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A mathematical model for the regulation of juvenile hormone titers

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Abstract

The titer of juvenile hormone (JH) is determined by three factors: its rate of synthesis, its rate of degradation, and the degree to which JH is protected from degradation by binding to a diversity of JH-binding proteins. All three of these factors vary throughout the life history of an insect and contribute to variation in the JH titer. The relative importance of each of these factors in determining variation in the JH titer is not known and can, presumably, differ in different life stages and different species. Here we develop a mathematical model for JH synthesis, degradation, and sequestration that allows us to describe quantitatively how each of these contribute to the titer of total JH and free JH in the hemolymph. Our model allows for a diversity of JH-binding proteins with different dissociation constants, and also for a number of different modes of degradation and inactivation. The model can be used to analyze whether data on synthesis and degradation are compatible with the observed titer data. We use the model to analyze two data sets, from *Manduca* and *Gryllus*, and show that in both cases, the known data on synthesis and degradation cannot account for the observed JH titers because the role of JH sequestration by binding proteins is greatly underestimated, and/or the *in vivo* rate of JH degradation is greatly overestimated. These analyses suggest that there is a critical need to develop a better understanding of the *in vivo* role of synthesis, sequestration and degradation in JH titer regulation.

Keywords: Juvenile hormone; Model; JH-binding proteins; JH titer regulation

1. Introduction

The juvenile hormones (JHs) of insects regulate an uncommonly broad diversity of developmental and physiological processes such as metamorphosis, reproduction, diapause, migration, seasonal polyphenism, and caste determination in social insects. Yet, in spite of more than half a century of studies on these ubiquitous insect hormones, their mode of action at the molecular level is still a mystery (Wheeler and Nijhout, 2003), and the mechanisms that control their level in the hemolymph and tissues remain incompletely understood.

It is generally agreed that the titer of JH is determined by a balance in the rate of its secretion and degradation, and by the degree to which JH is protected from degradation by binding to proteins in the hemolymph and in cells. There are several forms of JH in different species of insects, as well as a variety of binding proteins that differ in their abundance, in their localization, and in the strength with which they bind the different forms of JH (De Kort and Granger, 1996). An excellent and comprehensive recent review of the literature on the biology and biochemistry of JH is given by Goodman and Granger (2005).

Although secretion, degradation, and sequestration each must play a role in regulating the JH titer, it is not clear whether one of these factors is always more important than the others, or whether different factors control the JH titer at different times in an insect's life-cycle. One way to examine the relative roles of synthesis, degradation, and sequestration is by means of a quantitative mathematical description of the biochemical kinetics of these processes. A mathematical description makes explicit assumptions about how a process works, and is therefore useful for examining the consequences of those assumptions. The purpose of the present paper is to develop a quantitative theory for JH titers that can be used to study

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the significance of the various factors that are known, or suspected, to affect the levels of JH.

The kinetics of JH degradation and sequestration have been studied in many species of insects and are well enough understood for *Manduca* and *Gryllus* so that an explicit quantitative description is possible. During larval development of *Manduca sexta*, the JH titer fluctuates significantly, as does the rate of JH secretion, the activity of JH esterases (JHE), and the level of JH-binding proteins (Goodman, 1985; Hammock and Roe, 1985; Baker et al., 1987; Jesudason et al., 1990; Janzen et al., 1991; Hidayat and Goodman, 1994; Park et al., 1993). In adult *Gryllus firmus* there is a dramatic morph-specific daily cycle of JH titer that is accompanied by fluctuations in both JH synthesis and JHE activity (Zhao and Zera, 2004). We would like to find a method for calculating and predicting how fluctuating levels of JH synthesis, sequestration, and degradation control the JH titer and the half-life of JH in the insect.

