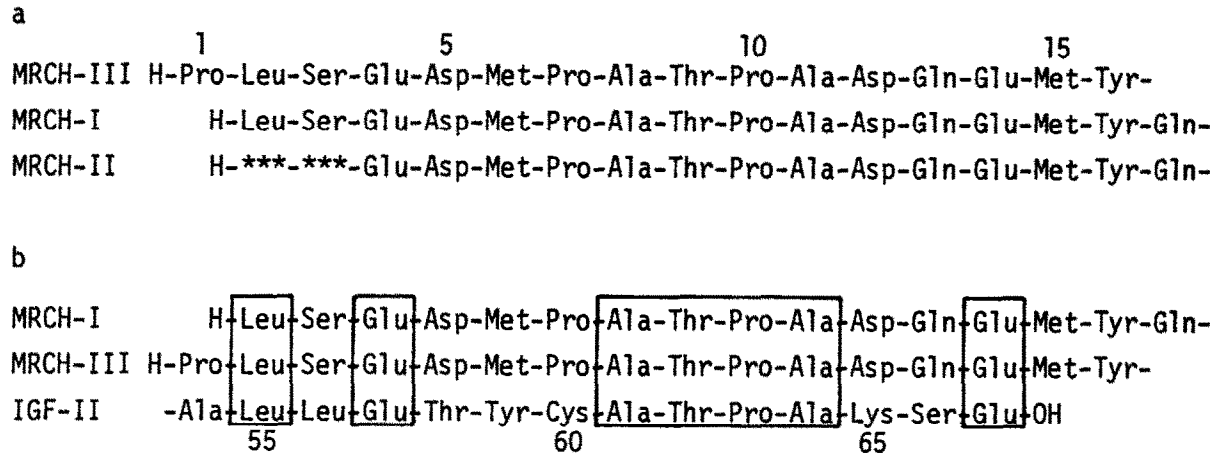

Fig.2. (a) N-terminal amino acid sequence of MRCHs. ***, Unidentified [2]. (b) Homology between MRCH-I, -III and IGF-II.

mones; 4 consecutive amino acids and 3 isolated amino acids are matched with no sequence gap due to insertions or deletions (fig.2b). This is an intriguing coincidence although limited sequences of the MRCHs are on hand. It is noteworthy that insect prothoracicotropic hormone (4K-PTTH) of the silkworm reveals a striking homology with insulin and IGFs [12]. The present findings may support the idea that a common ancestral peptide molecule evolved into peptides with different functions in insects and vertebrates [12].

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