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Acetylation of *S*-Substituted Cysteines by a Rat Liver and Kidney Microsomal *N*-Acetyltransferase

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1. An acetyl-CoA–*S*-substituted cysteine *N*-acetyltransferase in rat liver and kidney preparations was investigated, by using an assay involving incubations with *S*-benzyl-L-cysteine and [1-^{14}C]acetyl-CoA and extraction of the radioactive product with ethyl acetate. 2. The enzyme was associated with the microsomal fraction and could not be solubilized. Metal ions, EDTA and detergents did not significantly affect the enzyme activity. *p*-Chloromercuribenzoate and *N*-ethylmaleimide inhibited the enzyme. 3. Other *S*-substituted cysteines were acetylated at about the same rate as *S*-benzyl-L-cysteine. Acetylation of cysteine itself and of methionine, ethionine and tryptophan could be detected but was much slower. Acetylation of aspartic acid, glycine, phenylalanine and serine could not be detected. Palmitoyl-CoA was not a substrate. 4. The enzyme is presumably responsible for the acetylation step of mercapturic acid synthesis; a more physiological function is not yet known, except that the enzyme may be involved in acetylation of those amino acids which occur in elevated amounts in some disorders of amino acid metabolism.

Although many *N*-acetyltransferases have been described, no detailed study of the enzyme responsible for *N*-acetylation of *S*-substituted cysteines has previously appeared. The present paper reports the discovery and characterization of a liver and a kidney microsomal *S*-substituted cysteine *N*-acetyltransferase.

The excretion of *N*-acetyl-*S*-substituted cysteines (mercapturic acids) has been recognized for some time, but most of the reported work was carried out *in vivo* (Wood, 1970). Acetylation of *S*-substituted cysteines *in vitro* had previously been observed only in tissue-slice experiments (Elce, 1970). It was then shown that acetylation of *S*-(2-hydroxyoestradiol-1-yl)-L-cysteine was catalysed by the 8000*g* supernatant from rat liver and kidney only in the presence of acetyl-CoA (Elce, 1970).

For precise studies, the existing assays of mercapturic acid formation were inadequate, and a new assay was developed. It was found that the amount of radioactivity extracted from incubations containing [1-^{14}C]acetyl-CoA, *S*-benzyl-L-cysteine and the tissue sample was a sensitive measure of mercapturic acid formation, provided that [1-^{14}C]acetic acid was first removed by evaporation to dryness of the ethyl acetate extract.

With this assay method it was possible to locate and characterize the enzyme and to demonstrate that it did not correspond to any previously known *N*-acetyltransferase.

A preliminary account of some of this work has been published (Elce & Green, 1973).

Experimental

Materials

Adult male hooded rats (Charles River, Long Evans, outbred strain; 250–300g) were obtained from Canadian Breeding Farms and Laboratories, St. Constant, P.Q., Canada. [1-^{14}C]Acetyl-CoA (batch 2, 5.8μCi/μmol; batch 3, 3.3μCi/μmol; batch 4, 6.6μCi/μmol), [1-^{14}C]hexadecane (1.1μCi/g), assorted ^{14}C-labelled amino acids and Spectrafluor–PPO–POPOP [a commercial concentrated scintillator solution which contains 50g of 2,5-diphenyloxazole and 0.625g of 1,4-bis-(5-phenyloxazol-2-yl)benzene, in 500ml of toluene solution], were obtained from Amersham/Searle Corp., Don Mills, Ont., Canada. *N*-Acetyl-*S*-benzyl-L-cysteine, *S*-benzyl-L-cysteine and *S*-benzyl-D-cysteine were obtained from Cyclo Chemical, Los Angeles, Calif., U.S.A. All other materials were of commercial reagent grade.

Tissue preparation

After overnight starvation the animals were stunned by a blow to the head and killed by cervical dislocation. The tissue samples were homogenized in 4vol. of 0.25M-sucrose at 0°C and fractionated by the method of de Duve *et al.* (1955). The homogenate was diluted with an equal volume of 0.25M-sucrose

and filtered through four layers of cheesecloth. The filtrate was subjected to a succession of centrifugation steps at increasing speeds by using an SS-34 head in a Sorvall RC2 centrifuge for speeds up to 10000 rev./min and a 50Ti head in a Beckman model L ultracentrifuge for higher speeds. The subcellular fractions were defined as follows: the nuclear fraction sedimented on centrifugation for 10min at $1000g_{av.}$, the lysosomal fraction for 20min at $12500g_{av.}$ and the microsomal fraction for 90min at $106000g_{av.}$. The supernatant which then remained is referred to as the soluble fraction. For routine studies, fresh microsomal fractions were obtained daily from the post-mitochondrial supernatant in sucrose by the method of calcium precipitation (Kamath & Narayan, 1972). The final pellet of microsomal material was resuspended in 0.4M-Tris–HCl, pH7.4, containing 10% (w/v) glycerol, so that the concentration of protein was approx. 1mg/ml.

