Detection of Pheromone Biosynthetic and Degradative Enzymes in Vitro*

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Highly sensitive, luminescent assays have been developed to measure enzyme activities involved in the metabolism of a major class of insect pheromones which includes fatty aldehydes, alcohols, and their acetate esters. These assays have been applied to measure the in vitro biosynthesis and degradation of the sex pheromone of the eastern spruce budworm, Choristoneura fumiferana. Three activities were detected on analyses of extracts of the female moths: (a) an esterase that hydrolyzes both the cis and trans isomers of 11-tetradecenyl acetate, (b) an oxidase that converts fatty alcohols to aldehydes in the presence of O₂, and (c) an NAD-dependent aldehyde dehydrogenase. The coupled luminescence response of bacterial luciferase to long chain aldehydes was used to measure rates of reaction as low as 0.1 pmol/min since only low amounts of material can be analyzed. Specific activities of these enzymes were higher in the pheromone producing gland than in other parts of the moth, implicating these enzymes, and the oxidase in particular, in the pathway of pheromone biosynthesis.

The pathway was supported in vivo by demonstrating that topical application of [³H]-labeled tetradecenyl acetate onto the insect gland resulted in the formation of [³H]tetradecanal and [³H]tetradecanoic acid, thus providing evidence that all three enzymes were functional in the living insects.

Almost all insect species have highly effective communication systems that involve the release of special compounds known as pheromones. A pheromone is a chemical, or more generally, a precise blend of chemicals, that affects the behavior of insects of the same species (Weaver, 1978; Silverstein, 1981; Mayer and McLaughlin, 1975). In view of the potential contamination of the environment by insecticides, pheromones have practical importance since they can be used to monitor and/or control insect populations either as lures in trapping devices, or by disrupting the normal communication process (Silverstein, 1981).

The identification of the chemical structures of pheromones has advanced very rapidly since 10,12-hexadecadien-1-ol was shown in 1959 to be the sex pheromone of the silkworm moth, Bombyx mori (Butenandt et al., 1959). The pheromone components of moths are typically long chain unsaturated acetates, alcohols, and aldehydes (Mayer and McLaughlin, 1975). However, biochemical studies on the biosynthesis of pheromones are extremely limited (Bjostad and Roelofs, 1981; Dillwitz et al., 1981) and only in a few instances have in vitro enzyme activities been measured (Clearwater, 1975; Hedin, 1977; Inoue and Hamamura, 1972; Weatherston and Percy, 1976). The paucity of biochemical studies on insect pheromones can be at least partially traced to the low amounts of material available and the absence of rapid and sensitive approaches for measuring the degradation and biosynthesis of pheromones. Biochemical investigations of pheromone metabolism are relevant to the effective application of pheromones in controlling insect populations (Weaver, 1978; Silverstein, 1981) and could lead to alternative pest control methods.

The eastern spruce budworm, Choristoneura fumiferana, is one of the major insect pests in the United States and Canada, causing millions of dollars in damage annually to the forest industry. The sex pheromone consists of two long chain aldehydes, trans-11-tetradecenal (96%) and cis-11-tetradecenal (4%) (Sanders and Weatherston, 1976; Silk et al., 1980). The pheromone, which attracts male moths of the same species, is secreted from a specialized gland located at the end of the abdomen of the female moth (Roelofs and Feng, 1968). The gland contains little of the aldehyde, but relatively large amounts of 11-tetradecenyl acetate (96:4, trans:cis) which has led to the suggestion that the acetate ester is a stored precursor of the pheromone (Silk et al., 1980).

Recently, we have developed an extremely sensitive and rapid bioluminescence assay for long chain aldehyde pheromones found in insects (Meighen et al., 1981; Meighen et al., 1982; Grant et al., 1982). Since this analysis involves measurement of aqueous solutions of the compounds at the picomole level, it provides a readily applicable approach for studying the metabolism in vitro of aldehyde pheromones and their precursors.

The present paper reports the discovery of three enzyme activities involved in pheromone metabolism in extracts of the gland of the eastern budworm: acetate esterase, alcohol oxidase, and aldehyde dehydrogenase activities. Luminescence assays were applied to measure the production and removal of long chain aldehyde as well as to measure the production of long chain alcohol using a coupled enzyme system to convert alcohol to aldehyde. Since analogous enzyme activities may be present in other insects with pheromones of similar structure, including such major pests as the corn earworm, the tobacco budworm, and the navel orangeworm (Grant et al., 1982), the approaches and techniques developed in this paper may be generally applicable for future biochemical investigations on enzymes involved in pheromone biosynthesis and degradation in a variety of different insects.

