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# AMINE:ACETYL COENZYME A ACETYLTRANSFERASE FROM THE SOLUBLE FRACTION OF *HANSENULA CIFERRI*:

# ISOLATION AND PROPERTIES

YECHEZKEL BARENHOLZ, IRIT EDELMAN and SHIMON GATT

*Laboratory of Neurochemistry, Department of Biochemistry, Hebrew University–Hadassah Medical School, Jerusalem (Israel)*

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## SUMMARY

An enzyme (amine:acetyl coenzyme A acetyltransferase, EC 2.3.1.5) was partially purified from the 250 000 × *g* supernatant of extracts of the yeast *Hansenula ciferri*. It catalyzed the transfer of the acetyl group of acetyl-CoA to long-chain primary amines of 6–16 carbon atoms, as well as to water-soluble primary amines, such as glucosamine, histamine, tryptamine, serotonine, hydroxytyramine and noradrenaline, but not to polyamines, amino acids or sphingosine bases. The acetylation of the long-chain amines was inhibited by detergents, coenzyme A, butyryl or palmitoyl coenzyme A and by SH-inhibitors.

Hyperbolic curves were obtained when reaction rates were measured as a function of increasing acetyl-CoA concentrations, while maintaining a constant concentration of the amine. The curves were also hyperbolic when acetyl-CoA was maintained at a constant level and the concentrations of the water-soluble amines or normal amines of 6–10 carbon atoms were varied. With long-chain amines of 12–16 carbon atoms as the varying substrates, the $v/S$ curves were hyperbolic to a certain concentration of the amine, above this concentration ("inversion point") reaction rates decreased. The inversion points of the three amines were close to their "critical micellar concentration". Serum albumin increased the reaction rates as well as the $K_m$ values with the long-chain amines but not with the water-soluble compounds. These data suggest that the enzyme utilizes molecular solutions of the amines but is inhibited by their micellar aggregates.

## INTRODUCTION

The enzyme arylamine:acetyl-CoA acetyltransferase (EC 2.3.1.5) of animal tissues was studied in several laboratories [1–7]. A similar enzyme has now been

Abbreviations: PIPES, piperazine-*N*,*N*-bis(2-ethanolsulfonic acid); TES, *N*-tris(hydroxymethyl)methyl-2-aminoethane; CAPS, cyclohexylaminopropanesulfonic acid; Tricine, *N*-tris(hydroxymethyl)methylglycine; DTNB, 5,5′-dithiobis-2-(nitrobenzoic acid); CMC, critical micellar concentration.

purified from the 250 000 × *g* supernatant of the yeast *Hansenula ciferri*. This soluble enzyme differs from a previous enzyme, present in the microsomes of the same yeast, which catalyzes the transfer of the acetyl group of acetyl-CoA to sphingosine bases and long-chain amines [8, 9]. The soluble enzyme yielded biphasic *v/S* curves when $C_{12}$–$C_{16}$ normal amines were used as substrates. The shapes of these curves suggested that the enzyme utilized the long-chain amine when in true molecular solution, and was inhibited by its micellar aggregates (such a behaviour corresponds to Type III, as classified by Gatt et al. [10, 11]). The enzyme was very sensitive to heat; glycerol and acetyl-CoA protected against heat denaturation (unpublished).

## EXPERIMENTAL PROCEDURE

### *Materials*

Tritium-labelled acetyl-CoA and unlabelled acetyl-CoA, butyryl-CoA, sphingosine, dihydrosphingosine, phytosphingosine, *N*-acetylphytosphingosine and the scintillation fluid were prepared as described previously [9]. Sphingosine (of 14 carbon atoms) was prepared from the appropriate ceramide (a gift of Dr K. K. Karlsson from the Institute of Medical Biochemistry, University of Goteborg), according to Gaver and Sweeley [12]. Palmitoyl-CoA was synthesized similarly to acetyl- and butyryl-CoA; the palmitic anhydride was prepared according to Selinger and Lapidot [13]. *N*-acetylated derivatives of the primary aliphatic amines were prepared as described by Carter and Gaver [14]. Primary and secondary amines were purchased from Koch–Light; histamine, tryptamine, serotonin, hydroxytyramine and noradrenaline were purchased from Serva. The following compounds were purchased from Sigma: DEAE-cellulose; the sodium salts of cholic, glycocholic, taurocholic and deoxycholic acids; sodium dodecylsulfate, glucosamine, ATP, coenzyme A (85% pure), *p*-hydroxymercuribenzoate, dithiothreitol, amino acids and the buffers piperazine-*N*,*N*-bis(2-ethanolsulfonic acid) (PIPES), *N*-tris(hydroxymethyl)methyl-2-aminoethane (TES), cyclohexylaminopropanesulfonic acid (CAPS) and *N*-tris-(hydroxymethyl)-methylglycine (Tricine). Acetic anhydride, butyric anhydride, glucose, Triton X-100, methylamine, ethylamine, propylamine, butylamine and ethanolamine were purchased from BDH; [2-$^3H_3$]acetic anhydride from Amersham; 5,5′-dithiobis-2-(nitrobenzoic acid) (DTNB) from Aldrich; Sephadex G-50 (fine) from Pharmacia; albumin (fatty acid poor) from Pentex; glycerol from Frutarom; the polyamines were gifts of Professor Y. Bachrach of the Department of Molecular Biology, The Hebrew University.