A polyribosomal fraction and rough and smooth microsomal membrane fractions were prepared by centrifugation through a discontinuous sucrose gradient, and their RNA/protein ratios assayed, by the method of Brown & Jellinck (1971).

Assay of S-substituted cysteine N-acetyltransferase activity

Assay of reverse reaction

The formation of [^{14}C]acetyl-CoA in incubation mixtures containing CoA, *N*-[1-^{14}C]acetyl-*S*-benzyl-L-cysteine and enzyme sample was determined from the formation of water-soluble radioactivity by using the procedure of ethyl acetate extraction described above.

Acetylation of other amino acids

The incubation mixtures contained acetyl-CoA, ^{14}C-labelled amino acid and enzyme sample in a final volume of 0.4ml of 0.1M-Tris–HCl, pH7.4. Reaction was terminated by addition of 2.6ml of 0.5% (v/v) acetic acid. The resulting 3ml was passed through a column of about 3ml of Dowex 50W (X2; H^+ form) equilibrated with 0.5% acetic acid, followed by washes of 7ml and 10ml of 0.5% acetic acid. With neutral and acidic amino acids, the radioactivity observed in the eluted 0.5% acetic acid, in excess of controls, is associated with the *N*-acetyl derivatives (Yip & Liew, 1973).

Acetyl-CoA was synthesized as described by Stadtman (1957) and assayed by means of the arylamine transacetylase (EC 2.3.1.5) reaction (Decker, 1965). *N*-[1-^{14}C]Acetyl-*S*-benzyl-L-cysteine (2.5μCi/μmol) was synthesized by the method of Smith & Beeman (1970). *S*-Benzyl-L-cysteine (10.56mg, 50μmol) was dissolved in 1.0ml of hot acetic acid and added to a vial containing [1-^{14}C]acetic anhydride (5μCi/μmol, 50μmol). The mixture was heated at 100°C for 45min and then evaporated to dryness. The residue was redissolved in 4.0ml of 0.5M-acetic acid and the product purified by column chromatography on DEAE-Sephadex A-25 in a gradient of 0.5–4M-acetic acid (Elce, 1970).

$$[1\text{-}^{14}C]\text{Acetyl-CoA} + S\text{-benzyl-L-cysteine} \rightarrow \text{CoA} + N\text{-}[1\text{-}^{14}C]\text{acetyl-}S\text{-benzyl-L-cysteine}$$

S-Benzyl-L-cysteine, dissolved in 0.05M-NaOH and titrated with HCl to pH7–8, and [1-^{14}C]acetyl-CoA, dissolved in water, were added to 15ml conical glass centrifuge tubes and the volume was made up to 0.3ml with water. The final concentrations of the substrates were in the range 5–100μM for most purposes, bearing in mind the observed K_m values of 7.9μM for acetyl-CoA and 37μM for *S*-benzyl-L-cysteine. The incubation tubes and a sample of the microsomal suspension were preincubated for 4min at 37°C with shaking in a water bath. Reaction was initiated by addition of 0.1ml of the microsomal suspension to the incubation tubes and terminated after a suitable time, usually 1 or 2min, by addition of 2ml of 0.5M-H_2SO_4 with rapid mixing, followed immediately by addition of 4ml of ethyl acetate. After brief centrifugation, the ethyl acetate layer was removed, washed with 1ml of 0.5M-H_2SO_4 and transferred to a scintillation vial. The aqueous incubation mixture was re-extracted with a further 4ml of ethyl acetate, which was washed as before and added to the first extract in the scintillation vial. The combined extracts were evaporated to dryness under N_2, leaving the N_2 flowing for 1h in order to evaporate traces of [1-^{14}C]acetic acid. Scintillation fluid (10ml) was added and the radioactivity determined.

