Long Chain Base-Acetyl Coenzyme A Acetyltransferase from the Microsomes of Hansenula ciferri

I. ISOLATION AND PROPERTIES

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SUMMARY

Microsomes of the yeast, Hansenula ciferri NRRL Y-1031, F-60-10, contain an enzyme (long chain base-acetyl coenzyme A acetyltransferase, EC 2.3.1) which catalyzes the transfer of the acetyl moiety of acetyl coenzyme A to both the amino and hydroxyl groups of the sphingosine bases; a separation of these two transfer reactions could not be achieved. The enzyme also catalyzed an acetyl transfer to the hydroxyl groups of N-acetylated sphingosine bases and to the amino groups of normal aliphatic primary amines of 6 to 18 carbon atoms, but not to compounds such as p-nitroaniline, glucosamine, secondary amines, or primary alcohols. A similar enzyme was not found in rat organs nor in extracts of pigeon liver acetone powder.

The reaction rates were directly proportional to enzyme concentration and time of incubation. The optimal pH depended on the type of buffer used. The reaction rates could be correlated to the charge conferred upon the micelles of the bases by the various buffers. The reaction was not reversible and an exchange between the products and substrates could not be shown. It was inhibited by coenzyme A but not by the lipid products.

Several strains of the yeast Hansenula ciferri produce large quantities of sphingosine bases which are secreted into the growth medium (1). In a previous investigation (2), 25 strains of the genus Hansenula were compared and classified on the basis of the quantities of the LCB's produced. These ranged from 120 to 266 μmoles of LCB's per liter of growth medium ("high producers") to 2 to 5 μmoles per liter ("low producers"). These compounds (mostly phytosphingosine) occur not as free, but as partially or fully acetylated bases (1). The enzyme responsible for this acetylation was identified and some of its properties were the subject of a preliminary communication (3). This enzyme utilized acetyl coenzyme A for the transfer of the acetyl group to the LCB's. It was of interest that this enzyme was present in the high producers but was practically absent from those yeast strains (low producers) which produced small quantities of the bases. The implication of this to the "diseased state" of the yeast was discussed (2).

Several acetyltransferases are known and were classified according to the donors and acceptors of the acetyl group. The acceptor might be a hydroxyl or an amino group, but no enzyme has been described to date which transfers the acetyl group to both amino and hydroxyl groups. Also, none of the acetyltransferases utilized a sphingosine base as an acceptor of the acetyl group. The sphingosine bases are acylated at their amino groups by a long chain fatty acid (4, 5) or long chain fatty acyl coenzyme A (6, 7). This paper further describes the properties of the enzyme, present in the microsomes of H. ciferri, which transfers the acetyl group of acetyl-CoA to both the amino and hydroxyl groups of the sphingosine bases or to the amino group of long chain primary amines.

EXPERIMENTAL PROCEDURE

Materials

Acetyl and butyryl coenzyme A as well as [2-3H]acetyl coenzyme A, were synthesized with the respective anhydrides for acylation of the free coenzyme (8); these acyl coenzyme A derivatives contained no more than 1.5% of free coenzyme A and less than 0.1% of acetic or butyric acid, respectively.