### *Methods*

The critical micellar concentrations (CMC) of the long-chain primary amines were measured as previously described [9]. Dispersion of the long-chain primary amines, with and without albumin was described in former papers [8, 9]. Incubation mixtures in final volumes of 0.2 ml, contained the substrates (i.e., amine and acetyl-CoA), enzyme and 20 μmoles of buffer. The mixtures were incubated with shaking at 37 °C. When using long-chain primary amines the reaction rates were followed by measuring either the CoA released or the [$^3$H]-acetate bound to the lipid substrate [8]; the results were the same with or without mild alkaline hydrolysis. When using water-soluble amines as substrates, two alternative procedures were used for the

determination of the reaction rates. In the first (applicable for all water-soluble amines) the reaction was terminated by the addition of 0.3 μmole of DTNB and 0.2 ml of a mixture of ethanol–ether (2:1, v/v); the CoA released was calculated from the absorbance at 413 nm [15]. The second procedure (used for histamine, tryptamine, hydroxytyramine as well as for $C_6$–$C_{10}$ aliphatic amines) was that of Weissbach et al. [3]. The reaction was terminated by the addition of 0.17 ml of 0.5 M borate buffer, pH 10, in saturated NaCl and 1 ml of pentanol or isopentanol. After mixing and centrifugation, the upper, isopentanol phase was removed, washed once with 0.3 ml of the above borate–NaCl buffer and then transferred into counting vials containing 1 ml of ethanol and 10 ml of scintillation fluid, these were counted in a scintillation counter. Protein was determined according to Lowry et al. [16], using bovine serum albumin as standard.

## RESULTS

### *Enzyme Preparation*

Yeast were grown, harvested and the cells were disrupted as described [8]. The homogenate was centrifuged successively, for 10 min at 800 × *g* and at 1200 × *g*, and then twice, for 65 min at 250 000 × *g*. The sediments were discarded and the 250 000 × *g* supernatant was filtered through glass wool to remove a floating lipid layer. It was then concentrated to about one eighth of its volume by pressure filtration through a PM10 or UM 20E Diaflo membrane, using a nitrogen pressure of 25 lb/inch$^2$; all the activity was recovered on the filter. 12 ml of the concentrate, corresponding to 99 ml of the original high-speed supernatant were applied to a column of Sephadex G-50 (fine, 126 cm × 3 cm), previously equilibrated against 0.05 M phosphate buffer (pH 7.0) containing 10% glycerol (v/v). This buffer was used for elution and 6-ml fractions were collected at a rate of about 20 ml per h. Fig. 1 shows the elution pattern of the enzyme which suggests a molecular weight of about 24 000. Fractions

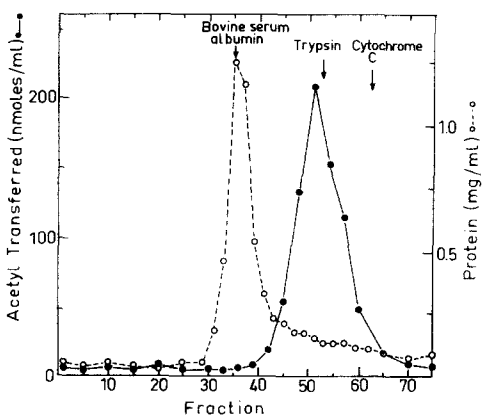


Fig. 1. Gel filtration on Sephadex G-50. For experimental conditions see text. The eluted fractions were assayed as follows: Incubation mixtures, in volumes of 0.2 ml each, contained 20 μmoles of potassium phosphate buffer, pH 7.5, 0.1 μmole of [2-$^3H_3$]acetyl-CoA (2500 dpm/nmole), 0.1 μmole of dodecylamine and 0.12 ml of the respective enzyme fraction. After 15 min at 37 °C, the products were determined as described under Methods. Bovine serum albumin, trypsin and cytochrome *c* were used as molecularweight markers.