2. Methods

2.1. Derivation of the kinetic equation for JH titers and half-life

The reaction system shown in Fig. 1 describes the synthesis and degradation of JH and its equilibrium binding to a protein that protects it from degradation. This system considers only one kind of JH, a single mode of JH degradation, and an indefinite number of JH-binding proteins with different affinities for JH. We note here that the binding protein term potentially includes other (yet unknown) sites like the fat body or cell membranes that bind JH and protect it from degradation. We develop a mathematical analysis of this system first, and then consider how it can be modified to deal with multiple modes of JH breakdown. The notational conventions we will use are JH_f for free JH (unbound to protein), BP for the free binding protein, and JHBP for the hormone–protein complex. We assume that the free JH is the active form of the hormone and that when JH is bound to a binding protein it is inactive. When JH titers in insect hemolymph are measured they refer to the total amount of JH_{tot} (free plus bound). Park et al. (1993) have shown that in *Manduca* only about 0.1% of the JH is free (i.e. about 99.9% of the JH is bound to protein), and the binding protein is in great excess so that 95% of the binding protein is free (i.e. has no JH bound). We assume that only free JH is subject to enzymatic degradation by JHE (Fig. 1). Although JH breakdown follows Michaelis–Menten kinetics, the K_m is much larger than the concentration of free JH (Hidayat and Goodman, 1994; Bonning et al., 1997), so the rate of JH breakdown is pseudo-first-order, and we assume it to depend on a single rate constant (k_2), which is approximately V_{max}/K_m. The derivation of k_2 is given in a separate section below.

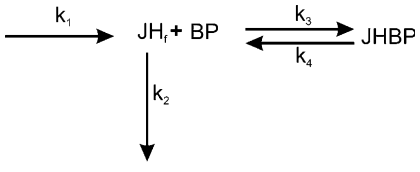

Fig. 1. Scheme of JH synthesis, degradation and sequestration. JH_f, free juvenile hormone; BP, free juvenile hormone-binding protein; JHBP, hormone–protein complex; k_1, rate of synthesis; k_2, rate of degradation; k_3, rate of binding to binding protein; k_4, rate of dissociation from binding protein. Although BP stands for any and all possible JH-binding proteins, this term also includes potential non-protein-binding sites such as plasma membranes and fat body inclusions.

2.2. Derivation of the kinetic equation for the JH titer

The concentration of each of the JH-binding proteins is generally much larger than the total concentration of JH, JH_{tot} (De Kort and Koopmanschap, 1989; Hidayat and Goodman, 1994; De Kort and Granger, 1996; Goodman and Granger, 2005), so that the concentration of free binding protein is not affected by JH_{tot}.

The concentration of free JH (JH_f) changes over time according to

$$\frac{\mathrm{d}}{\mathrm{d}t}(JH_f) = k_1 - k_2\,JH_f + \sum_{i=1}^{n}(k_4^i\,JHBP^i - k_3^i\,BP_{tot}^i\,JH_f), \tag{1}$$

where k_1 is the rate of synthesis, k_2 the degradation constant, so that $k_2 JH_f$ is the rate of degradation of free JH. Similarly, k_3^i and k_4^i are the association and dissociations constants, respectively, of JH with the ith binding protein, BP^i is the concentration of the ith binding protein, and $JHBP^i$ is the concentration of the ith hormone–protein complex. We note that it is possible for JH to bind and be sequestered by non-protein-binding sites. As long as this binding is reversible, it is accounted for in Eq. (1), and one can consider the ith binding protein in this equation (and all equations that follow) to represent a reversible non-protein sink for JH, with its own characteristic association and dissociation constants.

The concentration of each hormone–protein complex changes according to

$$\frac{\mathrm{d}}{\mathrm{d}t}(JHBP^i) = k_3^i\,BP_{tot}^i\,JH_f - k_4^i\,JHBP^i. \tag{2}$$

At equilibrium $\mathrm{d}/\mathrm{d}t(JH_f) = 0$, and $k_4\,JHBP^i = k_3\,BP^i\,JH_f$, thus, letting the dissociation equilibrium constants be $K_D^i = (k_4^i/k_3^i)$, we have

$$JH_f = \frac{k_1}{k_2} \text{ and } JHBP^i = \frac{BP_{tot}^i\,JH_f}{K_D}. \tag{3}$$

This means that at equilibrium, the concentration of free JH depends only on its rate of synthesis and degradation. Thus the binding proteins have no effect on the equilibrium