Sterol substrate specificity of acyl coenzyme A:cholesterol acyltransferase from the corn earworm, *Heliothis zea*

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Abstract The enzymatic activity and sterol substrate specificity of acyl coenzyme A:cholesterol acyltransferase (ACAT) were measured in microsomes of cells from *Heliothis zea*. Under standard assay conditions, the specific enzymatic activity of ACAT was highest in the intestine followed by the fat body and ovary (380.7, 30.7, 8.3 pmol/min per mg, respectively). The structure of the exogenous sterol used in the ACAT assay affected its rate of esterification. The relative rates of esterification of analogs of cholesterol with varidus modifications of the side chain were: 24-H > 24α-CH_3 > Δ^{22} > Δ^{24} > 24α-C_2H_5 > 24β-CH_3, Δ^{22}-24β-CH_3 and Δ^{22}-24α-C_2H_5. The number and position of double bonds in the B-ring of the sterol nucleus greatly affected the rate of esterification of sterols by ACAT. The average relative rates of esterification of sterols with differences in their B-rings were: Δ^7 >> Δ^8 > Δ^0 > Δ^5 > $\Delta^{5,7}$. The presence of a 9,14-cyclopropane group and/or methyl groups at the C-4 and 14 positions prevented significant esterification of such sterols. The formation of cholesteryl and lathosteryl esters was partially inhibited in microsomes from the intestine, fat body, and ovary by the addition of the ACAT inhibitor, 3-(decyldimethylsilyl)-N-[2-(4-methylphenyl)-1-phenylethyl]propanamide (Sandoz Compound 58-035). Therefore, since the structure of exogenous sterols affected the rate of ACAT activity in three tissues of *H. zea* and 58-035 inhibited the esterification process, further studies on the role of ACAT in insects and the effect of ACAT inhibitors on their development and biochemistry are warranted. — **Macauley, S. K., J. T. Billheimer, and K. S. Ritter.** Sterol substrate specificity of acyl coenzyme A:cholesterol acyltransferase from the corn earworm, *Heliothis zea*. *J. Lipid Res.* 1986. **27:** 64–71.

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Unlike mammals, insects are unable to synthesize sterols de novo and therefore acquire these molecules from their diet and/or symbionts in order to biosynthesize membranes and hormones (1). Species such as the corn earworm, *Heliothis zea*, may absorb and utilize a variety of dietary sterols as tissue sterols either directly (e.g., Δ^0-, Δ^5-, and Δ^7-24-desalkylsterols) or after dealkylation (e.g., Δ^0-, Δ^5-, and Δ^7-24-alkylsterols) (2). Some of these sterols may also be stored in *H. zea* as esters of fatty acids (2). In contrast, there is very little absorption, metabolism, and utilization of exogenous sterols other than cholesterol in mammals, which synthesize sterols endogenously from acetate (3). Therefore, the mechanisms for the uptake and utilization of dietary sterols in *H. zea* appear to be much less specific than those in mammals and so warrant further examination.

The enzyme, acyl coenzyme A:cholesterol acyltransferase (ACAT), is responsible for the intracellular esterification of cholesterol in mammals (e.g., rats and humans). In rat liver, in vitro experiments have demonstrated that cholesterol is preferentially esterified by ACAT relative to other endogenously synthesized sterols (e.g., lanosterol) or dietary sterols (e.g., sitosterol) (4). The substrate specificity of ACAT may help to explain the specificity of absorption of cholesterol versus these other sterols (5, 6). Since previous studies in this laboratory have shown that ACAT is also present in the microsomes of the fat body and intestinal cells of *H. zea* (7), it is of interest to further characterize the activity and substrate-specificity of this molecule in the insect, especially because *H. zea* can absorb and utilize a wide range of sterols. Such studies may determine whether there are any differences between the ACAT in rats and that in *H. zea* which may help to explain the selective uptake and esterification of sterols in mammals and the nonspecific uptake and storage of sterols in *H. zea*.

Therefore, the purpose of this study was to: *A*) further characterize the distribution and nature of ACAT activity in *H. zea*; *B*) examine the sterol substrate specificity of ACAT in some of those tissues of *H. zea* that exhibit ACAT activity (the fat body and intestine as well as ovary); *C*) test the effects of 3-(decyldimethylsilyl)-N-[2-(4-methylphenyl)-1-phenylethyl]propanamide (Sandoz Compound 58-035), which is a specific inhibitor of ACAT in mammals

Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; BSA, bovine serum albumin; GSH, glutathione; RPLC, reversed-phase liquid chromatography; TLC, thin-layer chromatography.

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(8), in order to determine whether such chemicals may inhibit steryl ester synthesis in microsomes of *H. zea*.

MATERIALS AND METHODS

H. zea

Larvae of *H. zea* were reared individually on an artificial diet that was supplemented with wheat germ, corn oil, and cholesterol, as described previously (9). Adults were fed, ad libitum, an artificial diet containing 10% sucrose and 2% ascorbic acid (9).

