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## The mechanism of isoniazid acetylation by human *N*-acetyltransferase

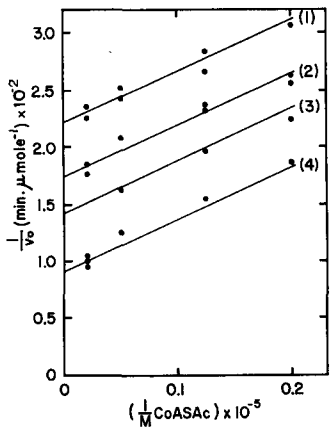
We have recently reported some kinetic properties of an *N*-acetyltransferase partially purified from rabbit liver[1]. A ping pong Bi-Bi mechanism was proposed for the acetylation of isonicotinic acid hydrazide (INH) from initial rate and product inhibition studies. This report describes the results of similar studies on human liver *N*-acetyltransferase from a rapid inactivator of INH.

The enzyme was isolated from liver obtained at autopsy about 2 h after death. Purification was carried out in 4 steps according to a procedure modified from that described for the purification of the enzyme obtained from rabbit liver[1]. The whole homogenate of liver (33% in cold 0.1 M potassium phosphate buffer, pH 7.2) was centrifuged at 10 000 × *g* for 20 min and the supernatant was centrifuged at 105 000 × *g* for 1 h. The 105 000 × *g* supernatant was treated with saturated ammonium sulfate solution adjusted to pH 7.2. The active fraction (40–50% saturation), concentrated by ultrafiltration to a final protein concentration of 140–170 mg/ml, was further fractionated by gel filtration on a Sephadex G-100 column (5.0 cm × 76.0 cm) using 0.005 M phosphate buffer, pH 7.2. The active fractions from this column were combined, treated with EDTA, tetrasodium salt (final concentration 0.001 M) and further fractionated on a DEAE-cellulose column (0.8 cm × 17.5 cm) at pH 7.2. Gradient elution from 0.005 M to 0.3 M was performed with KCl in phosphate buffer, 0.005 M containing EDTA, 0.001 M. The peak specific activity in the fractions from the DEAE-cellulose column was approx. 700-fold greater than that in the 40–50% saturation ammonium sulfate fraction. Enzyme activity was determined by measuring 2-acetyl-1-isonicotinyl hydrazide (acetyl-INH) production at 303 mμ in a 0.7-ml reaction mixture containing 0.1 ml of the enzyme preparation, 0.7 μmole of INH, 0.35 μmole of acetyl-CoA and 400 μmoles of borate, pH 9.0.

In studies of the kinetics of acetylation with the purified enzyme (DEAE-cellulose fractions) the rate of the reaction was determined by following the appearance of sulfhydryl groups from acetyl-CoA in an automatic recording spectrophotometer at 412 mμ and 27° by a procedure utilizing 5,5'-dithiobis-(2-dinitrobenzoic acid)[2]. These experiments were carried out in a 2.0-ml reaction mixture in 0.1 M borate buffer, pH 8.0. INH, acetyl-CoA and 0.12 μmole of 5,5'-dithiobis-(2-dinitrobenzoic acid) were added to each cuvette (1.0-cm light path) and the reaction was started by the addition of 0.1 ml of enzyme with an adder-mixer[3]. Initial velocities were determined by extrapolating the recorded curve to the time of addition of the enzyme and measuring the slope of the tangent to the curve at that point.

Reciprocal velocities were plotted against the reciprocal of substrate concentrations for each of the substrates, acetyl-CoA and INH. An occasional point which deviated markedly from a linear plot was discarded. The remaining data were fitted to Eqn. 1 in which A is acetyl-CoA and B is INH. $K_a$ and $K_b$ are the apparent Michaelis constants for acetyl-CoA and for INH defined as the concentration which gives half the maximum rate, $V$, in the presence of an infinite concentration of the other substrate. The calculations were performed by a digital computer utilizing the

Abbreviations: INH, isonicotinic acid hydrazide; acetyl-INH, 2-acetyl-1-isonicotiny hydrazide.



