Juvenile-hormone-binding protein from silk gland of *Galleria mellonella*

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Juvenile-hormone-binding protein (JHBP) has been detected in cytosol of *Galleria mellonella* silk glands. JHBP has been separated from juvenile hormone (JH) degrading enzymes using column chromatography on Sephacryl S-300. JHBP has a sedimentation coefficient of 4.1 S, an isoelectric point of pH 4.7 and exhibits one class of binding sites ($K_{d\ JH\ III} = 1.5 \times 10^{-8}$ M) with the highest specificity towards JH II. It was found that Zwittergent 3-10 increases 10-fold the JHBP activity and converts some higher molecular mass species of JHBP into a single, approx. 50-kDa component.

Juvenile hormone receptor *Juvenile hormone esterase* *Epoxide hydrolase* *Silk gland*
Galleria mellonella

1. INTRODUCTION

Juvenile hormones are powerful regulators of insect development. It is generally accepted that silk gland is the specific target tissue for JH [1]. When the proper dose of JH is administered in the early 7th instar, larvae undergo a supernumerary molting, the growth of their silk glands and RNA synthesis is lowered and silk synthesis is delayed [2].

Cytosolic juvenile-hormone-binding proteins (JHBP) have been detected in hypodermis [3], K_c cells (*Drosophila*) [4], ovaries (*Leucophaea*) [5], fat bodies of *Leucophaea* [6] and *Locusta* [7]. The aim of this work was to search for JHBP in *Galleria mellonella* silk glands.

2. MATERIALS AND METHODS

2.1. *Insects*

The larvae of *G. mellonella* were reared on an artificial diet [8] at 30°C. Posterior parts of silk glands were dissected from exactly timed last larval insects [9] and stored at −20°C.

2.2. *Cytosol preparation*

The silk glands were homogenized in a loosely fitting Potter-type homogenizer in stock buffer: 10 mM Tris–HCl, 50 mM KCl, 1 mM EDTA, 10% (v/v) glycerol, 14 mM 2-mercaptoethanol (pH 7.4) at 20°C. The homogenate was initially centrifuged for 10 min at 10000 × *g* and then the supernatant was centrifuged for 60 min at 100000 × *g*. The resulting supernatant was designated the cytosol preparation.

2.3. *JH-binding assay*

Two hundred μl fractions of cytosol or cytosol fractions were incubated in duplicate with 4.5 nM racemic [^{3}H]JH III (spec. act. 11 Ci/mmol) with or without 10 μM unlabeled JH III for 20 min at 30°C and subsequently for 20 min at 4°C. Following the incubation 50 μl of charcoal suspension [1% Norit A, 0.1% Dextran T-70, 0.25% bovine serum albumin in 10 mM Tris–HCl (pH 7.4)] was added and JHBP activity was determined as in [6]. The specific activity was considered to be the portion of total binding that was prevented by the excess of cold hormone.

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2.4. *JH degradative activity*

The JH esterase activity (JHE) was measured using JH III as a substrate at a final concentration of 5×10^{-6} M [10]. This assay was also used for JH III epoxide hydrolase activity, and degradation products were identified by thin-layer chromatography.

2.5. *Gel filtration*

Sephacryl S-300 (Pharmacia) columns (0.9 i.d. × 60 cm) were equilibrated with stock buffer with or without 0.1% Zwittergent 3-10 (Calbiochem). The columns were calibrated with appropriate molecular mass markers.

2.6. *Density gradient centrifugation*

The sedimentation coefficient was determined using a 5–20% sucrose gradient [11] formed in stock buffer. ^{14}C-labeled proteins were used as internal standards.

2.7. *Isoelectrofocusing*

Isoelectrofocusing and gel extraction were performed as in [12]. The gel extract was assayed for JHBP activity after overnight dialysis against the stock buffer.

3. RESULTS AND DISCUSSION

During development of *G. mellonella* the silk gland JHBP activity exhibits a sharp single maximum on the second day after ecdysis (fig.1). This activity peak occurs 2 days earlier than the increase of JH binding by hemolymph proteins [13]. Moreover, the peak of cytosol JHBP occurs when the JH titer in the hemolymph has dropped markedly [1,14]. One possible explanation is that a rapid increase of JHBP activity is directly associated with the reprogramming of biological events in the cytoplasm.

The glands of the second day of the 7th instar larvae were used for further experiments. Two JH degrading activities and one broad JHBP activity peak are revealed by gel filtration of cytosol proteins (fig.2A). Epoxide hydrolase (EH) is associated with a high-molecular-mass fraction over 1000 kDa whereas the majority of the JHE activity is eluted in the range 100–400 kDa. A broad heterogeneous JHBP activity peak appears at 50–120 kDa. Usually a satisfactory separation of JHE and JHBP activities was achieved, although some fluctuations in JHE and JHBP activities with respect to their molecular mass distribution were observed. The molecular mass variability in JHE and JHBP activities led us to

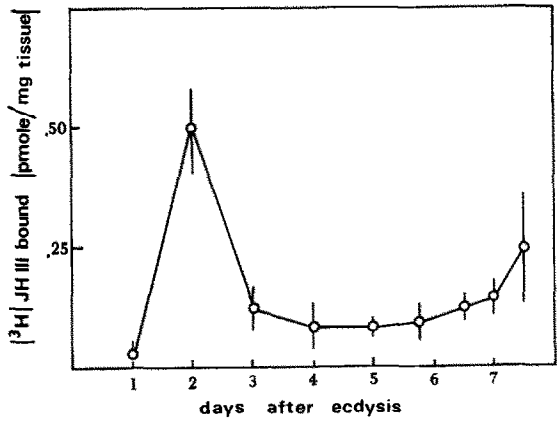

Fig.1. JH-binding activity in silk gland cytosol during the last larval instar. Each cytosol sample was prepared from 10 silk gland pairs. The vertical bars show ± SE.

