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THE INHIBITORY EFFECT OF SINEFUNGIN ON JUVENILE HORMONE BIOSYNTHESIS AND DEVELOPMENT IN LOCUSTS

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Abstract—The antibiotic fungal metabolite sinefungin is a potent inhibitor of S-adenosylmethionine-acceptor methyltransferases. Its effect on insect metabolism and especially on corpora allata farnesoic acid methyltransferase, which catalyzes the penultimate step of juvenile hormone biosynthesis, was investigated in Locusta migratoria. Injection of sinefungin results in a delay of imaginal molt and in suppression of ovary development. Isolated corpora allata are unable to synthesize juvenile hormone III in the presence of more than 1.0 mM sinefungin. In a cell-free system containing the S-adenosylmethionine-dependent farnesoic acid methyltransferase from corpora allata sinefungin is a competitive inhibitor of the synthesis of methylfarnesoate with $K_i$ of 1 $\mu$M.

Key Word Index: Locust, sinefungin, O-methyltransferase, juvenile hormone biosynthesis

INTRODUCTION

Sinefungin is an antifungal antibiotic isolated from Streptomyces griseolus (Hamill and Hoehn, 1973). It is a structural analog of S-adenosylmethionine (AdoMet) (Fig. 1). Sinefungin has been found to have activity against fungi, viruses and parasites. In various systems it has been shown to be a potent inhibitor of transmethylation reactions. The effect of sinefungin on insects has not been investigated. Of special interest is the farnesoic acid O-methyltransferase (OMT), an enzyme that is involved in the last steps of juvenile hormone (JH) biosynthesis in insect corpora allata (CA) (Weirich, 1985). It is well established that this reaction consists of the transfer of the methyl group from AdoMet to either farnesoic acid (FA) (Hamnett et al., 1981) or to 10,11-epoxy-farnesoic acid or higher homologues of the latter (Reibstein and Law, 1973; Weirich and Culver, 1979; Peter et al., 1979). Here we describe the effects of sinefungin on Locusta migratoria in vivo and on the inhibition of JH biosynthesis in CA in vitro as well as in CA extracts containing the enzyme OMT.

MATERIAL AND METHODS

Materials, in vivo experiments and in vitro assays of CA activity were as described previously (Ferenz et al., 1986; Ferenz and Diehl, 1983).

O-methyl transferase assay

Extracts of 40–50 pairs of CA were prepared as described by Feyereisen et al. (1981). The OMT containing extract was mixed with glycerol (final concentration 15%) and stored in several batches frozen at $-70^\circ$C. Aliquots of 10 $\mu$l, containing 1.7 gland pair equivalents (=5 $\mu$g protein) were incubated in 90 $\mu$l of phosphate buffer (100 mM, pH 7.0) containing 1 mM EDTA and 1 mM mercaptoethanol to which 0.4 $\mu$g (1.6 nmol) FA and 0.7 $\mu$g [methyl-$^{14}$C]-S-adenosylmethionine hydrogen sulfate (1.7 nmol, containing 0.1 $\mu$Ci) as well as varying amounts of sinefungin were added. The total volume was 100 $\mu$l. After incubation for 30 min at $30^\circ$C, the mixture was extracted once with 300 $\mu$l iso-octane containing 14 $\mu$g methyl farnesoate (MF) and aliquots of the iso-octane phase were removed for counting.

RESULTS

Effects of sinefungin on larval and adult development

Injecting fifth-instar larvae of Locusta with sinefungin resulted in a delay or suppression of metamorphosis depending on the dose applied (data not shown). Dose-dependent reductions in ovary weight, oocyte development and total haemolymph protein were caused by repeated injections of increasing amounts of sinefungin into adult female locusts (Table 1). Often resorption of partly matured oocytes

Fig. 1. Chemical structures of S-adenosylmethionine and sinefungin.
Inhibition of juvenile hormone biosynthesis in isolated corpora allata

The JH biosynthesis in isolated CA was measured with a short-term radiochemical assay. This assay utilizes the fact that the labelled methyl group of methyl-methionine is incorporated into the methyl-ester group of JH. Addition of sinefungin resulted in a dose-dependent decrease of incorporation of radioactivity into JH (Fig. 2). Hormone production fell to background levels at inhibitor concentrations higher than 1 mM. Transfer of such CA into fresh medium without sinefungin did not restore the JH production.

