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GLUTATHIONE CONJUGATION OF ARYLNITROSO COMPOUND: DETECTION AND MONITORING LABILE INTERMEDIATES IN SITU INSIDE A FAST ATOM BOMBARDMENT MASS SPECTROMETER[1]

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Summary: The glutathione (GSH) conjugation reaction of the active metabolite of a potent protein-pyrolysate carcinogen, 2-nitroso-6-methyldipyrido[1,2-a:3',2'-d]imidazole (NO-Glu-P-1), occurred in glycerol matrix inside a fast atom bombardment (FAB) mass spectrometer. The short lived GSH-conjugates were detected by in situ FAB analysis. The precursor-product relationship between the conjugates was indicated by following the reaction by measuring $[M+H]^+$ ions of the conjugates.

The activation and inactivation of carcinogenic nitrogen containing compounds by endogenous biological materials are an important problem for chemical carcinogenesis. The rapid conjugation reaction of carcinogenic-mutagenic arylnitroso compounds with GSH[2] yields the corresponding N-hydroxyarylamines, arylamines and short-lived intermediary GSH-conjugates (1,2). This conjugation reaction is regarded as both an activation and inactivation process (3-5). Although the complex mechanism of the reaction is proposed using several nitrosoarenes such as nitrosobenzene (1,2, 6,7), there has been little direct experimental evidence that clarifies the mechanism. The reason for this is that the postulated GSH-conjugates are very labile and that isolation and full characterization of the conjugates are difficult (3,6-9).

[1] Mass Spectrometry of Biological Labile Compounds. II.
[2] Abbreviations: GSH, reduced glutathione: NO-Glu-P-1, 2-nitroso-6-methyldipyrido[1,2-a:3',2'-d]imidazole: FAB, fast atom bombardment.

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The recently developed FAB mass spectrometry has been shown to be well-suited for non-volatile and thermally labile molecules (10,11). In this technique, samples are dissolved in a polar matrix, such as glycerol, on the target. Therefore, if a reaction may occur in glycerol as well as in the aqueous solution, reaction products can be analyzed by FAB technique *in situ* without isolation and purification (12). Especially, very labile products which prevent any purification and characterization may be analyzed easily *in situ* by this technique.

We applied FAB mass spectrometry to monitor the GSH conjugation reaction occurring inside the spectrometer. We report herein the detection of the short-lived intermediary GSH-conjugates of the active metabolite of a potent protein-pyrolysate carcinogen, NO-Glu-P-1, formed in FAB matrix and evidence for the precursor-product relationship of the two conjugates by monitoring their $[M+H]^+$ ions *in situ*.

MATERIALS AND METHODS

Chemicals --- NO-Glu-P-1 was synthesized by sequential oxidation and reduction from the parent amine as described previously (13). GSH was purchased from Sigma Chemical Co., St. Louis, MO.

Following GSH conjugation by in situ FAB mass spectrometry --- One µl of 10 mM GSH in water and 0.5 µl of 20 mM NO-Glu-P-1 in DMSO were mixed with 2 µl of glycerol as the matrix on the stainless steel target of the FAB probe. The probe was immediately inserted into the ion source of the mass spectrometer. FAB mass spectra were obtained using a JEOL JMS DX-300 instrument equipped with a JMA 3500 data-analysis system and FAB ion-gun. A neutral xenon beam was used at 6 KeV energy, and the accelerating potential of the ion was 3 KV. Magnetic field scanning from m/z 100 to 1000 was repeated at 12 sec intervals.

RESULTS AND DISCUSSION

We have already reported that the large excess amount of GSH compared to nitroso compounds gave almost quantitative amounts of the corresponding N-hydroxyarylamines and only trace amounts of arylamines and GSH-conjugates (3). The equimolar or less amount of GSH compared to NO-Glu-P-1 gave the substantial amounts of the

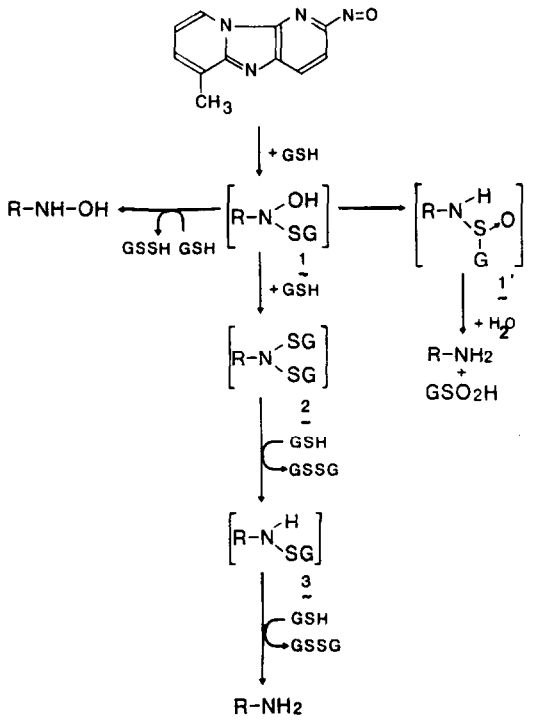

Fig. 1 The proposed mechanism of glutathione conjugation with arylnitroso compounds.