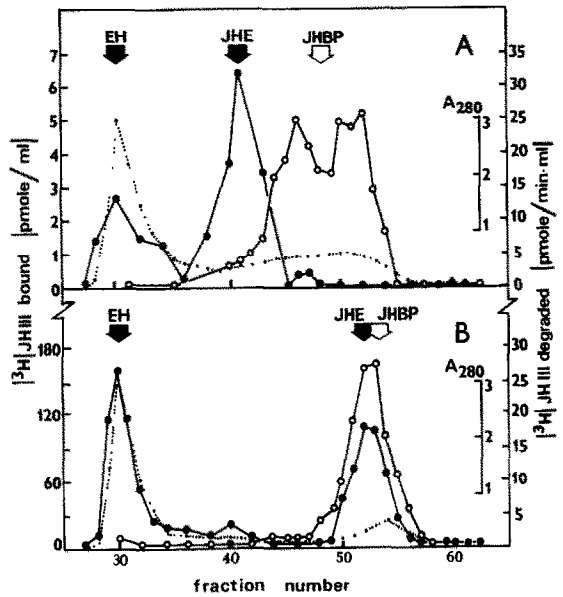

Fig.2. Sephacryl S-300 gel chromatography of silk gland cytosol. A 1-ml cytosol sample of 100 silk gland pairs was applied to the top of the column; 0.4–0.5 ml fractions were collected. Protein profile at A_{280} (•----•), JH degrading activity (●——●), JHBP activity (○——○). (A) No Zwittergent 3-10, (B) with 0.1% Zwittergent 3-10 present in cytosol and column equilibrating buffer.

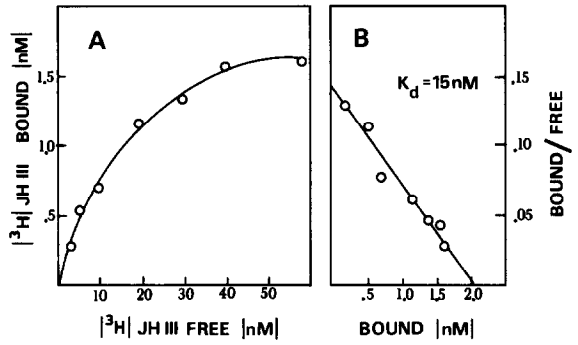

Fig.3. (A) [^{3}H]JH III binding to JHBP. The JHBP preparation was obtained from silk gland cytosol using gel filtration as described in section 3. (B) Scatchard plot of data from A.

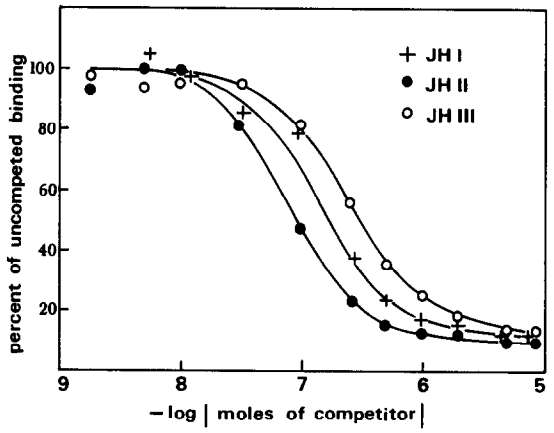

Fig.4. Competition by unlabelled JHs for [^{3}H]JH III binding sites in silk gland cytosol. Aliquots (0.2 ml) of cytosol were incubated with 4.5 nM [^{3}H]JH III alone or in the presence of 2 nM–10 μM unlabelled JHs.

believe that this phenomenon is associated with the aggregation process. In fact, the addition of Zwittergent 3-10 to the cytosol resulted in formation of a single peak of JHE and JHBP activities (fig.2B). In this case both JHE and JHBP activities elute at the same volume, corresponding approximately to 50 kDa. The detergent does not change the JHE activity. Surprisingly, the overall JHBP activity rises about 10-fold in the cytosol preparation incubated with Zwittergent 3-10. The fact that JHBP activity can be elevated by detergent suggests that a new regulatory process, based possibly on JHBP dissociation, may operate in the glands. In control experiments: (i) bovine serum albumin did not exhibit specific binding; (ii) in the JHE assay, no effect of 0.1% Zwittergent on the partition of JH III, JH III-acid and JH III-diol was observed.

Fractions from the descending portion of the JHBP activity peak were used for JH-binding studies (fig.3). The Scatchard plot [15] for JH III-specific binding yielded a straight line demonstrating a single type of high-affinity binding site with a dissociation constant of 1.5×10^{-8} M. This value is similar to that found in other insects [3–7]. Isoelectrofocusing showed that JHBP is a highly acidic protein with p*I* 4.7 (not shown). Density gradient centrifugation yielded an asymmetrical peak with 4.1 S (not shown). The competition binding experiment conducted with the unlabeled JHs indicates the highest specificity of JHBP toward JH II (fig.4). This finding correlates well with previous data [16] on the predominance of JH II over other analogs in this insect. The specific binding could not be demonstrated when [^{3}H]JH III-acid was used. This indicates that cytosol JHBP is a highly specific JH receptor protein.

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