EXPERIMENTAL PROCEDURES¹

¹ Portions of this paper (including "Experimental Procedures," Tables I and II, and Figs. 18-8S) are presented in miniprint at the

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RESULTS

Three enzyme activities involved in the metabolism of the aldehyde pheromone of the spruce budworm were detected in vitro in extracts of the gland of the female moth. The enzymes include an acetate esterase, a fatty alcohol oxidase, and a fatty aldehyde dehydrogenase catalyzing the reactions shown in the scheme below:

\[
RCH_2OCOCH_3 + H_2O \rightarrow RCH_2OH + O_2 \rightarrow RCHO + NAD + RCOOH
\]

Detection of these enzymes was achieved by the highly sensitive luminescent response of bacterial luciferase to long chain aldehydes enabling rates in the picomoles/min (nanomolar/min) range to be measured directly (see Miniprint). Low amounts of the gland extracts were used in the enzyme assays as high protein concentrations resulted in a decrease in luminescent response to aldehydes, and only limited quantities of enzyme were available.

Acetate Esterase—Acetate esterase activity was determined using the luminescence assay after conversion of the alcohol product into the corresponding aldehyde with NAD and horse liver alcohol dehydrogenase. The rates obtained with trans-11- and cis-11-tetradecenyl acetate as well as the saturated ester at different substrate concentrations are shown in Fig. 4S (see Miniprint). The enzyme showed a slight preference for the trans-11 isomer, having a \(K_m\) and \(V_{max}\) of 0.3 \(\mu M\) and 80 pmol/min/gland (4 pmol/min/\(\mu g\)), respectively, for this compound.

Alcohol Oxidase—The activity of alcohol oxidase in gland extracts was determined by the luminescence response of bacterial luciferase to the aldehyde product and also showed hyperbolic curves as a function of substrate concentration (Fig. 4S). This enzyme also appeared to have a preference for trans-11-tetradecanol over the cis-11 and saturated isomers.

The maximum oxidase oxidase activity is about 5-fold lower than the esterase activity in the gland, these rates are more comparable at low, nonsaturating substrate concentrations (~0.1 \(\mu M\)), since the \(K_m\) for alcohol is about 3-fold lower than that of the ester.

The activity of the oxidase was clearly dependent on the presence of molecular oxygen as saturation of the reaction buffer with \(N_2\) reduced the reaction velocity and rates were restored to normal when the mixture was exposed to the atmosphere (Fig. 1). This finding was in agreement with the inability of nicotinamide (NAD, NADP) or flavin (FMN, FAD) cofactors to stimulate the activity.

The metabolism of both tritium-labeled tetradecanoyl acetate and tritium-labeled tetradecanol catalyzed by the gland extract is illustrated in Fig. 2. In the absence of NAD, alcohol is produced from the tetradecanoyl acetate (lane 2), and aldehyde is produced from the tetradecanol (lane 4). In the presence of NAD, the aldehyde is further oxidized to the acid (lane 5), showing that an aldehyde dehydrogenase activity exists in the gland extract as well, and that the product of this reaction is a long chain carboxylic acid.

Aldehyde Dehydrogenase—Aldehyde dehydrogenase activity in gland extracts could be rapidly measured from the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83 M-0764, cite the authors, and include a check or money order for $4.40 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

NAD-dependent rate of disappearance of aldehyde using a luminescence coupled assay. Since changes of <25% in the initial aldehyde concentration could not be accurately measured, an integrated form of the Michaelis-Menten equation and not the initial velocity was used to determine the enzyme
activity (see Miniprint). The $K_m$ for trans-11-tetradecenal was found to be approximately 100 nM and the maximum velocity between 1 and 2 nmol/min/gland. The NAD was present at saturating concentrations (0.5 mM) since changing its concentration (0.01–1.0 mM) only resulted in a decrease in the activity at concentrations below 0.2 mM. Although activity was not observed if NADP replaced NAD, the aldehyde dehydrogenase was found to function equally well with both the 11-unsaturated and saturated 14-carbon aldehydes.