48–57 were pooled and stored at −20 °C. Trials to further purify the Sephadex effluent on columns of phospho- or DEAE-cellulose columns were unsuccessful.

The degree of purification using the above procedure could not be accurately assessed. The reason for this was a nonlinear response of rate against the concentration of the 250 000 × *g* supernatant. Furthermore, the specific activities of this supernatant were not constant, but increased upon dilution of the enzyme with glycerol-containing phosphate buffer (pH 7.0). This was probably caused by accompanying polynucleotides. Sephadex gel filtration of the supernatant yielded two polynucleotide peaks at $V_e/V_o$ ratios of 1.0 and 2.4 respectively, each exhibited a maximal absorbance at 258 nm. When added to the reaction mixtures they strongly inhibited the acetyltransferase reaction (70% inhibition was obtained using a quantity of inhibitor equivalent to 0.080 absorbance units at 258 nm). These inhibitors were removed from the enzyme during the gel filtration on the Sephadex. The Sephadex effluents yielded straight lines when the rates were plotted as a function of enzyme concentration.

*Stability of the enzyme*

The high-speed supernatant, in glycerol-containing phosphate buffer (pH 7.0), retained full activity for at least 3 years at −20 °C and the Sephadex effluent for at least 2 years at the same temperature. All enzymes were prepared and stored in media containing 10% glycerol, by vol. If the yeast cells were disrupted in phosphate buffer, free of glycerol, all activity was lost within 1–2 days at −20 °C or at +4 °C. Similarly, removal of the glycerol by dialysis of enzyme preparations against 50 mM phosphate, pH 7.0, for 36 h, resulted in a complete loss of activity. 0.5 M sucrose or mannitol could replace the glycerol during preparation of the enzyme; however the specific activities were only about half of those prepared in the presence of glycerol. Tris buffer could not replace the phosphate; dialysis against 0.05 M Tris, pH 7.4, containing 10% glycerol for 24 h resulted in a complete loss of the activity.

*Properties of the enzyme*

Using water-soluble amines or long-chain amines at substrate concentrations below the CMC, reaction rates were directly proportional to enzyme concentration (up to at least 10 μg) and to incubation time (up to 20–30 min). The reaction (using long-chain amines as substrates) was inhibited by the non-ionic detergent, Triton X-100 (0.5–5 mg/ml) and by several anionic detergents such as cholate, deoxycholate, taurocholate, glycocholate and taurodeoxycholate; each of these was tested at a concentration range of 0.25–5.0 mg/ml.

Fig. 2 shows the effect of pH and the type of buffer on the reaction, using dodecylamine as substrate; similar results were obtained using soluble amines. It is evident that the type of buffer markedly affected the reaction rates at the various pH values, as well as the shapes of the *v*/pH curves.

*Identification of the reaction products*

Reaction mixtures containing [2-$^3H_3$]acetyl coenzyme A and $C_7$–$C_{12}$ amines were terminated according to Folch et al. [17]. The lower phases were washed three times with a "pure solvents upper phase", evaporated and applied to thin-layer plates of silica gel. The plates were developed in chloroform–methanol (95:5, v/v). In each case 97% of the radioactivity migrated with the same $R_F$ as the N-acetylated amine.

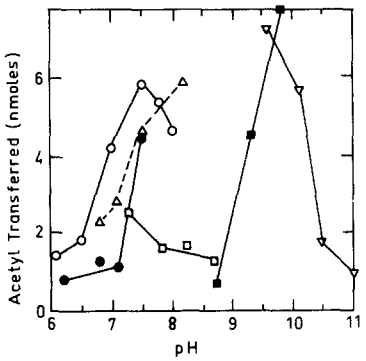


Fig. 2. Effect of the pH and the type of the buffer on the reaction rates. Conditions were similar to those of Fig. 1, except that 7.2 $\mu$g of enzyme and 20 $\mu$moles of each of the following buffers were used: ▽—▽, CAPS; ■—■, trisethanolamine; □—□, Tricine; △---△, TES; ●—●, PIPES; ○—○, potassium phosphate.

*Effect of substrate concentration on reaction rates*

Two sets of experiments were performed to test the effect of substrate concentration on the rates of the bisubstrate reaction. In the first, the concentration of acetyl-CoA was increased, while maintaining a constant concentration of the amine. Fig. 3 shows that, using dodecylamine, the $v/S$ curve was hyperbolic of the Michaelis–Menten type. The $v/S$ curves were also hyperbolic if the dodecylamine was replaced by $C_8$, $C_{10}$, $C_{14}$ and $C_{16}$ normal primary amines, or by the water-soluble amines, histamine, tryptamine, 5-hydroxytryptamine, hydroxytyramine and norepinephrine.