Sphingosine, dihydrosphingosine, and phytosphingosine were prepared according to the method of Barenholz and Gatt (9). N-Acetyl phytosphingosine was prepared by mild alkaline hydrolysis of the acetylated bases, isolated from the growth medium of H. ciferri. It was further purified with Silica Gel S columns (Riedel de Haen) from which it was eluted with 5% methanol in chloroform. Its purity was tested by thin layer silica gel chromatography, using chloroform methanol, 9:1 (10), and by gas-liquid chromatography of the trimethylsilyl derivative (11). N-Acetyl dihydrosphingosine and N-acetyl sphingosine were prepared according to the procedure of Gatt (4) or Carter and Gaver (11). N-Acetyl hexadecylamine and N-butyryl dihydrosphingosine were prepared according to the
method of Carter and Gaver (11), using acetic or butyric anhydrides, respectively. Tetraacetyl phytosphingosine was isolated from the growth medium of H. cijii and purified on a silicic acid (Mallinckrodt) column, from which it was eluted with 2% methanol in chloroform. It was characterized on thin layer silica gel plates, with chloroform-methanol, 30:1. DL-Erythro sphingosine, DL-erythro dihydrophospho- 
sphingosine, N-acetyl dihydrophospho-
sphingosine, N-octanoyl sphingosine, N-palmitoyl-
dihydrophospho-sphingosine, and 1-O-galactosyl sphingosine were gifts of Professor D. Shapiro of the Weizmann Institute for Science. The sodium salts of cholic, taurocholic, and deoxycholic acids; sodium lauryl sulfate, glucoseosamine, ATP, coenzyme A (95% pure), mercaptoethanol, dithiothreitol, p-hydroxymercurobenzoate, and “Sigma A” were purchased from Sigma. p-Nitroaniline, acetyl anhydride, butyric anhydride, glucose, and Triton X-100 were purchased from BDH. Primary and secondary long chain amines were purchased from Koch-Light; yeast extract, malt extract, and bactopeptone from Difco; DTNB from Aldrich; [2-3H]acetic anhydride from Amersham.

**Methods**

**Dispersion of Lipid Substrates**—A solution of the substrate in chloroform-methanol, 2:1, was evaporated to dryness under nitrogen at 60°. Water was added, the tube was placed in a boiling water bath for 15 s, and was then agitated on a Vortex cyclomixer. Stable and uniform, although somewhat opaque dispersions were obtained by this procedure. When testing the effect of detergents, a solution of the detergent was added instead of the water in the above procedure.

**Measurements of Electrophoretic Mobility**—The electrophoretic mobilities of dihydrophospho-sphingosine and hexadecylamine micelles were measured with the apparatus and methods of Bangham et al. (12). The distance between the electrodes was 16.7 cm and the voltage employed was 50 volts.

**Enzymatic Assay**—Incubation mixture, in final volume of 0.2 ml, contained 0.2 μmole of the lipid substrate, 0.15 μmole of acetyl coenzyme A, 0.2 μmole of DTNB, 20 μmoles of potassium phosphate buffer, pH 7.8, and 0.05 to 1.0 mg of microsomal or mitochondrial protein. The mixtures were incubated with shaking, at 37°. The reaction rates were determined by measuring either the coenzyme A released or the [3H]acetate bound to the lipid substrate. The determination of the acetylation of the hydroxyl group of N-acetylated LCB’s (“N-acetylation”) or of the amino and hydroxyl groups of the LCB’s (“N- and O-acetylation”) was done by measuring [2-3H]acetate bound to the lipid substrate as follows. The reaction was terminated by the addition of 1.6 ml of chloroform-methanol, 1:1, and 0.6 ml of water. After mixing, the lower phase was collected, the ether phase was evaporated, and the aqueous methanolic phase was again shaken with 1.2 ml of water, evaporated, and counted as above.

In an alternative procedure, 1.2 ml of ether were added to the hydrolysis mixture; after stirring, the ether phase was collected and the aqueous methanolic phase was again shaken with 1.2 ml of ether. The two ether extracts were combined, backwashed with 1 ml of water, evaporated, and counted as above.

One unit of activity is defined as a nanomole of [3H]acetate transferred or a nanomole of coenzyme A released during the transfer reaction.

**RESULTS**

**Preparation of Enzyme**

The yeast was cultured and disrupted according to the method of Braun and Snell (14). Care was taken to keep the growth temperature at no more than 27°. After harvesting, the packed cells were suspended in 0.05 M potassium phosphate, pH 7.0, which contained 20% glycerol (Buffer A) and frozen at -20°. The cells were disrupted within 24 to 40 hours and microsomes were prepared (3). In some experiments the 14,000 × g supernatant was centrifuged for 65 min at 350,000 × g, the sediment was suspended in Buffer A and recentrifuged as above. This sediment was suspended in Buffer A (0.5 ml per g of packed cells) and stored at -20°. Either microsomal preparation retained its acetyltansferase activity for at least 18 months at -20°. If suspended in phosphate buffer (pH 7.0) without glycerol, the enzymatic activity was lost within 1 week or less when stored at -20°.

