

SHORT COMMUNICATIONS

Reaction with electrophiles after enzyme-catalysed deacetylation of *N*-acetylcysteine

(Received 4 August 1973; accepted 15 September 1973)

It is now accepted that biosynthesis of mercapturic acids, which are derivatives of *N*-acetylcysteine, proceeds through initial conjugation of the precursor electrophile with the endogenous nucleophile, GSH.¹⁻⁷ This conjugation is usually enzyme-catalysed.^{6,8} The present paper describes the effect of hepatic cytosol on the reaction of *N*-acetylcysteine with electrophilic agents.

Experimental

Dialysed tissue supernatants were prepared as described by Boyland and Chasseaud.⁹ Equimolar amounts of *N*-acetylcysteine and the electrophilic agent were incubated in 0.2 M-orthophosphate buffer, pH 6.5 (total vol 4 ml), in the presence or absence of dialysed rat liver supernatant at 25°. Control incubations were also carried out using boiled dialysed rat liver supernatants. Parallel incubations were carried out using GSH or cysteine as the thiol in the presence or absence of dialysed rat liver supernatant. After incubation for 5 min, an equal volume of aq. 4% (w/v) sulphosalicylic acid was added to stop the reaction and to precipitate protein by lowering the pH to approx 2. Protein was separated by centrifugation and unreacted thiol estimated by the 5,5'-dithiobis-(2-nitrobenzoic acid) method.¹⁰ At equivalent concentrations in the range studied, standard curves for GSH and *N*-acetylcysteine were almost identical, whereas that for cysteine was about 10 per cent lower. Results in Table 1 are based on the standard curve for *N*-acetylcysteine, although cysteine was present because of deacetylation.

Deacetylase activity was indirectly assayed by preincubating *N*-acetylcysteine with dialysed liver supernatants for 30 min at pH 6.5 followed by reaction with diethyl fumarate for 5 min. Unreacted thiol was measured as before.

S-Acetylglutathione (2 mM) was incubated with dialysed rat liver supernatant in 0.2 M-orthophosphate buffer, pH 6.5, for 30 min at 25°. The free thiol formed (GSH) was measured as before.

All reaction mixtures were examined by chromatography by downward development (18 hr) on Whatman No. 1 paper with butan-1-ol:acetic acid:water (2:1:1, v/v). The dried chromatograms were treated either with ninhydrin (in acetone) or with the platonic iodide reagent.¹¹

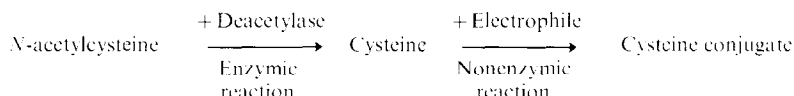
Results and discussion

The electrophilic agents chosen were those which undergo enzyme-catalysed conjugation with GSH,^{4,5,9,12-14} and the amount of dialysed rat liver supernatant used was sufficient to cause less than

TABLE 1. LOSS OF THIOL AFTER REACTION OF *N*-ACETYL-CYSTEINE WITH VARIOUS ELECTROPHILIC AGENTS IN THE PRESENCE OR ABSENCE OF DIALYSED RAT LIVER SUPERNATANT

| Electrophile | Nonenzymic reaction Loss of thiol (%) | Enzymic reaction Loss of thiol (%) |
|---|--|---------------------------------------|
| 2,3-Dimethyl-4-(2-methylenebutyl)-phenoxyacetic acid (2 mM) | 4 | 22 |
| Methyl vinyl sulphone (3 mM) | 4 | 20 |
| Benzylideneacetone (2 mM) | 1 | 19 |
| Diethyl fumarate (5 mM) | 9 | 18 |
| Methyl iodide (3 mM) | 2 | 17 |
| Benzyl chloride (3 mM) | 3 | 15 |
| 2,3-Epoxypropyl-phenyl ether (3 mM) | 2 | 15 |
| Diethyl malate (5 mM) | 1 | 7 |

20 per cent of the GSH to react. When these electrophiles were incubated with *N*-acetylcysteine, the reaction rate was increased in the presence of dialysed rat liver supernatant (Table 1). This result suggests that reaction of *N*-acetylcysteine with these electrophiles may be enzyme-catalysed. However, paper chromatography of reaction mixtures showed that the reaction product was chromatographically identical to the corresponding cysteine conjugate, not the *N*-acetylcysteine conjugate. The following sequence of reactions probably occurred:



Parallel experiments with cysteine instead of *N*-acetylcysteine supported this reaction sequence. The rate of reaction of cysteine with these agents was similar in the presence or absence of dialysed rat liver supernatant, in agreement with other work.^{4,5,12}

Since diethyl fumarate reacts readily with cysteine, an indirect measure of deacetylase activity in dialysed liver supernatants prepared from different species, was obtained by preincubating *N*-acetylcysteine with the supernatant for 30 min, adding diethyl fumarate, reacting for 5 min and measuring thiol loss. Deacetylase activity in different species was thus ranked as follows: Guinea pig (✓) > rabbit (✓) > rat (✓) > pigeon (✓) > ferret (✓) > mouse (✓) > dog (—).

Deacetylase activity in dialysed rat kidney supernatant was comparable to that in liver preparations.

S-Acetylglutathione was almost completely converted to GSH when incubated with dialysed rat liver supernatant for up to 30 min. This result shows that dialysed rat liver supernatant contains an enzyme(s) that deacetylates compounds containing *S*-acetyl as well as *N*-acetyl groups.

The work of Sheffner *et al.*¹³ has shown that *N*-acetylcysteine was converted to cysteine after incubation with a rat liver homogenate. The present studies indicate that the deacetylase(s) was present in hepatic cytosol and did not require diffusible cofactors, as dialysed preparations were active.

Of the three thiols, only GSH was enzymically conjugated to the electrophiles examined. Chemically, the reactivity of these thiols was generally in the order, cysteine > GSH > *N*-acetylcysteine.

Acknowledgements Part of this work was completed at the Chester Beatty Research Institute, during which period I was grateful to the Medical Research Council for a grant and to Professor E. Boyland for his interest. This investigation was supported by grants from the Medical Research Council and Cancer Research Campaign.

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