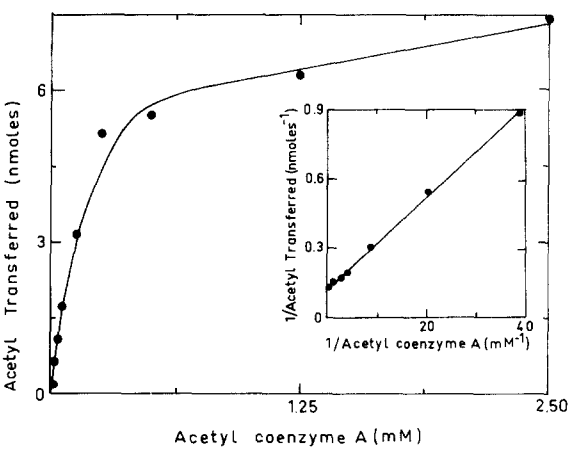


Fig. 3. Dependence of the rate of acetylation of tetradecylamine on the concentration of [2-$^3H_3$]-acetyl-CoA. Conditions were similar to those of Fig. 1 except that 7.2 $\mu$g of enzyme, 0.02 $\mu$mole of tetradecylamine and varying concentrations of acetyl-CoA were used.

In the second series of experiments acetyl-CoA was maintained at a constant level and the concentration of the amine was varied. Fig. 4 shows that tryptamine, serotonine, or hydroxytyramine yielded straight lines when $1/v$ was plotted against $1/S$. Using primary normal amines (Fig. 5), hyperbolic curves were obtained with

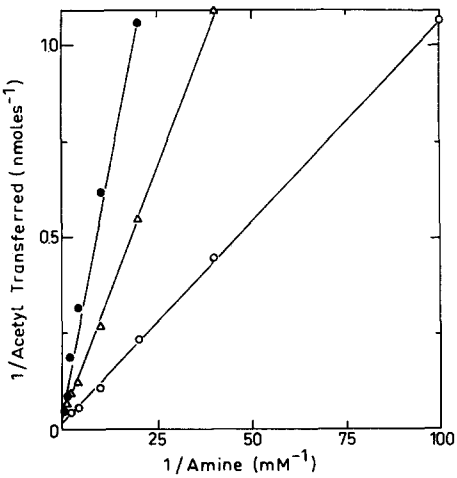


Fig. 4. $v^{-1}/S^{-1}$ curves at variable concentrations of amine and fixed concentrations of acetyl-CoA. Incubation mixtures, in volumes of 0.2 ml each, contained 20 $\mu$moles of buffer, 0.16 $\mu$mole of [2-$^3H_3$]acetyl-CoA (2500 dpm per nmole) and 7.2 $\mu$g of enzyme. After 15 min at 37 °C, the acetylated amines were isolated and counted as described under Methods. ○—○, 5-hydroxytryptamine (buffer $K_3BO_3$, pH 9.1); ●—●, hydroxytyramine (buffer $K_3BO_3$, pH 9.1); △—△, tryptamine (buffer potassium phosphate, pH 7.5).

hexylamine, octylamine and dodecylamine. With dodecyl, tetradecyl- and hexadecylamines, the reaction rates increased and the $v/S$ curves were hyperbolic up to a certain concentration of the amine. Above this concentration ("inversion point"*) the rates decreased with increasing substrate concentration. The respective inversion points were about 0.5 mM, 0.3 mM and 0.2 mM with $C_{12}$, $C_{14}$ and $C_{16}$ amines, respectively.

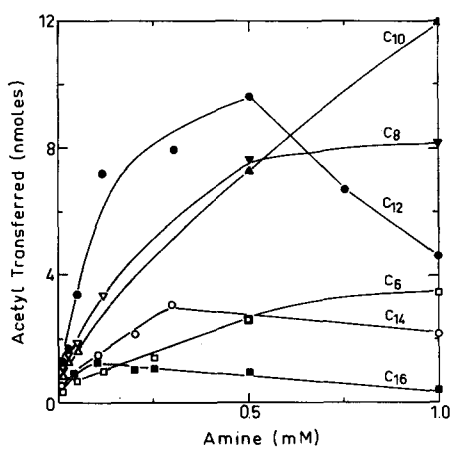


Fig. 5. Dependence of the rate of acetyltransfer on the concentration of long-chain primary amines of 6–16 carbon atoms. Conditions were similar to those of Fig. 4, except that 0.12 $\mu$mole of [2-$^3H_3$]-acetyl-CoA (2500 dpm/$\mu$mole) was used. The chain length of the amine is shown in the figure.