Preparation of microsomes

The fat bodies and intestines were isolated from prepupae of *H. zea* 1 day after they had ceased feeding. A dorsal incision was made through the integument of the larva, the hemolymph was washed from the hemocoel with cold 0.1 M phosphate buffer, pH 7.4, containing 2 or 5 mM glutathione (GSH), the fat body was removed by gentle suction, and then the entire intestine was removed from the cadaver. Ovaries were isolated from 3-day-old adults. The tip of the female's abdomen was removed, the egg-filled ovaries were exuded from the opening into cold buffer, and any fat body adhering to the ovaries was teased away.

The various tissues were homogenized (1 g tissue/2 ml buffer), the microsomes were collected by differential centrifugation, washed once with buffer, and stored at −70°C under nitrogen (7). Prior to use (within 2 weeks), the microsomes were thawed and rewashed with 0.1 M PO_4 buffer, pH 7.4, containing 2 mM GSH.

Control microsomes from rat liver were isolated as described previously (10).

Source of sterols

The exogenous sterols used in these experiments were: cholesterol (J. T. Baker Chemical Co.); brassicasterol, campestanol, 7-campestenol, 7-dehydrocholesterol, 22-dehydrocholesterol, 3-epicholesterol, lanosterol, lathosterol and spinasterol (Research Plus Steroid Laboratories); campesterol, cholestanol, desmosterol and sitosterol (Applied Science Laboratories, Inc); epicoprostanol, sitostanol (note: the sitostanol was purified further (2) using preparative reversed-phase liquid chromatography (RPLC)) and stigmasterol (Sigma Chemical Company); 24-dihydrolanosterol (Mann Research Laboratories); ergosterol (ICN Pharmaceuticals, Inc.); cycloartenol and 22-dihydrobrassicasterol (gifts from Mrs. J. R. Landrey, Drexel University); 22-dihydrospinasterol (gift from Dr. T. Salt, Drexel University); $\Delta^{8(14)}$-cholestenol (prepared by reduction of 5α-cholest-8,14-diene-3β-ol). The sterols were quantitated by gas–liquid chromatography and found to be >98% pure.

The steryl ester standards used in these experiments were: cholesteryl oleate (Sigma Chemical Company), lathosteryl oleate, and campesteryl oleate (synthesized by Dr. S. Avart, Drexel University (11)).

ACAT assay

ACAT activity in the microsomes was determined by calculating the rate of incorporation of [1-^{14}C]oleoyl CoA into steryl esters as described by Billheimer, Tavani, and Nes (10). The standard assay medium contained 200 μg of microsomal protein, when using either fat body or intestinal microsomes, and 500 μg of protein, when using microsomes isolated from the ovary. The protein concentration was determined by the method of Lowry et al. (12). In addition, the medium contained 1 mg of fatty acid-free bovine serum albumin (BSA), 20 μg of sterol suspended in 600 μg of Triton WR-1339, and 100 μM [1-^{14}C]oleoyl CoA (8-15,000 dpm/nmol). The volume was adjusted to 200 μl with 0.1 M PO_4 buffer (pH 7.4) containing 2 mM GSH. In those experiments that investigated the effect of 58-035 on ACAT activity, 200 ng of inhibitor (a gift from Dr. J. G. Heider, Sandoz) was added to the sterol solution.

The medium was preincubated for 30 min at 37°C and the reaction was initiated with the addition of the oleoyl CoA. The reaction was terminated with 4 ml of chloroform–methanol 2:1 (v/v) after 10 min (intestine), 15 min (fat body), or 20 min (ovary).

Cholesteryl oleate (5 μg) and [4-^{14}C]cholesterol (15,000 dpm) were added to serve as a carrier and internal standard, respectively. The mixture was allowed to stand for 30 min to ensure lipid extraction prior to separation into layers by the addition of 0.8 ml of 0.88% KCL. The chloroform layer was then collected and dried under nitrogen. The steryl esters were isolated from the residue by thin-layer chromatography (TLC), placed into scintillation vials, and counted.

The specific activity of ACAT (pmol/min per mg protein) was calculated using boiled microsomes as a control and the quantity of radioactivity present as background was subtracted from that of the experimental assays. All experiments were done in duplicate and each value for specific activity represents the average of two replicates (the average variation was <10%).

The percent esterification of sterols, relative to cholesterol, was determined as previously reported for rat liver (4) by using the equation: $(E_{c\ sterol} - E_{s\ sterol}/E_{c\ cholesterol} - E_{s\ cholesterol})$ (100). ($E_{c\ sterol}$ = esterification in the presence of 20 μg exogenous sterol, $E_{s\ sterol}$ = esterification in the absence of exogenous sterol, $E_{c\ cholesterol}$ = esterification in the presence of 20 μg exogenous cholesterol, and $E_{s\ cholesterol}$ = esterification in the absence of exogenous cholesterol.)