Attempts were made to solubilize the microsomes with detergents, proteases, lipase and phospholipases, and sonic or ultrasonic irradiation. None of these resulted in the formation of a soluble enzyme.

**Subcellular Localization of Enzymatic Activity**

Yeast cultures were grown, harvested, and disrupted as described elsewhere (14). The extract was separated into the following fractions: unbroken cells and debris were removed by two successive centri- fugations at 800 and 1200 × g, for 10 min each. The supernatant was centrifuged at 10,000 × g for 25 min and the sedimented mitochondria were suspended in Buffer A (0.5 ml per g of packed cells). Microsomes were then sedimented for 1 hour at 200,000 × g. The supernatant was retained and the microsomal sediment was suspended as above.

Each of the fractions was assayed for both N- and O-acetylation as described under “Methods.” The results were calculated in terms of “relative specific activity” (Table I).

**TABLE I**

<table>
<thead>
<tr>
<th>Subcellular Fraction</th>
<th>N-acetylation of phytosphingosine</th>
<th>O-acetylation of phytosphingosine</th>
<th>O-acetylation of N-acetyl phytosphingosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,300 × g supernatant</td>
<td>1.0</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>10,000 × g sediment</td>
<td>1.8</td>
<td>1.8</td>
<td>1.7</td>
</tr>
<tr>
<td>100,000 × g sediment</td>
<td>5.0</td>
<td>5.1</td>
<td>5.4</td>
</tr>
<tr>
<td>100,000 × g supernatant</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Relative specific activity is defined as the percentage of total activity divided by the percentage of total protein, in each fraction (15).
Fig. 1. Effect of pH and type of buffer on the reaction rates. Incubation mixtures were prepared and the products were determined as described under “Methods.” Micromosomal protein, 0.2 mg, and 20 μmoles of each of the following buffers were used: potassium acetate, pH 4.0 to 5.6; potassium phosphate, pH 5.8 to 8.0 (solid lines); potassium borate, pH 7.6 to 10.7 (dashed lines). Incubation time, 60 min. — hexadecylamine (N-acetylation); ••••, phytosphingosine (N-acetylation); O——O, phytosphingosine (N- and O-acetylation); □—□, N-acetyl phytosphingosine (O-acetylation).

Effect of Detergents

The effects of several detergents were tested on either N- or O-acetylation. The concentrations used were 0.025 to 0.2% of sodium lauryl sulfate and 0.05 to 0.5% of Cutexem, Triton X-100, and the sodium salts of cholic, deoxycholic, or taurocholic acids. Various degrees of inhibition were obtained depending on the type and concentration of the detergent. In no case was the enzymatic activity increased by the addition of these compounds.

Properties of Reaction

Effect of Protein Concentration and Reaction Time—The rates of N- or O-acetylation of phytosphingosine, O-acetylation of N-acetyl phytosphingosine, or N-acetylation of hexadecylamine, were directly proportional to enzyme concentration (up to at least 1 mg of microsomal protein) and to the reaction time (for at least 2 hours).

Effect of pH and Buffers—Fig. 1 shows the effect of three buffers at pH values between 4 to 10 on the rates of N-acetylation of hexadecylamine, N- and O-acetylation of phytosphingosine, and O-acetylation of N-acetyl phytosphingosine. None of the substrates used were acetylated with acetate buffer at pH 4 to 5.6. In phosphate buffer (at pH 5.6 to 8.0), reaction rates increased, reaching maximal values at pH 8. When borate replaced this buffer, reaction rates at pH 8 were lower than those obtained with phosphate at the same pH. The rates of N- or O-acetylation of phytosphingosine or N-acetyl phytosphingosine increased with increasing pH of the borate buffer, reaching a maximum above pH 9; the N-acetylation of hexadecylamine was practically constant in this buffer between pH 7.6 and 9.3.

Table II

<table>
<thead>
<tr>
<th>Buffer solution</th>
<th>Dihydrosphingosine</th>
<th>Hexadecylamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium acetate, pH 5.0</td>
<td>+11.00</td>
<td>+12.30</td>
</tr>
<tr>
<td>Potassium phosphate, pH 7.9</td>
<td>0.00</td>
<td>-1.42</td>
</tr>
<tr>
<td>Potassium borate, pH 9.3</td>
<td>-1.83</td>
<td>4.09</td>
</tr>
</tbody>
</table>

* Mobility units = μsec⁻¹ volt⁻¹ cm⁻¹.