* The inversion point is defined as the substrate concentration at which the peak of the biphasic $v/S$ occurs.

Expecting that the inhibition might be caused by substrate micelles [10] the CMC of dodecylamine and hexadecylamine values were measured [8] and found to be 0.6 and 0.2 mM, respectively. The inversion points therefore seem to reflect the transition of monomers to micelles and the curve is of Type III according to a proposed classification [10, 11]. Fig. 6 shows that the inversion point of the $v/S$ curve describing dodecylamine acetylation was not influenced by the quantity of enzymatic protein, even when the latter was increased 6-fold. This suggested that, dissimilar to the microsomal lysolecithinase [18, 19], the substrate was not removed, to a significant extent from the solution by non-specific absorption onto the enzymatic protein.

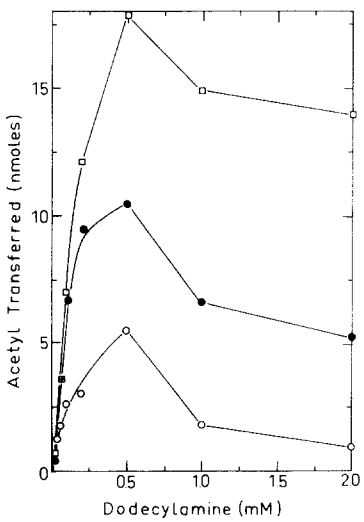


Fig. 6. The effect of enzyme concentration on the $v/S$ curves. Conditions were similar to those of Fig. 5, except that three enzyme concentrations were used. ○—○, 1.8 μg; ●—●, 5.4 μg; □—□, 10.8 μg.

*Effect of albumin*

Albumin affected the reaction rates when the variable substrate was a long-chain amine, it had however, no effect on the transfer reaction to the water-soluble amines. Albumin increased the reaction rates, using dodecyl, tetradecyl and hexadecylamines, the maximal stimulation was obtained at an albumin to amine molar ratio of 0.1–0.2 (Fig. 7). This ratio did not change when the protein concentration was varied. The concentrations of tetradecylamine and hexadecylamine used in this experiment (0.11 mM, each), were close to their CMC, while that of dodecylamine (2.5 mM) exceeded the CMC about 4-fold. At this high concentration, the latter substrate exerts a strong inhibitory effect (the reaction rate at 2.5 mM was only 10% that obtained using 0.4 mM; for comparison see Fig. 5). The degree of stimulation by albumin in the experiment of Fig. 7 was therefore 8-fold with dodecylamine but only 1.6- and 3-fold, using tetradecyl and hexadecylamine, respectively.

Fig. 8 shows a series of $v/S$ curves, each obtained at a fixed molar ratio of albumin to tetradecylamine. The reaction rates increased, at all substrate concentrations, up to a ratio of 0.4. At a ratio of 1 or more, the rates were lower than those

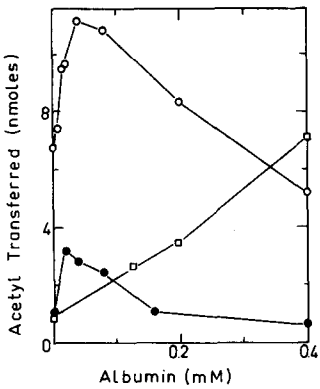


Fig. 7. The effect of albumin concentration on the rates of acetylation of dodecylamine, tetradecylamine and hexadecylamine. Conditions were similar to those of Fig. 5 except that the concentration of the amine was fixed and that of the albumin was varied. □—□, dodecylamine (2.5 mM); ○—○, tetradecylamine (0.4 mM); ●—●, hexadecylamine (0.4 mM).

obtained in the absence of albumin. At the optimal ratio of 0.2, the descending portion of the $v/S$ curve was practically absent.

Fig. 9 shows $v/S$ curves of $C_6$–$C_{16}$ amines, done in the presence of albumin, whose concentration was one tenth that of the amine. All curves were hyperbolic. This is in marked contrast with the biphasic shape of the $v/S$ curves obtained with $C_{12}$–$C_{16}$ amines, in the absence of albumin (Fig. 5). The reaction rates, in the presence of albumin were greater with all amines except of hexylamine.