Identification of steryl esters by RPLC

RPLC was used to confirm that the exogenously sup-

plied sterols were esterified by ACAT (i.e., to confirm that the radioactive label was actually associated with steryl ester). In these experiments 5- to 10-fold assays were conducted and the steryl ester band from TLC was extracted from diethyl ether. RPLC was carried out on a Perkin-Elmer Series 3B Liquid Chromatograph equipped with a Zorbax ODS (C_{18}) column, at 60°C, using 20 μl of sample in isopropanol and a mobile phase of acetonitrile–isopropanol 60:40 (v/v) at 2 ml/min. The α_{co}'s of the peaks were calculated using the k' of cholesteryl oleate (11). One-ml fractions of the eluent were collected and the quantity of radioactivity in each fraction was determined by scintillation counting.

RESULTS

ACAT activity

Effect of detergent, exogenous sterol, and GSH. ACAT activity was observed not only in the microsomes of the fat body and intestinal cells but also from the ovary of *H. zea.* Under standard assay conditions, with exogenous sterol added in Triton WR-1339 (using microsomes prepared in buffer containing 2 mM GSH), the specific enzymatic activity of ACAT was highest in the intestine, followed by the fat body and ovary (380.7, 30.7, and 8.3 pmol/min per mg, respectively) (**Table 1**). This addition of cholesterol in detergent resulted in at least a 2-fold increase in ACAT activity in microsomes isolated from the cells of all three of these tissues, because without this exogenous sterol, the activity was only 181.6, 16.5, and 4.2 pmol/min per mg, respectively (Table 1). This increase in enzymatic activity apparently was not a direct effect of the detergent per se because, in all three tissues, detergent alone resulted in at least a 71% reduction in the activity (52.9, 1.2, and 1.0 pmol/min per mg, respectively (Table 1)) obtained in its absence—probably due to an efflux of sterol from the microsomes (10). When fat body was rapidly collected and thoroughly washed with cold buffer containing 5 mM instead of 2 mM GSH (13), an increase in ACAT activity was observed (Table 1). In contrast, ACAT activity did not increase when the amount of GSH in the buffer for the intestines was increased from 2 to 5 mM.

Effect of pre-incubation time. When exogenous sterol is added to assay media, the formation of cholesteryl oleate is dependent upon the equilibrium between the exogenous and endogenous sterol. Therefore, in some experiments, the assay medium was preincubated for various times up to 60 min, prior to initiating the reaction, in order to determine the optimal preincubation period for the formation of cholesteryl oleate in microsomes of the fat body, ovary, and intestine. Maximal activity of ACAT was obtained in microsomes of these tissues when the assay medium was preincubated for at least 30 min (**Fig. 1**).

Effect of incubation time. The assay medium was incubated for various times up to 30 min after initiating the reaction, in order to determine the effects of different incubation periods on the formation of cholesteryl oleate in microsomes of the fat body, ovary, and intestine. The formation of cholesteryl ester was linear to at least 10 min for all three tissues (**Fig. 2**).

Effect of protein concentration. Cholesteryl oleate formation increased proportionally with increasing microsomal protein concentration up to at least 250 μg, for the fat body and intestine, and up to at least 500 μg for the ovarian microsomes (**Fig. 3**).

Effect of cholesterol concentration. ACAT activity increased linearly in microsomes of the fat body, intestine, and ovary with increasing exogenous cholesterol concentration up to 20 μg, when the concentration of Triton WR-1339 was kept constant at 0.3% (**Fig. 4**). The absence of further increase with amounts greater than 20 μg may be due to the inability of the detergent to adequately disperse cholesterol into a form that can be utilized by ACAT (10).

Effect of oleoyl CoA concentration. When the amount of BSA in the medium was 75 μM and the concentration of oleoyl CoA increased to 100 μM, the synthesis of cholesteryl oleate was linear for all three tissues; however, it decreased when the concentration was further increased to 150 μM (**Fig. 5**). (BSA was added to the assay medium to serve as a reservoir of oleoyl CoA in order to prevent its concentration from being inhibitory. In rat liver, the highest ACAT

TABLE 1. ACAT activity in microsomes of tissues isolated from *H. zea*

Origin of Microsomes	Amount of GSH (mM)	No. of Experiments	Specific Enzymatic Activity (pmol/min per mg)[a] with Additions to the Assay		
			None	Triton WR-1339	Cholesterol and Triton WR-1339
Fat body	2	7	16.5 ± 6.6	1.2 ± 0.9	30.7 ± 6.2
Fat body	5	6	36.7 ± 14.9	4.6 ± 3.1	107.3 ± 31.9
Intestine	2	13	181.6 ± 35.0	52.9 ± 20.5	380.7 ± 81.5
Intestine	5	5	137.4 ± 41.4	24.8 ± 14.0	309.1 ± 118.1
Ovary	2	19	4.2 ± 2.1	1.0 ± 0.9	8.3 ± 1.7

[a]The assays were carried out as described in Materials and Methods. The values represent the mean ± the standard deviation.