Glucose 6-phosphatase activity was determined by the method of Harper (1965). Protein was determined by Campbell & Sargent's (1967) modification of the Lowry method, deoxycholate being omitted since it was not observed to have any effect.

Scintillation counting

Radioactivity was determined in 10ml of a scintillation fluid prepared by adding 800ml of ethanol and 1120ml of toluene to 80ml of Spectrafluor–PPO–POPOP, by using a Nuclear–Chicago Unilux II liquid-scintillation counter. The observed c.p.m. were converted into d.p.m. by means of calibration curves of counting efficiency of [^{14}C]hexadecane standards against a channels ratio derived from the machine external standard. The specific radioactivity of [1-^{14}C]acetyl-CoA used when required to convert

d.p.m. into nmol was checked by means of the arylamine transacetylase reaction (Decker, 1965).

Initial-rate studies

Data were plotted in the double-reciprocal form without statistical evaluation, to obtain preliminary estimates of some kinetic constants. For more precise kinetic studies the data were subjected to non-linear regression analysis on an IBM 360 computer (Cleland, 1967; Hurst *et al.*, 1973), which provided values and standard deviations of some kinetic constants.

Statistics

A single rat was killed daily to provide microsomal material as required. In general, the experiments were repeated at least once with different animals and within the set of adult male hooded rats the results were found to be highly consistent. Where relevant, the standard deviation of multiple assays is given.

Results

Validity of the enzyme assay

The radioactive material extracted by means of ethyl acetate from enzyme incubations containing [1-^{14}C]acetyl-CoA and *S*-benzyl-L-cysteine was shown to be *N*-[1-^{14}C]acetyl-*S*-benzyl-L-cysteine by comparison with authentic material on t.l.c. and paper chromatography and on paper electrophoresis. Some of the radioactive material was recrystallized with added carrier, yielding values for successive crystallizations (c.p.m./mg, ±S.D. of three samples) of 999±14, 1032±43, 1018±38. On ion-exchange column chromatography as described by Elce (1970), the ethyl acetate extract was found to contain, in addition to *N*-[1-^{14}C]acetyl-*S*-benzyl-L-cysteine, two slightly more polar radioactive compounds. Ethyl acetate extraction of nmol quantities of *N*-[1-^{14}C]-acetyl-*S*-benzyl-L-cysteine with and without added carrier showed that the more polar material was an artifact of the extraction procedure. It is probable that the formation of the sulphoxide and sulphone was involved, but this was not investigated. The recovery of added *N*-[1-^{14}C]acetyl-*S*-benzyl-L-cysteine from the standard incubation medium in two 4 ml ethyl acetate extractions was 91.9±1% over a range from 0.13 to 1.0 μg. The addition of 100 μg of carrier *N*-acetyl-*S*-benzyl-L-cysteine (but not of 20 or 40 μg) or two further extractions with ethyl acetate raised the recovery to about 95%. For routine purposes no carrier was added and only two ethyl acetate extractions were performed. No corrections have been made in the calculations for the approximately 92% recovery. At pH 7.4 less than 1% of the added [1-^{14}C]-acetyl-CoA was converted into free [1-^{14}C]acetic acid, which was extracted into the ethyl acetate, and of this [1-^{14}C]acetic acid less than 2% remained after evaporation of the ethyl acetate extract under N_2. When [1-^{14}C]acetyl-CoA of specific radioactivity 6.6 μCi/μmol and in concentrations from 10 to 100 μM was used, the control radioactivity obtained from incubations lacking *S*-benzyl-L-cysteine, lacking enzyme, or containing boiled enzyme, varied from 20 to 100 c.p.m.

Subcellular distribution of S-substituted cysteine N-acetyltransferase

According to the criterion of glucose 6-phosphatase distribution, from the cell-fractionation scheme of de Duve *et al.* (1955), the *N*-acetyltransferase was concentrated in the microsomal fraction (Table 1). The membranes of nuclei, mitochondria and lysosomes were not broken for these assays, but in a separate experiment mitochondria were lysed by sonication (assayed by the appearance of glutamate dehydrogenase activity) and no additional *N*-acetyltransferase activity was detected. For sonication, the suspension of mitochondria was treated for 1 min in a 9 kHz water-cooled Fisher Ultrasonic Generator with a power output of 200 W. The rough and smooth microsomal membrane fractions of liver exhibited

Table 1. *Subcellular distribution of S-substituted cysteine N-acetyltransferase activity in rat liver*

Assays for *N*-acetyltransferase activity contained 1 mM-*S*-benzyl-L-cysteine, 0.1 mM-[1-^{14}C]acetyl-CoA (0.56 μCi/μmol), 0.1–0.38 mg of protein in 0.25 M-sucrose and 80 mM-Tris–HCl, pH 7.4, in a final volume of 1.0 ml, and were carried out for 15 min at 37°C. Data are the mean of duplicate determinations.