*Substrate and enzyme specificities*

The soluble acetyltransferase transferred acetyl groups to several primary amines other than the $C_6$–$C_{16}$ normal aliphatic amines. Among these were glucosamine, histamine, tryptamine, hydroxytyramine, serotonine and noradrenaline. The following amines were not substrates: *p*-nitroaniline, methyl-; ethyl-; propyl-; butyl- and ethanolamines; several polyamines of the spermidine group; amino acids and the fol-

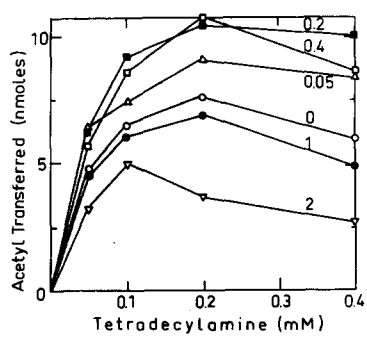


Fig. 8. The effect of the albumin to substrate ratios on the $v/S$ curves of tetradecylamine acetylation. Conditions were the same as in Fig. 5. Mixtures of albumin and tetradecylamine were prepared according to Methods. The numbers on the curves describe, in each case, the molar albumin to tetradecylamine ratio; this ratio was maintained throughout all substrate concentrations.

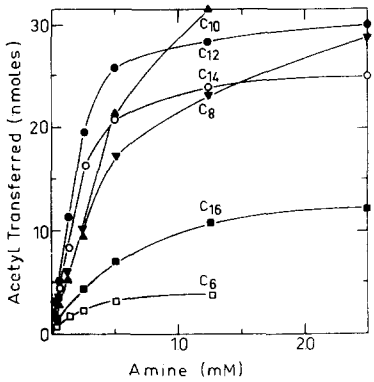


Fig. 9. The effect of albumin on the $v/S$ curves using long-chain primary amines. Conditions were similar to those of Fig. 5 except that a constant albumin to amine molar ratio of 0.1 was maintained throughout the entire experiment.

lowing secondary or tertiary amines: dioctylamine, didecylamine, methyloctaylamine, trihexylamine and trinonylamine.

Of special interest was the finding that the enzyme did not catalyze an acetyl transfer to sphingosine bases, even when tested at several pH values. The following bases were tested: dihydrosphingosine, phytosphingosine and the $C_{14}$-analog of sphingosine. Furthermore, addition of these bases (at 1 mM) to reaction mixtures containing 0.4 mM dodecylamine, did not affect the acetylation rates of the latter compound. This is in marked contrast with the microsomal enzyme which acetylated the sphingosine bases at both the amino and the hydroxyl groups [8]. In contrast to the variety of amines utilized by the enzyme, acetyl-CoA was the only donor of the acyl group. Butyryl-CoA and palmitoyl-CoA were not substrates, and when added to mixtures containing acetyl-CoA, inhibited the reaction. Acetylpantetheine, acetyl-AMP and acetyl phosphate were neither substrates nor inhibitors.

Table I presents the $K_m$ and $V$ values of $C_6$–$C_{16}$ normal amines. These were determined from the ascending, hyperbolic portions of the $v/S$ curves obtained under conditions similar to those of Figs 5 and 9. The $K_m$ values roughly paralleled the maximal velocities, i.e. the substrate with the highest turnover number had the lowest affinity to the enzyme. Addition of albumin increased both the $K_m$ and $V$ values. Table I also shows $K_m$ and $V$ values for tryptamine, serotonine, hydroxytyramine, noradrenaline and histamine. The $K_m$ values were very similar to each other and of the same order of magnitude as the corresponding values with the long-chain amines in the presence of albumin. From the data exemplified by tryptamine, it is evident that albumin did not affect the kinetic parameters with these water-soluble amines.

In a parallel experiment, the amine was maintained at a fixed concentration of 1 mM and the acetyl-CoA was varied. The $K_m$ values were as follows: 0.05 mM with dodecylamine, 0.15 mM with tetradecylamine, 0.09 mM with hexadecylamine, 0.15 mM with histamine and 0.25 mM with tryptamine. Albumin had no effect on any of these values. The corresponding maximal velocities were, 45, 75, 25, 165 and 200 nmoles $\cdot mg^{-1} \cdot min^{-1}$, respectively. It should be emphasized that the above $K_m$ values, obtained with $C_{14}$- and $C_{16}$-amines are "apparent" values [6]. This is a consequence

TABLE I

$K_m$ AND $V$ VALUES OF THE AMINES, WITH AND WITHOUT ALBUMIN

Incubation mixtures, in volumes of 0.2 ml, each, contained 20 $\mu$moles of potassium phosphate, pH 7.5, or $K_3BO_3$, pH 9.1 (using serotonine, hydroxytyramine and noradrenaline), 0.16 $\mu$mole of [2-$^3H_3$]-acetyl-CoA (2500 dpm/nmole), 7.2 $\mu$g of enzyme and variable concentrations of the amines. In those tubes which contained albumin, this was present at one tenth of the molar amine concentration. After 15 min at 37 °C, the reaction was terminated and the products were determined as described under Methods.