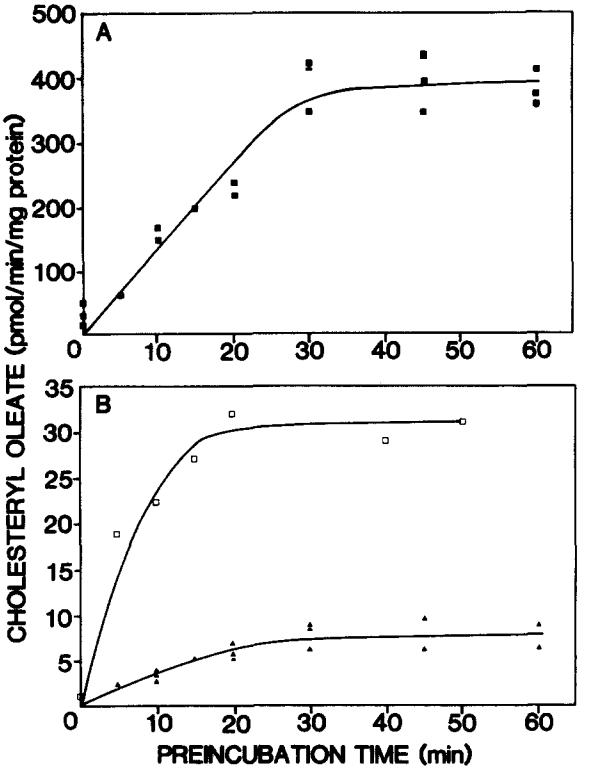

Fig. 1. Effect of preincubation time on ACAT activity in: panel A, intestinal microsomes (■); panel B, fat body microsomes (□) and ovarian microsomes (▲). (See Materials and Methods for procedure.)

activity is achieved when BSA and oleoyl CoA are present in a 1:1 molar ratio (10)).

Sterol specificity of ACAT

The structure of the exogenous sterol used in an ACAT assay affected its rate of esterification, relative to cholesterol, in the microsomes of the fat body, intestine, and ovary (**Table 2**). Alkylation at C-24 resulted in a decrease in esterification of sterols in all three tissues examined. The addition of a 24α-methyl group (campesterol) reduced the rate of esterification by an average of 51%, a 24α-ethyl group (sitosterol) by 81%, and a 24β-methyl group (22-dihydrobrassicasterol) by more than 90%. Unsaturation in the side chain at C-22 (22-dehydrocholesterol) or C-24 (desmosterol) also reduced the rate of esterification by an average of 55 and 75%, respectively. Unsaturation in the side chain at C-22, plus the addition of a 24α-ethyl or 24β-methyl at the C-24 (stigmasterol and brassicasterol, respectively), reduced the rate of esterification by more than 90%. Therefore, the relative rates of esterification of these sterols, with differences in their side chains, by ACAT in the microsomes, were: 24-H > 24α-methyl > Δ^{22} > Δ^{24} > 24α-ethyl > 24β-methyl, Δ^{22}-24β-methyl, and Δ^{22}-24α-ethyl.

The number and position of double bonds in the B-ring of the sterol nucleus also affected the rate of esterification of sterols by ACAT in the microsomes of the intestine, fat body, and ovaries of *H. zea*. The presence of a double bond at C-7 (lathosterol) resulted in an increase in esterification of over 9 times that of cholesterol. Similarly, at least a 9-fold increase was observed with the addition of a 24α-methyl group, 24α-ethyl group, or Δ^{22}-24α-ethyl group to lathosterol (7-campestenol, 22-dihydrospinasterol, and spinasterol, respectively), when the rates of esterification of these sterols were compared to those of the corresponding Δ^5-sterols (i.e., campesterol, sitosterol and stigmasterol). In contrast, no increase in activity, relative to cholesterol, was observed when control assays with Δ^7-sterols were performed using the microsomes from rat liver.

To confirm that Δ^7-steryl esters were actually synthesized in such large amounts in *H. zea* in these experiments, the steryl esters from some Δ^7-sterol assays were isolated and characterized by RPLC (**Table 3**). The large amount of radioactivity associated with the peaks in the Δ^7-sterol assays indicated that these sterols were esterified in significantly larger quantities than the Δ^5-sterols. When lathosterol was used, there was approximately a 7-fold increase in radioactivity in the peak with an α_{co} corresponding to that of lathosteryl oleate. However, since lathosteryl and cholesteryl oleate cannot be completely