	N-Acetyltransferase		Glucose 6-phosphatase		
Fraction	Relative specific activity	Percentage recovery	Relative specific activity	Percentage recovery	Protein Percentage recovery
Homogenate	1	100	1	100	100
Nuclei	0.57	10.4	0.79	17.1	21.7
Mitochondria	0.48	3.0	0.46	3.0	6.4
Lysosomes	1.74	8.6	2.46	13.6	5.6
Microsomal fraction	3.39	29.9	3.08	25.8	8.4
Soluble fraction	0	0	0	0	24.9
Totals		51.9		59.5	67.0

similar specific activities for *N*-acetylation of *S*-substituted cysteines, but the specific activity of the polyribosomal fraction was one-third to one-fifth as much.

Microsomal preparations obtained by calcium aggregation

In a series of three rats, it was found that the three parameters, mg of microsomal protein/g wet wt. of liver, glucose 6-phosphatase activity and *N*-acetyltransferase activity, did not differ significantly in microsomal material prepared from the same liver either by centrifugation for 90min at $100000g_{av.}$ or by calcium aggregation (Kamath & Narayan, 1972; Kamath & Rubin, 1972; Cinti *et al.*, 1972).

Stability of the enzyme

In 0.4M-Tris–HCl, pH7.4, containing 10% (w/v) glycerol, at a protein concentration of 1mg/ml the enzyme lost 60% of its activity in 24h at 0°C and 28% at −20°C. At a protein concentration of 5mg/ml the loss of activity was again 60% at 0°C in 24h, but at −20°C there was no significant loss.

Dependence of initial velocity on pH

The incubation mixtures contained 14.8μM-[1-^{14}C]acetyl-CoA (6.6μCi/μmol), 100μM-*S*-benzyl-L-cysteine, 2.5% (w/v) glycerol, 90μg of microsomal protein and either 0.1M-KH_2PO_4 adjusted with 2M-NaOH for the pH range 5–8 or 0.1M-Tris–HCl for the pH range 7–9. In phosphate buffer the greatest initial velocity was observed at pH6.5 and was very slightly but significantly higher than the optimum initial velocity in Tris buffer, which was at pH7.4.

Effects of metal ions and EDTA

Na^+, K^+ and Cl^- ions did not affect enzyme activity at concentrations up to 0.1M. At 0.5M these ions caused about 30% inhibition; 50mM-Na_2SO_4 caused 25% inhibition. $MgCl_2$ and $CaCl_2$ had no effect up to 10mM but inhibited the reaction by 35% at 50mM, and $MnCl_2$ almost abolished reaction at 50mM. Concentrations of EDTA from 0.1 to 50mM had no effect.

Effects of thiol reagents

In the standard assay 1mM-dithiothreitol and β-mercaptoethanol had no effect; 1mM-*p*-chloromercuribenzoate and 0.1mM-*N*-ethylmaleimide caused a 50% inhibition and 1.0mM-*N*-ethylmaleimide a 95% inhibition. An excess of dithiothreitol protected the enzyme from the effects of *N*-ethylmaleimide.

Effects of detergents and attempts to solubilize enzyme activity

Sodium deoxycholate had no effect in the range 0–0.5mM; sodium dodecyl sulphate inhibited the reaction by 50% at 0.1mM and abolished reaction at 0.5mM; Triton X-100 (average mol.wt. 646) showed a stimulation of about 10% at 0.5mM.