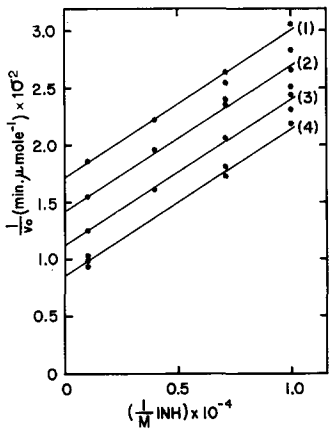



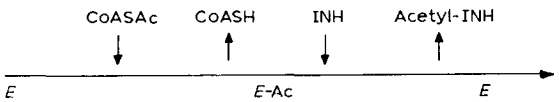

Fig. 1. Initial velocity pattern with acetyl-CoA as the variable substrate in borate buffer, 0.1 M, pH 8.0. INH concentrations: (1) 0.1 mM; (2) 0.14 mM; (3) 0.25 mM; and (4) 1.0 mM. The initial rate was calculated as μmoles of acetyl-INH formed per min per ml DEAE fraction.

Fig. 2. Initial velocity pattern with INH as the variable substrate in borate buffer, 0.1 M, pH 8.0. Acetyl-CoA concentrations: (1) 0.05 mM; (2) 0.08 mM; (3) 0.20 mM; and (4) 0.50 mM. The initial rate was calculated as μmoles of acetyl-INH formed per min per ml DEAE fraction.

Fortran "ping pong" program devised by CLELAND[4] which uses the least squares method and assumes equal variance for the velocities[5].

$$v_0 = \frac{VAB}{K_aB + K_bA + AB} \qquad (1)$$

The initial velocity pattern with acetyl-CoA as the variable substrate at 4 fixed concentrations of INH is shown in Fig. 1. Fig. 2 shows the pattern with INH as the variable substrate. These families of parallel lines indicate that human liver *N*-acetyltransferase may catalyze the acetylation of INH by a ping pong Bi-Bi mechanism[6]. The reaction may be written:




In this mechanism one would predict that the first product, CoA, would be a competitive inhibitor of INH acetylation with respect to INH and the second product, acetyl-INH, a competitive inhibitor with respect to acetyl-CoA[6]. The results of product inhibition studies were found to be consistent with these predictions.

Inhibition studies coupled with the initial velocity patterns indicate that INH is acetylated by human *N*-acetyltransferase according to a ping pong Bi-Bi mechanism similar to that found for the rabbit liver enzyme. However, additional product inhibition studies would be required to exclude an iso-ping pong mechanism for the human enzyme[1]. *N*-Acetyltransferase preparations purified from cynomolgus monkey and from rat (Sprague–Dawley) liver also catalyze acetylation by this mechanism (unpublished observations in our laboratory by Mr. D. J. HEARSE).

The kinetic constants in Eqn. 1 calculated for human liver *N*-acetyltransferase are given in Table I. The standard deviations shown indicate the precision of the experimental measurements with enzyme from a single individual and not the variation between individuals. Furthermore, the values of the constants cannot be compared with those reported for rabbit liver *N*-acetyltransferase[1] since the latter constants were determined by a different method under different experimental conditions.

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TABLE I

APPARENT MICHAELIS CONSTANTS FOR INH ACETYLATION AT 27° WITH DEAE-CELLULOSE FRACTIONS OF *N*-ACETYLTRANSFERASE FROM HUMAN LIVER

| | |
|---|---|
| $K_{CoASAc}$ | $5.3 \cdot 10^{-5} \pm 1.1 \cdot 10^{-5}$ M |
| $K_{INH}$ | $18.3 \cdot 10^{-5} \pm 0.4 \cdot 10^{-5}$ M |

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