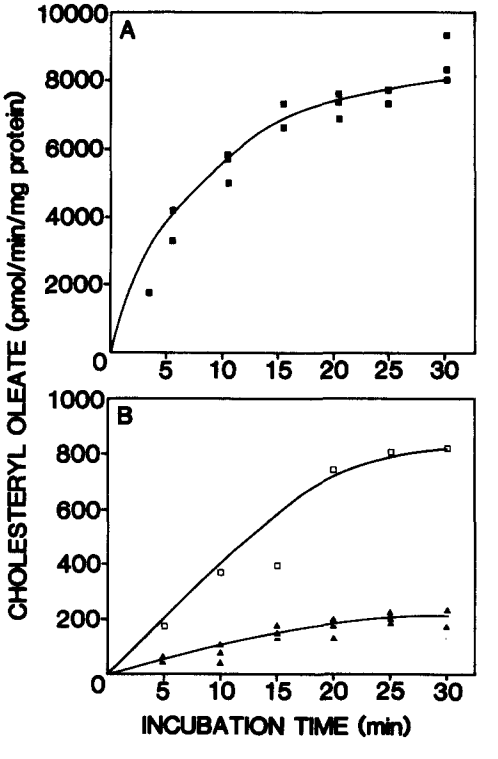

Fig. 2. Effect of incubation time on ACAT activity in: panel A, intestinal microsomes (■); panel B, fat body microsomes (□) and ovarian microsomes (▲). (See Materials and Methods for procedure.)

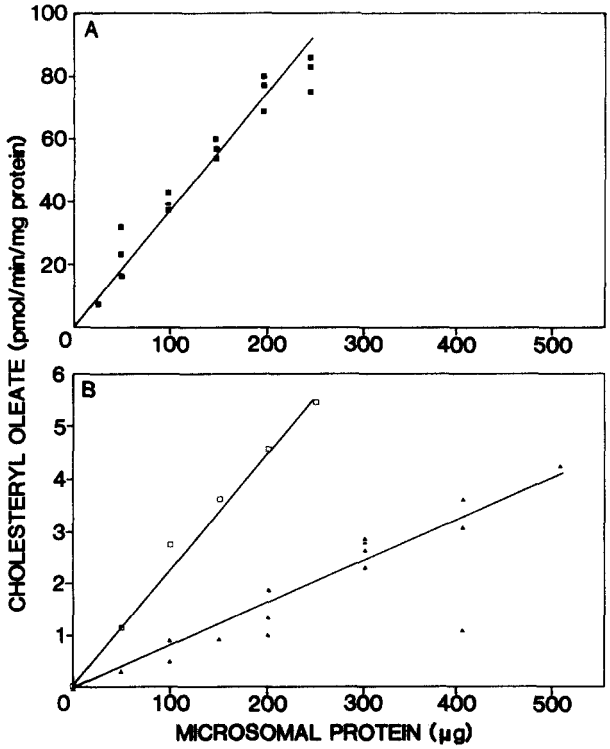

Fig. 3. Effect of protein concentration on ACAT activity in: panel A, intestinal microsomes (■); panel B, fat body microsomes (□) and ovarian microsomes (▲). (See Materials and Methods for procedure.)

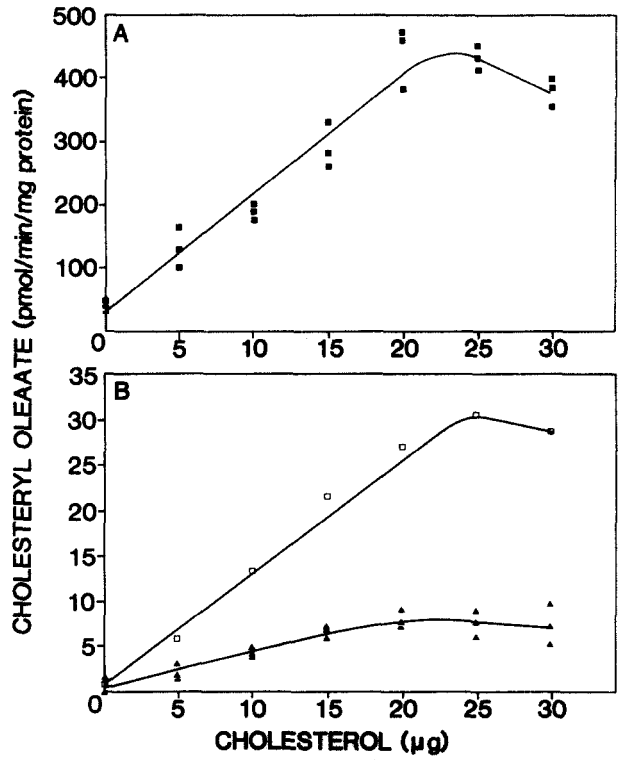

Fig. 4. Effect of cholesterol concentration on ACAT activity in: panel A, intestinal microsomes (■); panel B, fat body microsomes (□) and ovarian microsomes (▲). (See Materials and Methods for procedure.)

resolved in our RPLC system, in order to show that lathosterol was not increasing the esterification of cholesterol in microsomes, the esterification of a second Δ^7-sterol (7-campestenol) was also analyzed. In this case, the 24-methylsteryl ester was resolved from that of cholesteryl ester and the use of Δ^7-campestenol resulted in an 8- to 9-fold increase in activity over the corresponding Δ^5-sterol, campesterol. Therefore, these results confirmed that Δ^7-sterol was preferentially esterified over Δ^5-sterol.