Attempts to solubilize the enzyme by using a number of different methods (Penefsky & Tzagoloff, 1971) were unsuccessful. Extraction with butanol and treatment with phospholipases C and A destroyed activity. Alternate freezing and thawing, digestion with ficin and with phospholipase D had no effect. An acetone-dried powder of the microsomal fraction still had activity, but this was diminished and could not be extracted. Some of the activity could be solubilized by deoxycholate, Triton X-100 and Brij 35, but at the concentrations used these detergents inhibited the enzyme activity. In addition, when the deoxycholate and Triton X-100 in these solubilized preparations were removed by passing through XAD-2 resin (Schechter & Bloch, 1971) and Sephadex G-25 (Penefsky & Tzagoloff, 1971) respectively, the activity could be resedimented. A preparation of enzyme activity solubilized in the presence of 0.05% Brij 35 was adjusted to 50% saturation with $(NH_4)_2SO_4$. A protein pellet floating on top of the solution was found to contain some enzyme activity. The pellet was redissolved, dialysed and applied to a DEAE-cellulose column (37cm×2.5cm) in a buffer mixture containing 50mM-Tris–HCl, pH7.4, 5mM-β-mercaptoethanol and 10% (w/v) glycerol. Stepwise elution was carried out by using 0.1, 0.2 and 0.4M-NaCl in the same buffer mixture; protein but no enzyme activity was eluted at each step. Enzyme activity could be eluted with buffer containing 0.2M-NaCl and 0.05% Brij 35, but the yield was low, the activity was unstable and the preparation had a lower specific activity than that of the original membrane-bound enzyme.

Substrate specificity

Table 2 shows the specific activities of liver and kidney microsomal enzymes for *N*-acetylation of *S*-substituted cysteines. The method of ethyl acetate extraction fails for amino acids which do not have a large non-polar side chain; the recovery of authentic *N*-acetyl-L-methionine in the standard ethyl acetate extraction was about 63%, of *N*-acetylglycine about

Table 2. *Substrate specificity for S-substituted cysteines*

Incubation mixtures contained 200μM-*S*-substituted cysteine, 15.94μM-[1-^{14}C]acetyl-CoA (6.6μCi/μmol), 0.1M-Tris–HCl, pH7.4, 2.5% (w/v) glycerol and 90μg of microsomal protein from rat liver or 150μg of microsomal protein from rat kidney in a final volume of 0.4ml. Data are the mean specific activities in nmol acetylated/min per mg of protein, ±S.D. for four determinations.

Substrate	Liver	Kidney
S-Benzyl-L-cysteine	14.5±0.9	33.6±1.3
S-Butyl-L-cysteine	12.8±0.1	27.5±0.8
S-Diphenylmethyl-L-cysteine	11.0±0.4	19.0±1.0
S-Benzyl-D-cysteine	2.5±0.1	4.0±0.1

6% and of *N*-acetyl-L-serine about 2%. By using ethyl acetate extraction, no acetylation of amino acids was detectable in 1 min, but in 30 min with 0.5 mg of microsomal protein evidence was obtained for *N*-acetylation (in units of nmol acetylated/h per mg of protein) of tryptophan (10.6), ethionine (10.8) and methionine (1.5). The acetylation of some neutral and acidic amino acids was therefore investigated on the basis of elution in 0.5% (v/v) acetic acid from Dowex 50W (X2) (Yip & Liew, 1973). Incubations were performed for 1 h with 0.5 mg of microsomal protein, 1 mM-^{14}C-labelled amino acid, 250 μM-acetyl-CoA and 0.1 M-Tris–HCl, pH 7.4, in a final volume of 0.4 ml. In control experiments, it was shown that the *N*-acetyl derivatives of glycine, methionine and serine were not hydrolysed during the incubation, and that 85–95% of the acetylamino acids were recovered in the 0.5% acetic acid eluate of the Dowex column. Acetylation of aspartic acid, glycine, phenylalanine and serine could not be detected. The rate of acetylation of methionine was about 4.4 nmol/h per mg of protein and of cysteine about 39 nmol/h per mg of protein.

Reaction between [^{14}C]palmitoyl-CoA and *S*-benzyl-L-cysteine could not be detected. The assay was complicated by the highly active thiolase present in the microsomal fraction (Lands & Hart, 1965), so that the radioactive material present in the ether used to extract the incubations was largely free palmitic acid, but no trace of *N*-[^{14}C]palmitoyl-*S*-benzyl-L-cysteine could be detected by t.l.c. in the presence of authentic material.

Time-course of the reaction

With a variety of substrate concentrations, either being in excess, the reaction proceeded until 85±2% of the limiting substrate had been consumed, taking 30–60 min. Longer reaction times or increased amounts of enzyme did not increase the total percentage of reaction.