Table III

<table>
<thead>
<tr>
<th>Incubation mixture</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>4.0</td>
</tr>
<tr>
<td>Complete × 2</td>
<td>10.2</td>
</tr>
<tr>
<td>ATP omitted</td>
<td>0.04</td>
</tr>
<tr>
<td>CoA omitted</td>
<td>0.03</td>
</tr>
<tr>
<td>CoA and ATP omitted</td>
<td>0.01</td>
</tr>
<tr>
<td>Acetate, CoA, and ATP omitted, [2-3H]-acetly-CoA added</td>
<td>25.00</td>
</tr>
</tbody>
</table>

* In this tube N-acetyl phytosphingosine, acetate, A'TP', and CoA were each present at twice the above concentrations.

Effect of Micellar Charge on Reaction—The above results suggested that the charges of micelles of the lipid substrates might depend on the buffer and that this might be a possible cause for the differences in the reaction rates.

Table II shows the electrophoretic mobility of hexadecylamine and dihydrosphingosine at certain pH values of the above three buffers. The results show that when reaction rates were low or nil, the micelles migrated to the cathode. This applied to either dihydrosphingosine or hexadecylamine in acetate buffer, pH 5.0, and to the latter compound in borate buffer, pH 9.3. In phosphate buffer, pH 7.9, either substrate was negatively charged and in the borate buffer, dihydrosphingosine was similarly charged. This can be well correlated with acetylation of these two substrates, as shown in Fig. 1.

Substrate Specificity—Table III shows that acetyl-CoA could be replaced by a mixture of acetate, CoA, and ATP, although reaction rates were lower. Acetate “activation” depended on acetoalkokinase, this enzyme was present in the fresh yeast microsomes or in aged preparation stored in the presence of 1 mM dithiothreitol. If stored for a few days at -20° in the absence of the latter —SH reagent, the mixture of acetate, CoA,
Amine, glucosamine, p-nitroaniline; normal primary aliphatic and N-acetylation of hexadecylamine: phosphatidylethanolamine inhibitors of the N- or O-acetylation of sphingosine bases or of 1 mM, maximal reaction rates were determined as described under "Methods." Microsomal protein, 0.2 mg, was used; incubation time, 60 min. and ATP could no longer replace acetyl-CoA as substrate. Butyryl-CoA was neither a substrate for the reaction nor an inhibitor for acetyl group transfer from acetyl-CoA.

Table IV shows an acetyl transfer to several sphingosine bases and derivatives. Only the free bases or their N-acetylated derivatives were substrates for the enzyme. The N-butyryl, N-octanoyl, N-palmitoyl, 1-O-galactosyl, or N-acetyldichloro derivatives of the bases were not acetylated.

Normal aliphatic amines of 6 to 18 carbon atoms (but not secondary amines, such as didecyl- or didodecylamine) were also acceptors of the acetyl groups of acetyl-CoA (Fig. 2). In this experiment, where the concentration of each of the amines was 1 mM, maximal reaction rates were obtained with C16 to C18 amines (Fig. 2).

The following compounds were neither substrates nor inhibitors of the N- or O-acetylation of sphingosine bases or of the N-acetylation of hexadecylamine: phosphatidylethanolamine, glucosamine, p-nitroaniline; normal primary aliphatic

**Table IV**

Acetylation of sphingosine bases and their derivatives

<table>
<thead>
<tr>
<th>Substrate</th>
<th>N-Acetylation</th>
<th>O-Acetylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphingosine</td>
<td>8.3</td>
<td>8.9</td>
</tr>
<tr>
<td>Dihydrosphingosine</td>
<td>6.0</td>
<td>8.3</td>
</tr>
<tr>
<td>Phytosphingosine</td>
<td>7.3</td>
<td>7.6</td>
</tr>
<tr>
<td>N-Acetyl sphingosine</td>
<td>28.1</td>
<td></td>
</tr>
<tr>
<td>N-Acetyl dihydrosphingosine</td>
<td>16.8</td>
<td></td>
</tr>
<tr>
<td>N-Acetyl phytosphingosine</td>
<td>21.5</td>
<td></td>
</tr>
<tr>
<td>N-Acetyl dichlorodihydrosphingosine</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>N-Butyryl dihydrosphingosine</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>N-Octanoyl dihydrosphingosine</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>N-Palmitoyl dihydrosphingosine</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>1-O-Galactosyl sphingosine</td>
<td>&lt;0.1</td>
<td></td>
</tr>
</tbody>
</table>

* O-Acetylation was derived from the difference between the total acetylation and the N-acetylation.