The presence of a double bond at C-8 ($\Delta^{8(14)}$-cholestenol) instead of C-5 resulted in an average 4-fold increase in esterification in the fat body and intestinal microsomes. The presence of a conjugated diene in ring B (7-dehydrocholesterol) reduced the rate of esterification of this sterol, in the microsomes from the intestine and ovary, by an average of 80% of that of cholesterol; interestingly, though, the rate of esterification of this sterol in microsomes of the fat body was increased over 1.5-fold. The addition of a 24β-methyl group, as well as a Δ^{22}, to 7-dehydrocholesterol (ergosterol) decreased the rate of esterification on average over 90% for ergosterol as compared to cholesterol. The small amount of radioactivity found for ergosteryl ester with microsomes from the fat body was determined to be insignificant by RPLC analysis.

The absence of double bonds in the B-ring of the desalkylsterol cholestanol resulted in over a 2-fold increase in esterification when compared to cholesterol. A similar

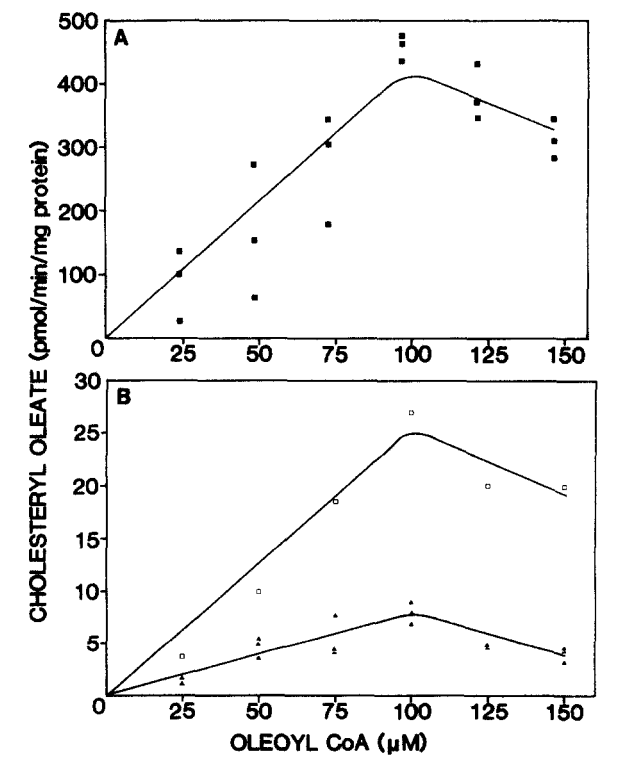

Fig. 5. Effect of oleoyl CoA concentration on ACAT activity in: panel A, intestinal microsomes (■); panel B, fat body microsomes (□) and ovarian microsomes (▲). (See Materials and Methods for procedure.)

TABLE 2. The percent esterification of various sterols relative to cholesterol by ACAT in microsomes of *H. zea*[a]

Sterol	Structural Differences from 5α-Cholestan-3β-ol	% Activity Relative to Cholesterol in Microsomes[b] from Intestine	Fat Body	Ovary
Lathosterol	Δ^7	1107 ± 346	997 ± 60	647 ± 144
7-Campestenol	Δ^7-24α-Methyl	376 ± 17	573 ± 157	226 ± 53
22-Dihydrospinasterol	Δ^7-24α-Ethyl	270 ± 32	221 ± 82	190 ± 43
Spinasterol	$\Delta^{7,22}$-24α-Ethyl	107 ± 14	92 ± 26	98 ± 11
Cholestanol		316 ± 94	252 ± 32	192 ± 54
Campestanol	24α-Methyl	65 ± 12	86 ± 37	65 ± 11
Sitostanol	24α-Ethyl	40 ± 18	30 ± 14	47 ± 13
Epicoprostanol	5β-H,3α-ol	12 ± 11	33 ± 12	34 ± 13
Cholesterol	Δ^5	100	100	100
22-Dehydrocholesterol	$\Delta^{5,22}$	50 ± 17	37 ± 9	48 ± 7
Campesterol	Δ^5-24α-Methyl	33 ± 6	57 ± 2	53 ± 10
Sitosterol	Δ^5-24α-Ethyl	19 ± 7	19 ± 12	19 ± 2
Desmosterol	Δ^{24}	19 ± 9	26 ± 13	30 ± 6
22-Dihydrobrassicasterol	Δ^5-24β-Methyl	<10[c]	<10	<10
Brassicasterol	$\Delta^{5,22}$-24β-Methyl	<10	<10	<10
Stigmasterol	$\Delta^{5,22}$-24α-Ethyl	<10	<10	<10
3-Epicholesterol	3α-ol	<10	12 ± 7	<10
7-Dehydrocholesterol	$\Delta^{5,7}$	22 ± 14	186 ± 29	18 ± 8
Ergosterol	$\Delta^{5,7,22}$-24β-Methyl	<10	15 ± 15	<10
$\Delta^{8(14)}$-Cholestenol	$\Delta^{8(14)}$	311	525	
24-Dihydrolanosterol	Δ^8-4,4,14-Trimethyl	<10	<10	<10
Lanosterol	$\Delta^{8,24}$-4,4,14-Trimethyl	<10	<10	<10
Cycloartenol	Δ^{24}-4,4,14-Trimethyl-9,19-cyclopropane	<10	<10	<10

[a] Assays were carried out as described in Materials and Methods.