GSH-conjugates. The proposed mechanism of the reaction was shown in Fig. 1. FAB mass spectrum of the intermediary GSH-conjugates was obtained by measuring the reaction mixture which consisted of equimolar amounts of GSH and NO-Glu-P-1 (Fig. 2). The protonated molecular ions $[M+H]^+$ of **1** or **1'** and **3** in Fig. 1 were detected at m/z 520 and 504, respectively. The ions at m/z 558 and 542 could be accounted for by potassium containing species of these molecules. The quasi-molecular ions of oxidized glutathione (GSSG) were also detected at m/z 613 $[M+H]^+$, 635 $[M+Na]^+$, and 651 $[M+K]^+$. Since these seven ions could be detected neither in the spectrum of only NO-Glu-P-1 nor in that of only GSH, it is clear that they were derived from the products of the conjugation reaction. Some transient intermediates of the reaction of GSH with arylnitroso compounds such as 3-nitroso-1-methyl-5H-pyrido[4,3-b]indole (3,-14), 2-nitrosofluorene (15), nitroso derivative of chloramphenicol

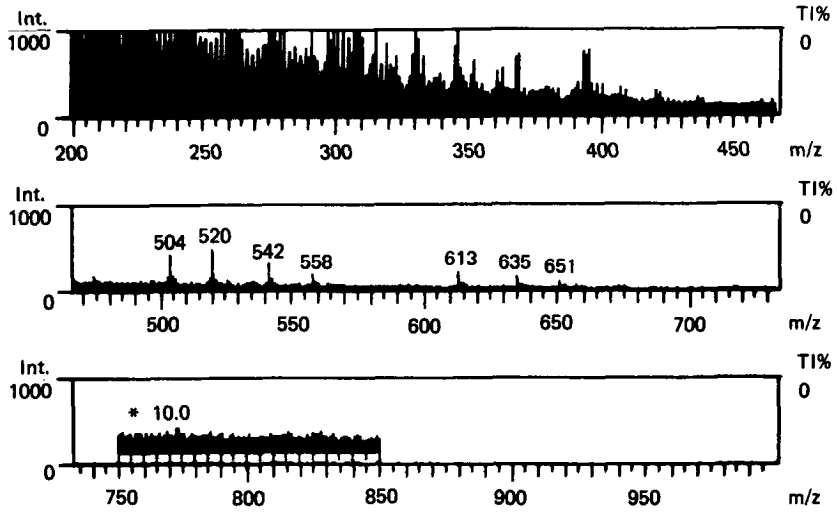

Fig. 2 FAB mass spectrum of the reaction mixture containing GSH (10 nmole) and NO-Glu-P-1 (10 nmole) in water-DMSO-glycerol matrix. The spectra were obtained using a JEOL JMS DX-300 instrument equipped with a JMA 3500 data-analysis system and FAB ion-gun. A neutral Xe beam was used at 6 KeV energy, and accelerating potential of the ion was 3KV.

(8), trans-4-nitrosostilbene (7), 4-nitrosobiphenyl (7) and nitrosobenzene (6) were reported. These intermediates may be the same conjugates such as **1**, **1'** and **3**, for the only well-characterized products were their 2-nitrosofluorene derivatives (15).

The intense peaks at m/z 504 and 520 were monitored by magnetic field scanning. The ratio of the ion intensity of m/z 504 to 520 was increased linearly to the reaction time (Fig. 3).

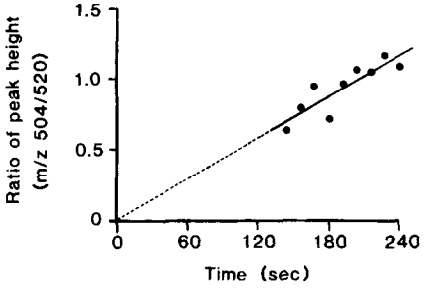

Fig. 3 Time course of the peak height ratio of m/z 504 to 520 of the reaction mixture containing GSH and NO-Glu-P-1. Magnetic field scanning was repeated at 12 sec intervals from 140 sec after starting the conjugation reaction on the FAB target.

This result showed the precursor-product relationship between 1 or 1' and 3. The ions corresponding to 2 were not detected throughout. Two explanations could possibly account for the absence of these expected ions: (1) this conjugate might exist for too short a time to be detected, (2) the direct reaction from 1' to 3 might occur, though this route has not been postulated previously.

This study indicated the existence of the intermediary GSH-conjugates and the reaction sequence of the conjugates. Thus, FAB mass spectrometry is applicable not only to the study of static biochemistry such as identification of postulated labile intermediates but also to that of dynamic processes of biochemical reactions.

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