**Fig. 2.** Effect of the chain length of the amine on the reaction rates. Incubation mixtures were prepared and the products were determined as described under "Methods." Microsomal protein, 0.2 mg, was used; incubation time, 60 min.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Incubation time</th>
<th>CoA released</th>
<th>P-Hexosylate bound to LCB</th>
<th>CoA released to acetyl bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Hexadecylamine</td>
<td>180</td>
<td>192</td>
<td>170</td>
<td>1.12</td>
</tr>
<tr>
<td>2 Phytosphingosine</td>
<td>120</td>
<td>177</td>
<td>132</td>
<td>1.34</td>
</tr>
<tr>
<td>3 N-acetyl phytosphingosine</td>
<td>120</td>
<td>207</td>
<td>171</td>
<td>1.21</td>
</tr>
</tbody>
</table>

**Table V**

Stoichiometry of reaction

Incubation mixtures contained, in volumes of 1 ml, 100 μmoles of potassium phosphate buffer, pH 7.8, 1 μmole of DTNB, and 1.5 mg of microsomal protein. Experiment 1 also contained 0.5 μmole of hexadecylamine and 0.5 μmole of [2-3H]acetyl-CoA (4600 dpm per n mole). Experiments 2 and 3 also contained, each, 1 μmole of [2-3H]acetyl-CoA and 1 μmole of lipid substrate (phytosphingosine or N-acetyl phytosphingosine, respectively). After the time specified for each substrate, the reaction was terminated and the products were determined as described under "Methods."

Inhibition of Reaction—The lipid products of the reaction (i.e. acetylated bases) had no effect on the acetylation, either of the same base or of the other LCB’s. Coenzyme A, the second product of the reaction, inhibited both N- and O-acetylation. At 1 mM each of CoA, LCB, and [2-3H]acetyl CoA, reaction rates were only 15 to 35% of those obtained in the absence of the CoA. DTNB or p-hydroxymercuribenzoate (each at a concentration of 1 mM) relieved this inhibition by binding the CoA. The inhibition was relieved even when these reagents were added after mixing the enzyme and CoA, thus suggesting that the inhibition by the coenzyme could be reversed. Addition of the two reagents to reaction mixtures containing LCB and acetyl-CoA (but no free CoA) increased reaction rates about 50%. Kinetic experiments of CoA inhibition are presented in the following paper (17). Other thio compounds, such as dithiothreitol and mercaptoethanol (each at 1 mM) had no effect on reaction rates; this suggests a specific, inhibitory effect of coenzyme A.
Fig. 3. Effect of heat on the rates of N- and O-acetylation. A suspension of microsomes in Buffer A (0 mg per ml) was heated in a 47.5°C bath. At the specified times, aliquots (0.06 ml) were removed and stored in ice. Each of these aliquots was then incubated for 135 min with either N-acetyl phytosphingosine or with phytosphingosine, and the degree of the O- or N-acetylation, respectively, was determined as described under "Methods." O—O, O-acetylation of N-acetyl phytosphingosine (left ordinate); I—I, N-acetylation of phytosphingosine (right ordinate).

Trials to Separate N- and O-Acetylation

Numerous experiments were conducted with the purpose of separating the microsomal preparation into two separate enzymes (or activities), one catalyzing an acetyl transfer to the amino and the other to the hydroxyl groups of the LCB's. These experiments are categorized as follows.