[b] Each value represents the mean of at least three experiments (except in the case of $\Delta^{8(14)}$-cholesterol) ± the standard deviation of the mean.

[c] <10 indicates that there was no significant increase between the amount of esterification in the presence of added sterol and that in the absence of additional sterol.

increase was observed with the addition of a 24α-methyl or 24α-ethyl group to cholestanol (campestanol and sitostanol, respectively), when the rates of esterification of these sterols were compared to those of the corresponding Δ^5-sterols (i.e., campesterol and sitosterol). Therefore, the effect of various modifications of the B-ring on the relative rates of esterification of sterols by ACAT in the microsomes was: $\Delta^7 >> \Delta^8 > \Delta^0 > \Delta^5 > \Delta^{5,7}$ (with the exception of 7-dehydrocholesterol in the fat body microsomes, i.e., $\Delta^{5,7} > \Delta^5$).

3-Epicoprostanol, which differs from cholestanol because it has a hydroxyl group in the 3α-position (instead

TABLE 3. Characterization of steryl esters by RPLC[a]

Exogenous Sterol in WR-1339 Added to Assay	Origin of Microsomes[b]	Cholesteryl and/or Lathosteryl Oleate Peak[c] α_{co}	No. of dpm	% of Total Steryl Ester	Campesteryl or 7-Campestenyl Oleate Peak[d] α_{co}	No. of dpm	% of Total Steryl Ester	Ratio of Δ^7- to Δ^5-Sterol
None[e]	I	1.00	982	100				
Cholesterol	I	1.00	4022	100				
Lathosterol	I	0.94	26585	100				7/1
Campesterol[e]	I	0.97	824	32	1.08	1772	58	
7-Campestenol	I	0.96	1866	11	1.05	15266	89	9/1
Campesterol[e]	FB	0.99	675	41	1.05	959	59	
7-Campestenol	FB	0.96	1346	15	1.08	7389	85	8/1

[a] In these experiments, fivefold assays (except where noted) were carried out and the steryl ester band was isolated and chromatographed by RPLC. One-ml fractions of the eluent were collected and the dpm's were determined by scintillation counting. The values were corrected for percent recovery of total dpm from the RPLC column. The α_{co}'s of the peaks of radioactivity were calculated using the k' of cholesterol oleate. (See Materials and Methods for further details.)

[b] I, intestine; FB, fat body.

[c] The α_{co} of a standard of cholesteryl oleate, using UV detection, was 1.00. The α_{co} of a standard of lathosteryl oleate, using UV detection, was 0.94. (These two peaks were not completely resolved by RPLC.)

[d] The α_{co} of a standard of campesteryl oleate, using UV detection, was 1.07. Theoretically, the α_{co} of a standard of 7-campestenyl oleate should be similar to that of campesteryl oleate (i.e., 1.07) (11).

[e] Tenfold assay.

of the 3β-position) as well as a hydrogen in the 5β-position (instead of the 5α-position), was esterified about 23% as well as cholesterol. 3-Epicholesterol, which has a hydroxyl group in the 3α-position, instead of the 3β-position, was esterified at about 10% or less the rate of cholesterol in the ovary, intestine, and fat body. (In some assays, using fat body microsomes, a slight increase in radioactivity was found in the TLC steryl ester band when compared to controls; however, when the ester fraction was analyzed by RPLC, little or no radioactivity was found in the region corresponding to 3-epicholesteryl oleate).

Esterification of the 4,4,14-trimethylsterols lanosterol, dihydrolanosterol, and cycloartenol was not detectable under the conditions of the assay.

Inhibition of ACAT activity

Formation of cholesteryl and lathosteryl esters was inhibited in microsomes, isolated from the fat body, intestine, and ovary, by the addition of the ACAT inhibitor, 58-035 (**Table 4**). This molecule may compete with oleoyl CoA for the enzyme (5, 14).

DISCUSSION

Previous studies in this laboratory (7) demonstrated that ACAT activity was present in the intestine and fat body of prepupae of *H. zea*. The present study corroborates these results and demonstrates that ACAT activity is also present in the ovary of the adult. In addition, the activity of this enzyme is affected by the structure of the sterol substrate.