Morphological Distribution of Enzymes—Since the luminescence coupled assays gave activities proportional to the amount of extract for not only aldehyde dehydrogenase but also for the oxidase and esterase (Figs. 2S and 3S, Miniprint), it was thus possible to examine the morphological distribution of the enzymes in the female moth of the eastern spruce budworm. All three enzymes had their highest specificity (units/µg of protein) in extracts of the gland with lower levels in the head, thorax, legs, and abdomen (Table I). Since the percentage of the wet weight as protein was essentially identical (7.2–8.6%) for all tissues investigated, the specific activity based on units/µg of wet weight gave similar conclusions. The alcohol oxidase appeared to have the greatest specific activity in the gland in terms of its morphological distribution, consistent with it being the enzyme directly responsible for the biosynthesis of the pheromone released from the gland. Mixing of the gland extract with extracts of other tissues showed that the lower levels of enzyme in other parts were not due to inhibitory factors present in these extracts (see Miniprint, Table IS). The polypeptide distributions by SDS2 gel electrophoresis of the extracts from the different body parts were very similar, indicating that the oxidase is not a major protein component in the gland.

Enzyme Activities in Vivo—To demonstrate the activity of all three enzymes in vivo, radiolabeled tetradecanyl acetate was applied to the glands, and the products formed were analyzed by thin layer chromatography. As shown in Fig. 3, the tetradecanyl acetate was readily metabolized by the gland into both tetradecanol and tetradecanoic acid, indicating that the esterase, oxidase, and dehydrogenase could function in series in the intact gland. The absence of tetradecanol, the intermediate between the alcohol and acid, is presumably a reflection of the presence of NAD in the tissue. As shown in Fig. 2, addition of NAD to in vitro assays converts the fatty aldehyde to fatty acid. When tetradecanoyl acetate was applied to either the head or the abdomen in vivo, tetradecanoyl was observed with the production of tetradecanol and being significantly lower than that obtained for the gland (Fig. 3). This in vivo specificity is in agreement with the morphological

**Table I**

| Oxidase | Esterase | Dehydrogenase |
|---------|----------|-------------|
| Head    | 0.14 ± 0.06 | 1.5 ± 2.0 | 21 ± 8 |
| Thorax  | 0.14 ± 0.12 | 0.4 ± 0.5 | 22 ± 22 |
| Legs    | 0.07 ± 0.08 | 1.0 ± 1.0 | 16 ± 21 |
| Abdomen | 0.15 ± 0.02 | 1.7 ± 1.3 | 18 ± 18 |
| Gland   | 1.1 ± 0.7  | 4.2 ± 4.8 | 38 ± 17 |

**Fig. 3.** Metabolism of tetradecanoyl acetate in vivo. Tritium-labeled tetradecanoyl acetate was applied topically to living insects as described (Bjostad and Roelofs, 1981). The distribution of radioactivity was determined by fluorography after thin layer chromatography of the hexane extracts obtained from the tissues after a 15-min incubation in vivo with the glands (lane 2), the heads (lane 3), and the abdomens (lane 4). The dimethyl sulfoxide solution of tetradecanoyl acetate before application is shown in lane 1.

**DISCUSSION**

Studies on identifying and resolving the enzymes involved in pheromone metabolism have been primarily limited by the low amounts of material and the lack of suitable assays. Thus, the development of the bioluminescence assay for aldehyde pheromones, and its application in the present study to measure long chain acetate esterases, fatty alcohol oxidases, and fatty aldehyde dehydrogenases, provided a significant increase in sensitivity so that enzyme activities involved in sex pheromone metabolism could be detected in vivo. The assays are sensitive enough to measure all three activities in a homogenate from a single gland.

Two of the enzyme activities, an esterase and an oxidase, which catalyze the formation of the aldehyde pheromone (trans-11-tetradecenal; 96:4) from trans-11-tetradecenyl acetate, are soluble enzymes. Partial resolution of the enzymes by anion exchange chromatography had no effect on their kinetic properties even though multiple molecular forms of the esterase were observed (see Miniprint). Both enzymes had higher specific activities in extracts from the pheromone-releasing gland than in extracts from the other parts of the insect. Tetradecenoyl acetate has been shown by Silk et al. (1980) to be present in the same isomeric ratio and in significantly higher amounts (10–40-fold) in the spruce budworm gland compared to the aldehyde pheromone. Furthermore, an increase in the acetate ester levels (~2-fold) in the gland during the calling period of the insect has led to the proposal that the acetate ester may be the precursor of the aldehyde pheromone (Silk et al., 1980). Although a daily rhythm in aldehyde levels was not observed by these workers, recent experiments have demonstrated that aldehyde levels in the gland are also significantly higher in the calling period (Morse et al., 1982). The detection of the esterase and oxidase activi-
Pheromone Metabolism in Vitro