Physical and Chemical Separation—This included treatment with 1 M KCl, buffers at a pH range of 4 to 10, 1-butanol, and acetone, proteases (trypsin or chymotrypsin), phospholipases A or C, pancreatic lipase, or various detergents. Microsomal suspensions were also subjected to sonic irradiation at either 10 or 20 kc. After each of the above treatments, the suspensions were centrifuged for 30 min at 40,000 × g, the supernatants were retained and the sediments were dispersed in Buffer A. In most cases the activity was recovered in the sediments and the ratio of N- to O-acetylation was identical with that of the original microsomes. The use of the proteases or acetone resulted in a complete loss of the enzymatic activity. Treatment with 5% cholate, ammonium hydroxide at pH 10 or 0.1% Triton X-100, resulted in recoveries of 20, 5, and 5%, respectively, in the 1.2 × 10⁶ g × min supernatant. The ratio of O-acetylation of N-acetyl phytosphingosine to N-acetylation of hexadecylamine was 2.1 prior to these treatments and 1.9 to 2.15 in the supernatants.

Effect of Heating—Heating for 10 min at 60°C (not shown in the figures), resulted in complete inactivation of both activities. Fig. 3 shows the effect of heating at 47.5°C. After half a minute, the decrease of the N- and O-acetylations were 34 and 38%, respectively. After 5 min, about 40% of either activity remained.

Effect of Chemical Agents—The effect of several compounds on the O- and N-acetylation was tested. These included several detergents (Triton X-100, 0.5 to 5%; sodium cholate, deoxycholate, and taurocholate, 0.5 to 5%; each; sodium dodecyl sulfate, 0.25 to 2%); mono- and divalent ions (KCl or NaCl, 5 to 100 mM; CaCl₂ or MgCl₂, 1 to 10 mM) and EDTA, 1 to 10 mM. In all these cases the ratio of N- to O-acetylation remained constant. Table VI shows the effect of several thiols and —SH inhibitors. The effects of these compounds varied. Thus coenzyme A inhibited either reaction while p-hydroxymercuribenzoate increased the reaction rates. However, the ratio of N- to O-acetylation remained practically constant with all agents.

Kinetic Experiments—Fig. 4 describes two parallel experiments. Experiment 1, in the experiment presented by the solid line, increasing concentrations of N-acetyl phytosphingosine (i.e. a substrate for O-acetylation) were added to reaction mixtures containing fixed quantities of hexadecylamine (as a substrate for N-acetylation). Experiment 2, in the experiment presented by the dashed line, increasing concentrations of hexadecylamine were added to reaction mixtures containing fixed...
quantities of N-acetyl phytosphingosine. The total acetylation (i.e., the sum of N- and O-acetylation, see "Methods") was determined in each of these experiments. The values in Fig. 4 therefore represent, in each case, the sum of N- and O-acetylations. These results show that the total acetylation depended on the relative concentrations of the above two substrates. However at any substrate concentration, the combined acetylation of the two substrates was always smaller than the arithmetic sum of the acetylation of each individual substrate. This again supports the conclusion that both the N- and O-acetylation are catalyzed by the same protein (18).

**Reaction Mechanism**—As shown in the following paper (17), either the N- or O-acetylation are of the Sequential Bi Bi type.

**Identification of Products of Reaction**

When using hexadecylamine or the various sphingosine bases, the following compounds might be obtained as products of the acetylation reaction: (a) N-acetyl hexadecylamine; (b) N-acetyl dihydro- or phytosphingosine; (c) partially or fully acetylated LCB's (i.e., mono-, di-, and triacetyl dihydrosphingosine or tetraacetyl phytosphingosine). [2-3H]Acetyl-CoA was incubated with each of the free or N-acetylated bases, the reaction was terminated, and the products were identified with thin layer silica gel chromatography (10, 19) or gas-liquid chromatography of the silylated products (11). Suitable markers were available for the free, N-acetylated, or fully acetylated bases, but not for the intermediate stages where the bases were only partially acetylated. It was of interest that in spite of the many possibilities of the above intermediates only three radioactive spots were obtained on the thin layer plates and only three peaks on gas-liquid chromatography column, with dihydrosphingosine as substrate (these are herewith designated as "mono-, di-, and triacetyl dihydrosphingosine"). With phytosphingosine, four radioactive spots or peaks were obtained (herewith designated as "mono-, di-, tri- and tetracetyl phytosphingosine"). Fig. 5 shows the results of an experiment in which dihydrosphingosine, phytosphingosine, and N-acetyl phytosphingosine were incubated with [3H]acetyl-CoA. The positions of the numbers correspond to the relative RF values of the products, and their values represent disintegrations per min. With each of the three substrates, the intermediate products (i.e., the diacetyl dihydrosphingosine or di- and triacetyl phytosphingosine) contained the highest radioactivity. In those lanes, where the products were subjected to mild alkaline hydrolysis (i.e., Lanes 8, 9, and 10), the monoacetoylated product represented the combined N-acetylation values of the N- and N- plus O-acetylated bases. With hexadecylamine as substrate (not presented in the Fig. 5), only one radioactive spot of N-acetyl hexadecylamine was obtained; as expected, the radioactivity (31,000 dpm) was the same without or with mild alkaline hydrolysis.