In all experiments, highest ACAT activity was found in the intestine, followed by the fat body and the ovary (Table 1). The presence of ACAT activity in the intestine and fat body of prepupae corroborates the active metabolic role these tissues play in insects. Since the ovaries of insects may synthesize ecdysteroids (15) and the eggs of some insects have been shown to contain large quantities of steryl esters (16), it is not surprising to find ACAT activity present in this tissue of *H. zea*. The corpus luteum of the ovary of rats also stores large quantities of cholesteryl esters (17). Why the activity of the *H. zea* ovary is low compared to that of the intestine and fat body is not clear. The low activity may, in part, be due to the heterogeneity of the cells associated with the reproductive tract. In addition, because this tissue was removed from the 3-day-old adult, after eggs had already formed in the ovarioles (older ovaries are more easily separated from contaminating fat body), perhaps enzyme activity had already peaked by this time.

TABLE 4. Inhibition of the formation of cholesteryl and lathosteryl esters in microsomes by the ACAT inhibitor, 58-035

Exogenous Sterol	Origin of Microsomes	No. of Experiments	% Inhibition of ACAT Activity by 58-035[a]
Cholesterol	Intestine	3	74 ± 12[b]
	Fat body	3	73 ± 17
	Ovary	4	57 ± 26
Lathosterol	Intestine	2	57 ± 3[c]
	Fat body	3	78 ± 13
	Ovary	3	38 ± 8

[a] In each experiment, 200 ng of ACAT inhibitor, 58-035, was added to the exogenous sterol solution used in the standard assay medium (see Materials and Methods) and the resulting ACAT activity was compared to that obtained in the absence of inhibitor.

[b] Except where noted, the values represent the mean ± the standard deviation of the mean.

[c] This ± value denotes the actual range of the results.

In this study, the relative sterol substrate specificity of ACAT was similar in the microsomes of the intestine, fat body, and ovary of *H. zea* but differed in some respects from that characterized in the microsomes of the liver of the rat (4). In *H. zea*, ACAT esterified Δ^7- and Δ^0-desalkylsterols 900 and 200%, respectively, as well as Δ^5-desalkylsterol, whereas ACAT in rats esterified Δ^7- and Δ^0-desalkylsterols 42 and 70%, respectively, as well as Δ^5-desalkylsterol. Increases similar to those for the desalkylsterols were observed in the esterification of Δ^0- and Δ^7-24α-methylsterols and Δ^0- and Δ^7-24α-ethylsterols, as compared to the corresponding Δ^5-sterols, in *H. zea*; analogous studies were not conducted in the rat. Interestingly, 8(14)-cholestenol was also esterified on average 400% as well as cholesterol in *H. zea*. Since the presence and position of unsaturation in the B ring plays a significant role in the rate of esterification of sterols by ACAT in *H. zea*, this may indicate a subtle difference in the active site of the corn earworm enzyme relative to that of the rat. Why the Δ^7-sterols were preferentially esterified over Δ^5-sterols is not known. However, since many plants predominately contain Δ^5-sterols, if Δ^7-sterols are selectively esterified for storage or for conversion to Δ^7-steroids such as ecdysteroids by *H. zea*, another acyl CoA-dependent enzyme may be present which esterifies the Δ^7-sterols.

Despite this major difference between the substrate specificity of ACAT in *H. zea* and that in rats, there were similarities in the relative esterification of other sterols. When compared to cholesterol, Δ^5-24-methylsterol and Δ^5-24-ethylsterol were esterified to a lesser extent in both *H. zea* (an average of 48% and 19%, respectively) and the rat (23% and <5%, respectively). Perhaps the absolute amount of esterification of these Δ^5-24-alkylsterols was greater in *H. zea* because these sterols are the typical dietary sterols of the phytophagous insect. In addition, desmosterol was not esterified as well as cholesterol in either animal (25% in *H. zea* and 62% in the rat). The presence of a 14α-methyl group as well as 4,4-dimethyl groups, associated with a $\Delta^{8,24}$-sterol or a Δ^{24}-sterol containing a 9,19-cyclopropane group, prevented significant esterification of the sterol. Neither 3-epicholesterol nor

$\Delta^{5,7,22}$-24β-methylsterol was esterified in significant quantities by either organism.

The esterification of a variety of Δ^0-, Δ^5-, and Δ^7-sterols by ACAT in microsomes of *H. zea*, in vitro, corroborates the results from in vivo studies in which a variety of Δ^0-, Δ^5-, and Δ^7-steryl esters were identified in larvae of *H. zea* fed different dietary sterols (2). It is not known whether ACAT activity in insects is responsible only for regulating the amount of dietary sterol that is stored as steryl ester or whether the esterification process also helps to regulate the uptake of dietary sterols, which may occur in some other animals (5, 14). Since we have found that ACAT activity is inhibited by 58-035 in microsomes of *H. zea*, just as it is in microsomes of rat liver (5), further studies on the effects of this inhibitor on the development and biochemistry of *H. zea* are warranted.

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