activities in the extracts of the budworm and the morphological specificity for the gland both in vivo and in vitro provides direct support to the proposal that the biosynthesis of the aldehyde pheromone occurs via a fatty alcohol acetate ester. Some caution must be exercised in this interpretation, however, as it is possible these enzymes, and the esterase in particular, may have a more general role in the insect in addition to or instead of pheromone metabolism. It is of interest that the reactions catalyzed by the esterase and oxidase require no substrates other than H₂O and O₂, and are highly favored thermodynamically (i.e. irreversible), thus making the acetate ester an ideal precursor and storage form for the aldehyde pheromone. Conversely, the irreversibility of these reactions clearly indicates that the acetate ester is synthesized in a different set of reactions.

A NAD-dependent fatty aldehyde dehydrogenase was also found in extracts of the budworm. This enzyme, in contrast to the esterase and oxidase, appears to be membrane-bound which suggests that it may be located in a different subcellular location than the esterase and oxidase. The dehydrogenase could function to remove high concentrations of aldehydes that would be toxic to the cell. This enzyme has also been found in the male budworm including the antennae and could serve in a pheromone-clearing role which may be essential for the continuous response to pheromone molecules by the male moth. The dehydrogenase activity is high in gland homogenates and could explain why aldehyde was not observed in the in vivo labeling studies when tetradecanoyl acetate was applied topically to the gland in dimethyl sulfoxide. The in vivo labeling technique could not be used to show incorporation of radiolabel into the volatile pheromone, as the aldehyde release rates, in the calling period (Morse et al., 1982), dropped to very low levels after application of dimethyl sulfoxide (data not shown). Nevertheless, the labeling studies did indicate that all three enzymes could function sequentially in vivo.

A wide variety of insects have been shown to have long chain aldehydes as their pheromones (Mayer and McLauughlin, 1975; Grant et al., 1982). Although the luminescent assay is not advantageous for studying the specificities of enzymes for different aldehydes since luciferase only gives a high response to low concentrations of 14- to 16-carbon aldehydes (Meighen et al., 1982), fortunately, the major insect pests such as the corn earworm, the tobacco earworm, the tobacco budworm, and the navel orangeworm all have unsaturated aldehyde pheromones of this chain length. Similarly, an even greater number of insects have been shown to have acetate esters of 14- and 16-carbon alcohols as their major pheromone component (Mayer and McLauughlin, 1975). Consequently, the luminescent assays should be generally applicable for studying pheromone biosynthesis and degradation in a wide variety of insects. Recent experiments have shown that these esterase, oxidase, and dehydrogenase activities can be readily detected in gland extracts of the corn earworm Heliothis zea. These investigations have the potential for identification of a common pathway for pheromone metabolism in insects using long chain alcohols, acetate esters, or aldehydes as pheromones.

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Pheromone Metabolism in Vitro

DETECTION OF PHEROMONE BIOSYNTHETIC AND DEGRADATIVE ENZYMES IN VITRO

by

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EXPERIMENTAL PROCEDURES

Materials - Luciferase was purified from Photoreus pyrophoricus (now Chiropterae) by a procedure similar to that of Bolen and Velez (1983). L-3,5,7,9-Tetradecanoyl-adipic acid (New England Nuclear) was dithiothreitol (Sigma) and were activated for 1 h at 20°C and then developed in a substrate solution. Trifluoroacetic acid (TFA) was used for extraction of lipids. Thin layer chromatography (TLC) plates were used. DEAE-Sephadex A-25 for anion exchange chromatography was obtained from Pharmacia.

Female eastern hemlocks (Choristoneura fumiferana) were reared from NOSA (the mirror) and allowed to emerge under natural lighting. Extracts were prepared by excision of the glands from 2- to 3-day-old female moths and homogenization of the glands for 2 min at 5°C with a motor driven pestle in 0.05 M phosphate buffer, pH 7.0. Solutions were clarified by centrifugation at 10,000 g. Protein concentration was approximately 0.5 mg/ml. The assays described below yield 9 to 10% of the extract per ml of assay mixture corresponding to 0.1 gland. Protein buffers were prepared by diluting appropriate amounts of 1.0 M NaPO4 and 1.0 M K2HPO4.

