COMPARISON OF RAT PINEAL GLAND AND RAT LIVER SEROTONIN-N-ACETYLTRANSFERASE

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SUMMARY

The kinetic and molecular weight characteristics of the non-inducible rat liver and the inducible rat pineal gland N-acetyltransferases were determined and compared. Both enzymes exhibited initial rate kinetics suggestive of a ping-pong mechanism implying the existence of an acetylated enzyme intermediate. The elution characteristics of the enzyme activities on Sephadex G-100 indicate an apparent molecular weight for the liver enzyme of 26,000 while the pineal gland N-acetyltransferase eluted at an apparent weight of 39,000. Molecular weight determinations performed in the presence of β-mercaptoethylamine suggest active subunit molecular weights of 10,000 - 12,000 daltons for the pineal gland and liver enzymes, respectively.

INTRODUCTION

The activity of the rat pineal gland enzyme N-acetyltransferase (EC 2.3.1.5) may be induced 20 - 100-fold by the administration of β-agonists to the animals (1). A similar enzyme activity found in rat liver is, however, not inducible by β stimulation (2). There is evidence that the inducible pineal gland enzyme is not the same as the non-inducible N-acetyltransferase found in other tissues. This is based on widely different pH optima (3,4) for enzyme activity, different relative substrate specificities (2,5,6) and differential inhibitor effects (4-6). There has been no real comparison between the pineal gland and non-pineal N-acetyltransferases with respect to kinetic or physical parameters. The enzyme of rabbit liver has been found to proceed by a ping-pong mechanism (7) and the human liver enzyme was found to have an apparent molecular weight of 26,500 (8). The present investigation determines and compares the kinetic properties and molecular weight characteristics of the inducible rat pineal gland and the non-inducible rat liver N-acetyltransferase.

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MATERIALS AND METHODS

Preparation of enzymes: Male Sprague-Dawley rats 175-200 gm (Zivic Miller, Allison Park, Pa.) were injected with 5 mg/kg l-150 proterenol (Sigma Chemical). The animals were sacrificed 3 hours later and the pineal glands and livers excised. The tissues were homogenized in four volumes of 50 mM potassium phosphate pH 6.5. In some experiments the homogenizing buffer also contained 4 mM β-mercaptoethylamine (Aldrich Chemical). The homogenates were centrifuged at 105,000 g for 30 minutes and the supernatants retained for analysis. The liver enzyme was further purified and concentrated by ammonium sulfate precipitation (2).

Determination of enzyme activity: The activity of N-acetyltransferase was determined by the method of Deguchi and Axelrod (3). The activity for both the liver and pineal gland enzymes was determined at pH 6.5.

Determination of molecular weight: Enzyme samples (50 - 100 µl) were applied to 0.6 x 25 cm Sephadex G - 100 columns equilibrated with 50 mM potassium phosphate pH 6.5 or with 50 mM potassium phosphate pH 6.5 -4 mM β-mercaptoethylamine. The columns were calibrated with bovine serum albumin (68,000), ovalbumin (45,000), pepsinogen (35,000), chymotrypsinogen (25,000) and cytochrome c (12,500). During column calibration and enzyme molecular weight determinations, fractions of 0.1 ml were collected. In experiments comparing enzyme elution in the presence and absence of β-mercaptoethylamine. Fractions of 0.3 ml were collected.

RESULTS

The rabbit liver enzyme was previously found to proceed with a ping-pong mechanism (7). Double reciprocal plots of the initial rates of inducible pineal gland N-acetyltransferase activity also display parallel lines (Figure 1A and 1B) indicative of a ping-pong mechanism. The rat liver enzyme was also found to have similar kinetic characteristics (data not shown). It is evident that the K_m value of the varied substrate will depend upon the concentration of the fixed substrate. To obtain a calculated K_m for each substrate in the presence of an infinite amount of the other substrate, secondary plots of the intercepts derived from the primary plots were used (9). The calculated K_m for the substrates tryptamine and acetyl coenzyme A for the non-inducible rat liver and the inducible rat pineal gland N-acetyltransferases are presented in Table I. It is evident that the calculated Michaelis constants for the two enzymes are significantly different. The K_m for the acetate acceptor, tryptamine, of pineal gland N-acetyltransferase (530 µM) is approximately twice that of the liver enzyme (240 µM) while the K_m of the acetate donor, acetyl coenzyme A, of the pineal gland enzyme (50 µM) is essentially half that found