| Variable substrate | Without albumin | | With albumin | |
|---|---|---|---|---|
| | $K_m$ (mM) | $V$ (nmole/mg per min) | $K_m$ (mM) | $V$ (nmoles/mg per min) |
| Hexylamine | 0.145 | 28 | 0.18 | 40 |
| Octylamine | 0.08 | 70 | 0.38 | 259 |
| Decylamine | 0.77 | 167 | 0.79 | 410 |
| Dodecylamine | 0.29 | 193 | 0.36 | 424 |
| Tetradecylamine | 0.07 | 57 | 0.30 | 286 |
| Hexadecylamine | 0.03 | 23 | 0.13 | 87 |
| Tryptamine | 0.53 | 269 | 0.50 | 260 |
| Serotonine | 0.42 | 380 | — | — |
| Hydroxytyramine | 0.72 | 145 | — | — |
| Noradrenaline | 0.53 | 157 | — | — |
| Histamine | 0.71 | 161 | — | — |

of the biphasic shape of the $v/S$ curves (Fig. 5) which precludes the use of the amines at concentrations greater than the inversion points of these curves.

The enzyme utilized two types of substrates, normal primary amines of six to sixteen carbon atoms and primary amines derived from aromatic amino acids. The following evidence suggested that one enzyme utilizes both types of substrates. (1) The ratio of activities using either tryptamine or a normal amine (i.e. octylamine or dodecylamine) was constant in all fractions eluted from columns of Sephadex (Fig. 1) or DEAE-cellulose. (2) The degree of loss of activity on DEAE- or phosphocellulose columns, or upon heating of the enzyme was the same using either type of substrate. (3) The $V$ using a mixture of tryptamine and octylamine did not exceed that of tryptamine alone [20]. (4) Inhibitors (i.e. $AgNO_3$, *p*-hydroxymercuribenzoate and coenzyme A) affected similarly both types of amines.

*Effect of inhibitors*

Table II shows the effects of several inhibitors on the acetyltransfer reaction using octylamine as substrate. Coenzyme A, a product of the reaction, was an inhibitor. Under the conditions employed in this paper, between 5 to 20 nmoles of coenzyme A were released (i.e. 25–100 $\mu$M). The maximal inhibition to be expected by this product was therefore about 30%. The second product, *N*-acetyloctylamine (as well as other N-acetylated amines), had no effect on the reaction rates. Addition of 2 mM $CaCl_2$ or 2–4 mM $MgCl_2$ did not affect the reaction rates. The enzyme did not require addition of metal ions, however, 5 mM of EDTA decreased the rate by about 70%; $MgCl_2$ (2.5 mM) partly reversed this inhibition. Butyryl-CoA and palmitoyl-CoA, as well as several SH-inhibitors inhibited the reaction. This suggested that an

TABLE II

EFFECT OF INHIBITORS

Incubation mixtures in volumes of 0.2 ml each, contained 0.3 μmole of octylamine, 0.1 μmole of [2-$^3H_3$]acetyl-CoA (2500 dpm/nmole), 20 μmoles of CAPS buffer, pH 9.9, 7.2 μg of enzyme and the inhibitors as specified in the table. After 20 min at 37 °C, the reaction was terminated and the products were determined as described under Methods.

| Inhibitor | Concentration (mM) | % of activity |
|---|---|---|
| Coenzyme A | 0.1 | 69 |
| Coenzyme A | 0.5 | 47 |
| Coenzyme A | 1.0 | 27 |
| *N*-acetyl octylamine | 1.0 | 100 |
| *N*-acetyl dodecylamine | 1.0 | 100 |
| Butyryl-CoA | 0.6 | 60 |
| Palmitoyl-CoA | 0.1 | 0 |
| EDTA | 5.0 | 36 |
| EDTA | 20.0 | 26 |
| EDTA (plus 2.5 mM $MgCl_2$) | 5.0 | 79 |
| *p*-Hydroxymercuribenzoate | 0.01 | 64 |
| *p*-Hydroxymercuribenzoate | 0.1 | 11 |
| *p*-Hydroxymercuribenzoate | 1.0 | 0 |
| $AgNO_3$ | 0.001 | 59 |
| $AgNO_3$ | 0.01 | 21 |
| $AgNO_3$ | 0.1 | 6 |
| DTNB | 1.0 | 25 |

SH-group is necessary for optimal activity of the enzyme. However, dithiothreitol or mercaptoethanol (1 mM each) did not affect the reaction and had no effect when added during enzyme purification.