Stock solutions of aldehydes, alcohols, and fatty acid esters were prepared from the non-solvent fraction of the extract. Purified solutions of long chain compounds (generally 5-10 mg) were made up to 1 ml of a 0.05 M phosphate buffer, pH 7.0, under a controlled flow of N2. For 10 min before addition to buffer (generally 0.5 ml). Although similar results can be obtained if the long chain compounds are transferred directly across media by using a solvent miscible with water (i.e. isopropanol, diethylformamide), the amounts described below yield 9 to 10% of the extract per ml of assay mixture corresponding to 0.1 gland. Protein buffers were prepared by diluting appropriate amounts of 1.0 M NaPO4 and 1.0 M K2HPO4.

Luminescence Assay for Long Aldehydes - Aldehydes were measured by excision of the glands from 2- to 3-day-old female moths and homogenization of the glands for 2 min at 5°C with a motor driven pestle in 0.05 M phosphate buffer, pH 7.0. Solutions were clarified by centrifugation at 10,000 g. Protein concentration was approximately 0.5 mg/ml. The assays described below yield 9 to 10% of the extract per ml of assay mixture corresponding to 0.1 gland. Protein buffers were prepared by diluting appropriate amounts of 1.0 M NaPO4 and 1.0 M K2HPO4.

Alcohol Oxidase Assays - Reactions were initiated by the addition of enzyme to a 5 ml solution containing 2000 units of alcohol oxidase, 10 μg of fat-free albumin, 0.05 M phosphate, or 1000 units of luciferase in the presence of 0.5% of all alcohol esters. Alcohol oxidase was incubated at 37°C for 1 h, and was then added to the reaction mixture. The tissue extract was added to the reaction mixture and incubated at 37°C for 1 h. The reaction was stopped by the addition of 0.05 M phosphate buffer, pH 7.0. The presence of only low amounts of alcohol oxidase activity in parts of the extract other than the glands was not due to inhibitory substances present in these extracts, as shown by trials.

Tissue Extract Assays - Tissue extracts were prepared as follows: 1) 100 pupae from the Forest Pest Management Institute, St. Mary, Ontario and allowed to emerge under natural lighting. Extracts were prepared by excision of the glands from 2- to 3-day-old female moths and homogenization of the glands for 2 min at 5°C with a motor driven pestle in 0.05 M phosphate buffer, pH 7.0. Solutions were clarified by centrifugation at 10,000 g. Protein concentration was approximately 0.5 mg/ml. The assays described below yield 9 to 10% of the extract per ml of assay mixture corresponding to 0.1 gland. Protein buffers were prepared by diluting appropriate amounts of 1.0 M NaPO4 and 1.0 M K2HPO4.

All tissue extracts were assayed alone at a protein concentration of 0.4 mg in 10 ml of the reaction mixture. The tissue extract was added to 0.1 ml of hexane to dilute the extract. Alcohol Oxidase Assays - Reactions were initiated by the addition of enzyme to a 5 ml solution containing 2000 units of alcohol oxidase, 10 μg of fat-free albumin, 0.05 M phosphate, or 1000 units of luciferase in the presence of 0.5% of all alcohol esters. Alcohol oxidase was incubated at 37°C for 1 h, and was then added to the reaction mixture. The tissue extract was added to the reaction mixture and incubated at 37°C for 1 h. The reaction was stopped by the addition of 0.05 M phosphate buffer, pH 7.0. The presence of only low amounts of alcohol oxidase activity in parts of the extract other than the glands was not due to inhibitory substances present in these extracts, as shown by trials.


table 5

| Tissue          | Minus Gland | Plus Gland |
|-----------------|-------------|------------|
| Head            | 0.1 ± 0.2   | 3.2 ± 0.4  |
| Abdomen         | 0.2 ± 0.3   | 3.4 ± 0.6  |
| Leg             | 0.0 ± 0.2   | 3.6 ± 0.6  |
| Thors           | 0.0 ± 0.2   | 3.6 ± 0.6  |
| Gland           | 2.4 ± 0.8   | 8.4 ± 0.6  |