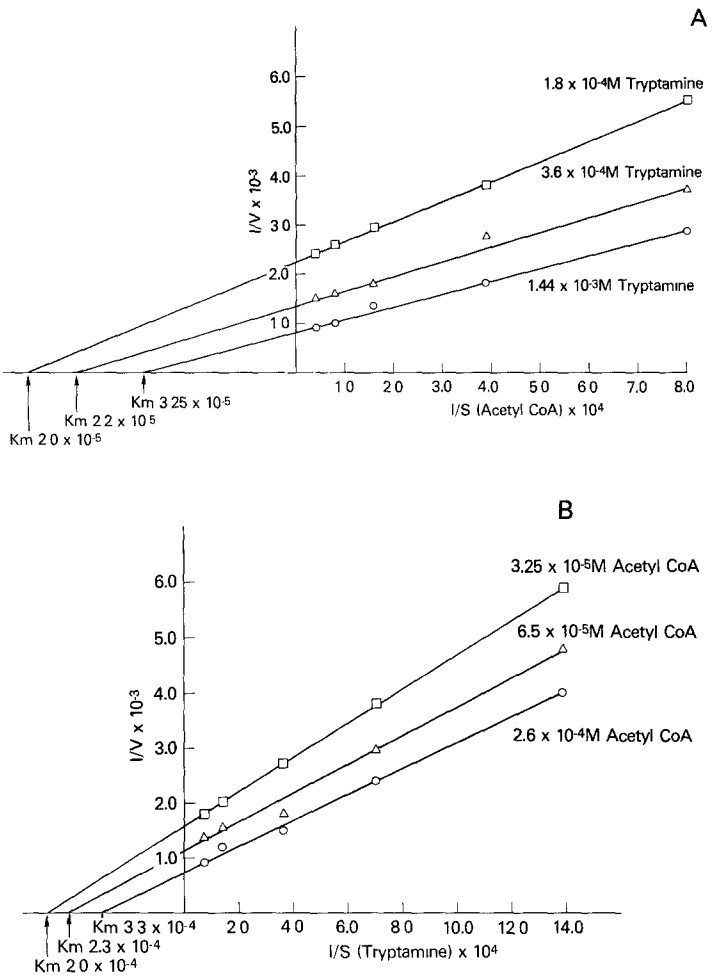

Figure 1. Double reciprocal plots of initial rates of inducible pineal gland N-acetyltransferase activity as a function of acetyl coenzyme A (A) or tryptamine (B) concentrations.

for the liver enzyme (125 μM). An earlier study (2) had found an apparent K_m of 600 μM for tryptamine and 30 μM for acetyl coenzyme A for the pineal gland enzyme using only one non-varied substrate concentration. These values are in agreement with the present investigation.

The apparent non-identity of the inducible pineal gland N-acetyltransferase and the non-inducible liver enzyme is further exemplified by the differences in molecular weight between the two activities (Table I and Figure 2A).

TABLE I

APPARENT MICHAELIS CONSTANTS' AND MOLECULAR WEIGHT OF N-ACETYLTRANSFERASES

ENZYME SOURCE	K_m[1] TRYPTAMINE	K_m[1] ACETYL COENZYME A	MOLECULAR WEIGHT
Liver	240 ± 30 μM	125 ± 20 μM	26,000 ± 1,000
Pineal	530 ± 40 μM	50 ± 10 μM	39,000 ± 3,000

[1] K_m values were determined by secondary plots of intercepts obtained from primary plots (9) of enzyme assays at pH 6.5.

All data represents the average of four determinations ± S.E.M.