*Mechanism of the reaction*

Other investigators, using *N*-acetyltransferases of rabbit [4] or pigeon [21] liver, showed that the mechanism is of the "simple, ping pong, Bi-Bi" type. Fig. 10 shows that the curves which described $1/v$ as a function of 1/tryptamine, at several concentrations of acetyl-CoA were parallel. When these values were replotted as $1/v$ as a function of 1/acetyl-CoA, at several concentrations of tryptamine, the curves were again parallel. This suggests that the yeast enzyme operates in a mechanism similar to that of the liver enzymes.

## DISCUSSION

The acetyltransferase from the soluble fraction of the yeast *H. ciferri* differs from the base:acetyl CoA acetyltransferase from the microsomes of the same yeast [8, 9] in several respects: (1) The soluble enzyme was present in all the 25 strains of the genus *Hansenula* which were examined, while the microsomal enzyme was found only in those strains of *H. ciferri*, NRRL Y-1031, which produced large quantities of acetylated phytosphingosine [22]. (2) The soluble enzyme utilized water-soluble amines but not sphingosine bases, the reverse was true for the microsomal enzyme. (3) Using long-chain normal amines as substrates, the optimal chain length was 12 with the soluble

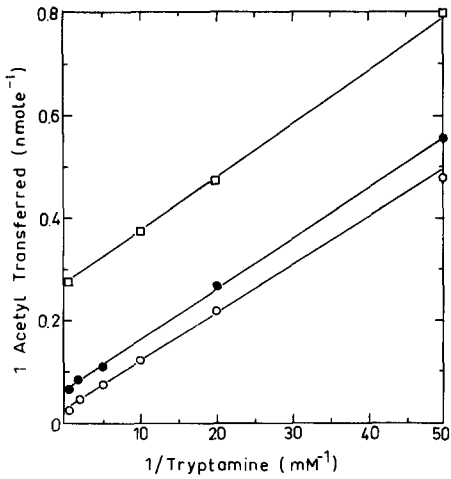


Fig. 10. $V^{-1}/S^{-1}$ curves at variable amine and several fixed concentrations of acetyl-CoA. Conditions were similar to those of Fig. 4, except that variable concentration of tryptamine and three fixed concentrations of acetyl-CoA were used. □—□, 0.02 mM [2-$^3H_3$]acetyl CoA; ●—●, 0.2 mM [2-$^3H_3$]acetyl CoA; ○—○, 2.0 mM [2-$^3H_3$]acetyl CoA.

enzyme and 16–18 with the microsomal preparation. (4) The soluble enzyme was inhibited by SH-inhibitors, while the microsomal preparation was not affected by these compounds. (5) The soluble enzyme operated in a "ping-pong, Bi-Bi" mechanism while the microsomal enzyme was of the "Sequential, Bi-Bi" type [9]. (6) The $v/S$ curves, obtained when acetyl-CoA was maintained at a constant level while the concentration of an amine having 12, 14 or 16 carbon atoms was varied, differed using the two enzymes. Using the soluble enzyme they were of Type III, according to the classification of Gatt et al. [10, 11] (Type III is characterized by a biphasic $v/S$ curve, where a hyperbolic portion is followed by a descending part). With the microsomal enzyme they were of Type IV (which is characterized by a nonsymmetrical sigmoid). This suggested that the microsomal enzyme utilized micellar aggregates of the amines [9], while the soluble enzyme utilized amine monomers and was inhibited by its micellar aggregates. The above establishes that the two yeast acetyltransferases are two separate enzymes.

According to the theoretical consideration [11], the inversion points in Type III curves should coincide with or be very close to the CMC of the substrate, this being the concentration where the inhibitory micelles start forming. A good correlation between the inversion points and the CMCs was found using dodecyl-, tetradecyl- and hexadecylamines. The normal amines of 6–10 carbon atoms, as well as other soluble amines (which do not form micellar aggregates) yielded hyperbolic $v/S$ curves throughout the entire range of substrate concentrations. This further supported the conclusion that the descending portions of the $v/S$ curves, obtained with long-chain amines, result from inhibition by substrate micelles.

Similar to its effect on other enzymes [23], albumin increased the reaction rates when a long-chain amine was used as substrate, but had no effect when hexylamine or the amines derived from the aromatic amino acids were substrates. It also affected the $v/S$ curves and the $K_m$ and $V$ values with $C_8$–$C_{16}$ normal amines, converting them to

rectangular hyperbolas. This is probably due to removal of inhibitory micelles by binding of the amine to the albumin.

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