Inhibition of O-Methyltransferase by sinefungin

Synthesis of MF by locust farnesoic acid. S-Adenosyl-methionine methyltransferase (OMT) prepared from CA was linear with time for at least 45 min, increased with temperature and was dependent on substrate concentrations (FA and AdoMet) (Fig. 3). The enzyme manifested classical Michaelis-Menten kinetics. It had an apparent $K_m$ for AdoMet of $1.2 \times 10^{-6}$ M and a $V_{max}$ of $14.3 \times 10^{-8}$ M/min ($K_m$ for FA $29 \mu$M). Sinefungin strongly inhibited the incorporation of radioactivity from [methyl-$^{14}$C]AdoMet into MF (labelled MF was

| Table 1. Effects* of sinefungin on sexual maturation during the first gonotrophic cycle of Locusta migratoria |
|---|---|---|---|---|---|---|
| Sinefungin injected† (μg) | Haemolymph protein (mg/ml) | Ovary wt (mg) | Oocyte length (mm) | Mortality (%) | N |
| 0 | 67.7 ± 3.9 | 233.3 ± 33.3 | 4.3 ± 0.6 | 14 | 12 |
| 6 | 67.2 ± 5.2 | 211.8 ± 34.0 | 3.9 ± 0.3 | 7 | 14 |
| 60 | 59.3 ± 3.9 | 100.9 ± 22.8 | 2.6 ± 0.4 | 13 | 13 |
| 150 | 36.9 ± 2.3 | 15.6 ± 1.9 | <1.3 | 9 | 10 |
| 300 | 24.3 ± 4.5 | 8.3 ± 1.8 | <1.3 | 75 | 4 |

*All determinations were made on day 15 after adult emergence. Data are mean values ± SEM.
†The amounts are the sum of six equal doses applied by repeated injections every second day.
AdoMet was the variable substrate for locust OMT, sinefungin exhibited competitive inhibitory kinetics with a $K_i$ of 0.5-1.0 x $10^{-4}$ M (Fig. 4).

**DISCUSSION**

The biosynthesis of juvenile hormone occurs in *Locusta migratoria* by the AdoMet-dependent methylation of FA to give MF which then is oxidized at the terminal double bond to (10R)-2E,6-E-3,7-dimethyl-1,5-dodecadienoate methyl ester (epoxyfarnesoid acid methyl ester, JH III) (Hamnett et al., 1981; Feyereisen et al., 1981; Weirich, 1985).

The results presented in this paper show that the enzyme farnesoid acid O-methyltransferase (OMT) was inhibited by sinefungin in a cell-free enzyme preparation from locust CA. The incorporation of [methyl-14C]SAM into FA in the presence of sinefungin decreased drastically with increasing concentrations of the inhibitor. Although the enzyme was not completely purified some of its properties could be determined in a crude extract of the CA. For conversion of AdoMet and FA apparent $K_m$ values of 1.2 and 29 $\mu$M, respectively, were found. Kinetics of inhibition showed that sinefungin acted as competitive inhibitor with a $K_i$ of 0.5-1.0 $\mu$M. This inhibition constant is comparable to those found with a number of methyltransferases inhibited by sinefungin (e.g. Borchardt et al., 1979; Smith and Norton, 1980; Robert-Gero, 1980; O'Dea et al., 1982). The inhibition of JH synthesis in cultured locust CA may be due to several effects, including disturbance of regulatory processes in enzyme synthesis and transportation phenomena. The observations on OMT in the cell-free system suggest, however, that sinefungin may also act in organ culture to inhibit the same enzyme. The irreversibility in the short-term assay may in part be attributed to the greater affinity of sinefungin for OMT than AdoMet. Many of the in vivo effects are similar to those observed after alactatomy at early stages of sexual maturation (Chen et al., 1976; Lazarovici and Pener, 1977; Goltzene and Porte, 1978). The low haemolymph protein concentration may in part be caused by a lack of vitellogenin synthesis which is regulated by JH (Chen et al., 1976; Ferenz and Kaufner, 1981). It was, however, not possible to revert these effects by topical application of the JH analog (RS)-methoprene. Though the animals were not lethally affected by small doses of sinefungin, it may be expected that at least larval development and fertility of adult females will be severely impaired. Most likely, sinefungin acts in locusts as a generally toxic agent and the inhibition of JH biosynthesis may be only one of the reasons for the inhibition of ovarian development. Sinefungin may prove of importance in further development of a new class of insect growth regulators.

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