For gas-liquid chromatography, the total product was silylated with the Sigmasil-A reagent and chromatographed on 4% SE-30 on Chromosorb, at 230° (11). The results were similar to those obtained by the use of thin layer chromatography.

In a separate experiment, dihydrosphingosine was incubated with a mixture of [2-3H]acetate, ATP, CoA, MgCl₂, and microsomes which had been stored in the presence of 1 mM DTT. The products were isolated either by thin layer chromatography or by gas-liquid chromatography. The results were similar to those obtained with the same base and [3H]acetyl-CoA.

**Distribution of Enzyme**—The microsomal LCB transacetylase was not found in rat organs (i.e., liver, brain, kidney, or spleen) nor in acetic powder of pigeon livers, although these contained arylamine acetyltransferase. The distribution of the enzyme in 25 strains of the genus *Hansenula* was presented in a former report (2).

**DISCUSSION**

This paper describes the properties of a novel enzyme present in the microsomes of the yeast *H. ciferri*. It exhibited two unique properties: first, it transferred the acetyl group of acetyl coenzyme A to both the amino and hydroxyl groups of the sphingosine bases; none of the other acetyltransferases described to date catalyze both an O- and N-acetyl transfer. Various experiments were performed attempting to distinguish between, and possibly separate the N- and O-acetylating moieties of this enzyme into two individual entities; they were however unsuccessful. It should however be emphasized that the enzyme is not a pure, soluble protein but a microsomal preparation. The possibility that the N- and O-acetylations are catalyzed by two separate membranous proteins can therefore not be excluded. The second unique property of the enzyme was its utilization of the sphingosine bases as acceptors. The enzyme exhibited a rather strict specificity, transferring the acetyl group of acetyl coenzyme A to several sphingosine bases (i.e., sphingosine, dihydrosphingosine, and phytosphingosine) and their N-acetyl derivatives as well as to long chain primary amines. p-Nitroaniline, glucosamine, phosphatidylethanolamine, secondary aliphatic amines, primary aliphatic alcohols, or 1-lyssolecithin were neither substrates nor inhibitors of the acetylation of the above substrates.
The strict specificity is further emphasized by the finding that among numerous sphingolipids tested as possible substrate, only the above three sphingosine bases and their N-acetyl derivatives were utilized by the enzyme. As the donor, only acetyl coenzyme A was utilized; butyryl-CoA or higher fatty acyl-CoA derivatives were not substrates. This contrast with the acylation of sphingosine in animal systems, where free fatty acids (4, 5) or fatty acyl-CoA derivatives (6, 7) were used to produce N-acetyl sphingosine (ceramide). The enzymes which catalyzed these reactions did not acetylate the hydroxyl groups of the bases.

The enzyme could not be detected in animal organs. Furthermore, it was present only in those strains of the yeast which, because of a metabolic aberration of the biosynthetic route, produced excessive quantities of sphingosine bases (2). Its production in these strains is probably a protective mechanism against possible toxic effects of the excessive quantities of the bases produced. Kinetic analyses and conclusions on the mode of interaction of the enzyme with its lipid substrates are presented and discussed in a second, accompanying paper (17).

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REFERENCES

Long Chain Base-Acetyl Coenzyme A Acetyltransferase from the Microsomes of *Hansenula ciferri*: I. ISOLATION AND PROPERTIES
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