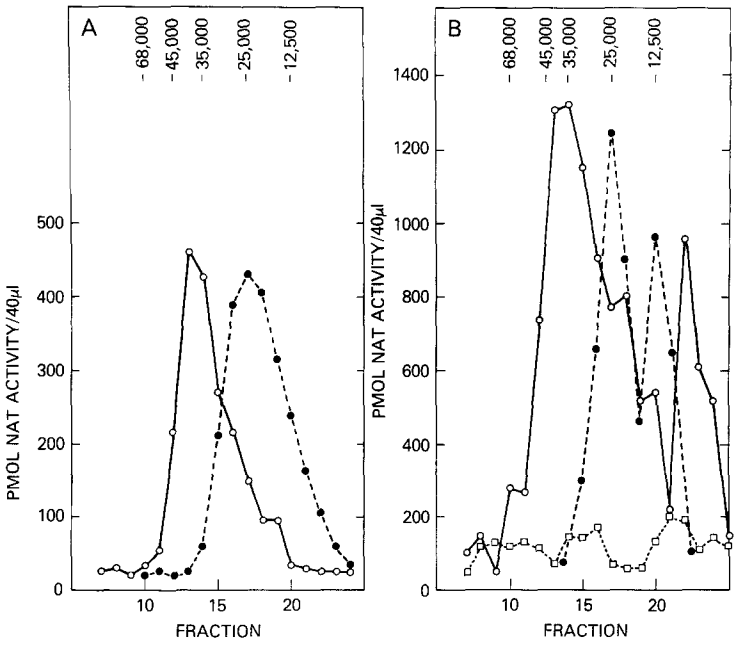

Figure 2. Sephadex G-100 chromatography of inducible pineal gland (O——O) or non-inducible liver (●——●) N-acetyltransferase activity in 50 mM potassium phosphate pH 6.5 (A) or 50 mM potassium phosphate pH 6.5 - 4 mM β-mercaptoethylamine (B). In both (A) and (B), material from 30 pineal glands were utilized. In (B) twice as much liver enzyme was applied as in (A). A homogenate obtained from 30 uninduced pineal gland (□ .... □) was also chromatographed (B).

The liver enzyme (26,000 daltons) is approximately two-thirds the size of the pineal enzyme (39,000 daltons). These values were obtained in phosphate buffer. The compound β-mercaptoethylamine was found to protect the activity of the inducible pineal gland enzyme which is normally very unstable (10). Elution characteristics of the liver or the pineal gland N-acetyltransferases were examined in the presence of 4 mM β-mercaptoethylamine (Figure 2B). It is seen that there is considerably more pineal gland N-acetyltransferase activity retained when the glands are homogenized and chromatographed in the presence of the β-mercaptoethylamine (cysteamine). In addition to the activity peaks at 39,000 for the pineal gland enzyme and 26,000 for the liver enzyme there are additional activity peaks at the 10,000 to 12,000 molecular weight location for each enzyme. The appearance of enzyme activity at a molecular weight of 10,000 to 12,000 may not be due to the activation of a low molecular weight form of the enzymes since cysteamine treatment of semipurified 26,000 dalton liver enzyme also yields a 12,000 dalton activity when rechromatographed. A homogenate of pineal glands from rats not injected with l-isoproterenol yielded no enzyme activity when prepared and chromatographed in the presence of cysteamine (Figure 2B). This suggests that the pineal gland N-acetyltransferase activity seen in the presence of β-mercaptoethylamine is solely due to that arising from isoproterenol induction and not due to the stabilization of an endogenous enzyme activity.

DISCUSSION

The serotonin-N-acetyltransferases, regardless of tissue source, seem to display ping-pong kinetics. This implicates the existence of an acetylated enzyme intermediate. This mechanism has been proposed for the pigeon liver (11), rabbit liver (7) and, in the present study, the non-inducible rat liver and inducible rat pineal gland N-acetyltransferase. It is also apparent that accurate Michaelis constants for a varied substrate cannot be obtained by using a single concentration of the non-varied substrate.

The molecular weight of the rat liver enzyme was found to be on the order

of 26,000 daltons. This is in agreement with the size of the human liver enzyme (8). The inducible pineal gland enzyme, however, is larger (39,000 daltons) than the liver N-acetyltransferase. Gel filtration in the presence of β-mercaptoethylamine ($HS-CH_2-CH_2-NH_2$), which mimicks the sulfhydryl end of the product coenzyme A, appears to partially dissociate the liver enzyme into 12,000 dalton subunits. The pineal gland enzyme is partially dissociated to 10,000 dalton subunits. This would tend to indicate that the rat liver N-acetyltransferase exists naturally as a dimer of 12,000 dalton subunits while the pineal gland enzyme is naturally a tetramer of 10,000 dalton subunits. The β-mercaptoethylamine is capable of partially dissociating both enzymes into active subunits. Whether this dissociation has physiologic significance is not known.

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