Hydrolysis of N-[1-^{14}C]acetyl-S-benzyl-L-cysteine

By using an amount of tissue extract equivalent to 20 mg wet wt. of tissue and 17.1 μM-*N*-[1-^{14}C]acetyl-*S*-benzyl-L-cysteine, incubated at 37°C for 30 min, about 16% and 65% of the substrate was hydrolysed by enzymes present in the post-mitochondrial supernatant of liver and kidney respectively. No hydrolysis was detectable in the presence of calcium-aggregated microsomal material.

Reverse reaction

In quadruplicate tubes, CoA (0.13 mM) and *N*-[1-^{14}C]acetyl-*S*-benzyl-L-cysteine (2.5 μCi/μmol, 0.122 mM) were incubated with 80 μg of microsomal protein in a final volume of 0.4 ml of 0.1 M-Tris–HCl, pH 7.4, containing 2.5% (w/v) glycerol at 37°C for 1 min and 5 min. Control incubations were carried out in the absence of CoA, in the absence of enzyme sample and in the presence of a boiled enzyme sample. The radioactivity remaining in the aqueous layer after ethyl acetate extraction corresponded to about 2 nmol, but was identical in the complete system and in all the controls. It was concluded that in the routine incubation conditions used for study of the forward reaction, a reverse reaction was not detectable.

Kinetic studies

The initial velocity of *N*-acetylation was proportional to the amount of protein present in the incubation mixture up to at least 180 μg; routine incubations contained 70–100 μg of microsomal protein, corresponding to 4 mg wet wt. of liver. The percentage reaction in 1 min with respect to the substrate present in lower concentration varied from 2 to 25%; the linearity of the reaction for 1 min, especially for the conditions of high percentage reaction, was carefully checked.

Typical results of initial-rate studies in the absence of added products are shown in Fig. 1. Product-inhibition and other kinetic studies have also been performed, and confirmed the values of some kinetic constants obtained in the absence of added products, but this work is not described in this paper, since some details of the reaction mechanism are not yet clear. However, the values of kinetic constants obtained so far by non-linear regression of the available data are unlikely to be much affected by further clarification. These values are: K_m (acetyl-CoA) = 7.93±0.91 μM; K_m (*S*-benzyl-L-cysteine) = 36.87±3.25 μM; for 90 μg

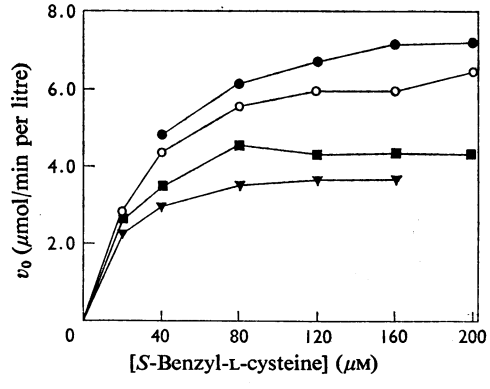

Fig. 1. *Initial velocity in the absence of added products*

The incubation mixtures contained [1-^{14}C]acetyl-CoA (3.3 μCi/μmol) at concentrations of 8.5 μM (▼), 17.0 μM (■), 34.1 μM (○) and 95.1 μM (●), *S*-benzyl-L-cysteine, 80 μg of microsomal protein, 0.1 M-Tris–HCl, pH 7.4, and 2.5% (w/v) glycerol in a final volume of 0.4 ml. Incubations were performed for 1 min. Each point represents a single observation.

of microsomal protein in 0.4ml, $V_{max.} = 6.51 \pm 0.23$ μmol of product formed/min per litre.

Discussion

The use of [1-^{14}C]acetyl-CoA permitted a more sensitive assay of mercapturic acid formation than previously available (Bray *et al.*, 1958; Barnsley *et al.*, 1969). The validity of the process of ethyl acetate extraction as an assay method was established by suitable controls. It appeared that the formation of [1-^{14}C]acetic acid occurred only slowly and that the [1-^{14}C]acetic acid was removed during evaporation of the extract. The formation of artifacts during extraction did not appear to affect the recoveries of radioactivity. A continuous assay is theoretically preferable, but 5,5′-dithiobis-(2-nitrobenzoic acid), used for example by Weber *et al.* (1968) to assay the release of CoA, was found to inhibit the microsomal *N*-acetyltransferase.