94% Tissue extracts were assayed alone at a protein concentration of 0.4 mg in 10 ml of the reaction mixture. The tissue extract was added to 0.1 ml of hexane to dilute the extract. Alcohol Oxidase Assays - Reactions were initiated by the addition of enzyme to a 5 ml solution containing 2000 units of alcohol oxidase, 10 μg of fat-free albumin, 0.05 M phosphate, or 1000 units of luciferase in the presence of 0.5% of all alcohol esters. Alcohol oxidase was incubated at 37°C for 1 h, and was then added to the reaction mixture. The tissue extract was added to the reaction mixture and incubated at 37°C for 1 h. The reaction was stopped by the addition of 0.05 M phosphate buffer, pH 7.0. The presence of only low amounts of alcohol oxidase activity in parts of the extract other than the glands was not due to inhibitory substances present in these extracts, as shown by trials.


table 6

| Tissue          | Minus Gland | Plus Gland |
|-----------------|-------------|------------|
| Head            | 0.1 ± 0.2   | 3.2 ± 0.4  |
| Abdomen         | 0.2 ± 0.3   | 3.4 ± 0.6  |
| Leg             | 0.0 ± 0.2   | 3.6 ± 0.6  |
| Thors           | 0.0 ± 0.2   | 3.6 ± 0.6  |
| Gland           | 2.4 ± 0.8   | 8.4 ± 0.6  |

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table 7

| Tissue          | Minus Gland | Plus Gland |
|-----------------|-------------|------------|
| Head            | 0.1 ± 0.2   | 3.2 ± 0.4  |
| Abdomen         | 0.2 ± 0.3   | 3.4 ± 0.6  |
| Leg             | 0.0 ± 0.2   | 3.6 ± 0.6  |
| Thors           | 0.0 ± 0.2   | 3.6 ± 0.6  |
| Gland           | 2.4 ± 0.8   | 8.4 ± 0.6  |

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A plot of $\log V_f$ vs time is thus linear (Fig. 35) with the slope $I_{Vf}(1-V_m)$ proportional to the activity of the substrate concentrations. Units of activity are reported in pmol/min calculated for saturating substrate concentrations ($I_{max}$) using a $V_m$ of 0.25. A plot of $\log V_f$ vs time is thus linear (Fig. 35) with the slope $I_{Vf}(1-V_m)$ proportional to the activity of the substrate concentrations. Units of activity are reported in pmol/min calculated for saturating substrate concentrations ($I_{max}$) using a $V_m$ of 0.25.

Dependence of aldehyde dehydrogenase activity on protein concentration. Assays were conducted with 0.5 M NAD and 0.5 M tetradecenal in 0.05 M phosphate buffer, pH 7.0. Each activity was averaged with the bars representing the standard deviation.

To determine the effect of varying substrate concentrations on the activity of the dehydrogenase, the initial activity (1) was employed. This equation can be rearranged to a form analogous to the Lineweaver-Burk plot used in initial velocity kinetics:

$$\frac{1}{I_{max}} = \frac{1}{V_m} + \frac{1}{I_{max}}$$

Data for initial aldehyde concentrations ranging from 4 to 300 nM are plotted according to this equation in Figure 50. Assays of higher substrate concentrations were conducted for longer periods of time to obtain significant changes in aldehyde concentration. The time axis has a slope corresponding to $V_m$, and an intercept of $1/V_m$.

Figure 50. Dependence of aldehyde dehydrogenase activity on protein concentration. Assays were conducted with 0.5 M NAD and 0.5 M tetradecenal in 0.05 M phosphate buffer, pH 7.0. Each activity was measured in duplicate at five different time points and the results averaged with the bars representing the standard deviation.

The aldehyde dehydrogenase could be separated from the alcohol oxidase and acetate esterase activities by purification at 300,000g (90'1 indicating that the dehydrogenase is membrane bound. The addition of a detergent (Triton X-100) to the extract prevented the dehydrogenase from sedimenting indicating that it could be isolated from the membrane by this treatment.

As shown in Table III, the aldehyde dehydrogenase could be separated from the alcohol oxidase and acetate esterase activities by purification at 300,000g (90'1 indicating that the dehydrogenase is membrane bound. The addition of a detergent (Triton X-100) to the extract prevented the dehydrogenase from sedimenting indicating that it could be isolated from the membrane by this treatment.
Detection of pheromone biosynthetic and degradative enzymes in vitro.
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