The microsomal location of the *S*-substituted cysteine *N*-acetyltransferase makes it clear that it differs from the soluble *N*-acetyltransferases which catalyse acetylation of many endogenous and foreign amines (Weber & Cohen, 1967; Schloot *et al.*, 1969; Hearse & Weber, 1973), and distinguish it also from the soluble glucosamine 6-phosphate *N*-acetyltransferase (Davidson *et al.*, 1957). The acetylation of putrescine by a liver microsomal preparation has been reported (Seiler & Al-Therib, 1974). In the field of naturally occurring amino acids, an aspartate *N*-acetyltransferase has been described in rat brain microsomal fractions (Goldstein, 1969) and a glutamate *N*-acetyltransferase in rat and mouse liver mitochondria (Shigesada & Tatibana, 1971). *N*-Acetylaspartate and *N*-acetylglutamate occur in vertebrate brain (Auditore & Wade, 1971, 1972), and *N*-acetylaspartate occurs in the urine of alloxan-treated rats and pancreatectomized dogs (Berlinguet & Laliberté, 1970). The *N*-acetyl derivatives of methionine sulphoxide and sulphone were excreted by rats, but it was postulated that the acetylation occurred at the level of the sulphoxide (Smith, 1972). Kodama *et al.* (1974) detected *N*-acetylcysteine in roughly equal amounts in the urine of normal and of cystinuric patients. Several other α-*N*-acetylamino acids have been isolated only from the urine of patients with disorders of amino acid metabolism, and Strandholm *et al.* (1971) suggested that acetylation of many amino acids occurred when there was a sufficient elevation of their plasma and tissue concentrations. The rates reported in the present work for acetylation of free amino acids by the microsomal enzyme are very low compared with the rate of acetylation of *S*-benzyl-L-cysteine, but are of the same order of magnitude as those reported elsewhere for the free amino acids [aspartate, 55nmol/h per mg of protein (Goldstein, 1969); glutamate, 12nmol/h per mg of protein (Shigesada & Tatibana, 1971); in the present work, cysteine, 39nmol/h per mg of protein; aspartate, zero; and *S*-benzyl-L-cysteine, 871nmol/h per mg of protein].

It is therefore possible that this microsomal *S*-substituted cysteine *N*-acetyltransferase might be the enzyme responsible for the acetylation of amino acids observed in patients with disorders of amino acid metabolism.

On the basis of chain-length specificity it is unlikely that the *N*-acetyltransferase could be confused with the *N*- and *O*-acyltransferases also present in the microsomal fraction (Lands & Hart, 1965; Sribney, 1966). In one experiment, it was shown that [1-^{14}C]-palmitoyl-CoA was not a substrate for the *S*-substituted cysteine *N*-acetyltransferase, although the rapid hydrolysis of the thioester made the assay insensitive.

Investigation of the enzyme in terms of more specific protein chemistry was impossible, since no effective means of solubilization could be found. The destructive effects of phospholipases A and C, but not of D, suggest a specific lipid dependency, now known for a number of membrane-bound enzymes (Coleman, 1973). The slight solubilization achieved with deoxycholate or Triton X-100 was reversed on removal of the detergent, suggesting that true solvation had not occurred, but only a dispersion of the microsomal material into macromolecular particles (Holtzman *et al.*, 1973).

On incubation with comparatively large amounts of tissue extract from the soluble portion of the cell, *N*-acetyl-*S*-benzyl-L-cysteine was partly hydrolysed. If the microsomal *N*-acetyltransferase described here is indeed responsible for the *N*-acetylated amino acids and substituted cysteines observed in the urine, they must be protected *in vivo* from cytoplasmic hydrolysis. This would imply that the *N*-acetylated products pass through the membrane at a time very close to that of their formation.

Although most of the work reported here was carried out with liver preparations, the *S*-substituted cysteine *N*-acetyltransferase had a higher specific activity in rat kidney microsomal preparations. Since the most obvious physiological function of the enzyme is in the catalysis of the last step of mercapturic acid biosynthesis, it is noteworthy that two other enzymes of that pathway are largely confined to kidney microsomal fraction, namely γ-glutamyltranspeptidase (Suga *et al.*, 1966; Meister, 1973), and cysteinylglycinase (cysteinylglycine dipeptidase; EC 3.4.13.6) (Semenza, 1957). With very few exceptions the mercapturic acid pathway is restricted to the terminal metabolism and excretion of foreign compounds, including many drugs (Wood, 1970).

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