Biochemical Pharmacology, Vol.31, No.11, pp.2137-2140, 1982.
Printed in Great Britain.

0006-2952/82/112137-04$03.00/0

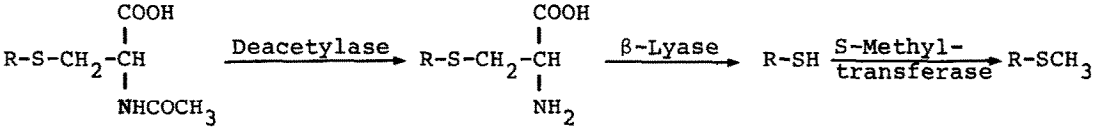
A C-S BOND CLEAVAGE ENZYME OF CYSTEINE CONJUGATES IN INTESTINAL MICROORGANISMS

S. Suzuki, H. Tomisawa, S. Ichihara, H. Fukazawa and M. Tateishi

Department of Biochemistry, Nippon Roche Research Center
200 Kajiwara, Kamakura, Japan

(Received 3 March 1982; accepted 31 March 1982)

During the past few years, the in vivo formation of Methylthio (SCH_3)-containing metabolites has been reported for numerous compounds, including drugs, herbicides, aromatic hydrocarbons and environmental contaminants (1-4). The pathway we have proposed for the formation of the methylthio-containing metabolites of xenobiotics in mammalian tissues is the following (5,6):



Cysteine conjugate β-lyase (E.C.4.4.1.13) in the liver was suggested by us to play an important role in the formation of thiols.

On the other hand, several microorganisms such as Pseudomonas ovalis have been reported to possess C-S lyase activity catalyzing α,γ- and α,β-elimination reactions of L-methionine, several derivatives of L-methionine and L-cysteine, e.g. L-ethionine, DL-methioninesulfone, L-homocysteine and S-methyl-L-cysteine (7-9). Recently, Bakke et al. (10) speculated that a C-S lyase in gastrointestinal microorganisms cleaved the C-S linkage of cysteine conjugates of xenobiotics.

During the course of a study on the mechanism of formation of methylthio-containing metabolites in the body of whole animals, we obtained evidence for the presence of C-S lyase(s) in microorganisms found in large intestinal contents of rats. The enzyme possessed high activity towards the cysteine conjugate of bromobenzene to produce the corresponding thiophenol but no activity towards the N-acetyl-L-cysteine or glutathione conjugates of bromobenzene.

MATERIALS AND METHODS

Chemicals: S-p-(Bromophenyl)-L-cysteine, N-acetyl-S-(p-bromophenyl)-L-cysteine and S-p-bromophenylglutathione were synthesized by the method described by Saunders (11). p-Bromothiophenol and p-fluorothiophenol were obtained from Aldrich. Pyridoxal phosphate and dithiothreitol were purchased from Wako (Tokyo). Bacitracin, neomycin and tetracycline were purchased from Sigma Chemical Co. All the other reagents used in the present study were of reagent grade.

Animals: Sprague-Dawley rats (male, 8 weeks old, 240-260g) were purchased from Shizuoka Experimental Animal Co-op (Shizuoka). The animals were killed by decapitation and the contents of stomach and small and large intestines were separately washed out with about three volumes of 10 mM potassium phosphate buffer, pH 7.4, containing 0.5 mM dithiothreitol and homogenized with a glass homogenizer. The intestinal mucosa was homogenized in 2 volumes of the buffer.

Antibiotic treatment: Rats were treated twice daily with 400 mg/kg of neomycin and bacitracin and 200 mg/kg of tetracycline in aqueous suspension by oral intubation. Control animals were given an equal volume (1.2-1.3 ml) of 0.9% NaCl solution.

Quantitative analysis of thiophenol formation: For the assay of C-S cleavage activity, S-(p-bromophenyl)-L-cysteine, N-acetyl-S-(p-bromophenyl)-L-cysteine or S-p-bromophenylglutathione was used as the substrate, and the formed thiophenol was quantitated with a high pressure liquid chromatograph. The reaction mixture contained in a final volume of 0.40 ml: substrate, 0.2 μmol; dithiothreitol, 2 μmol; pyridoxal phosphate, 0.02 μmol, potassium phosphate buffer, pH 7.4, 20 μmol and gutcontents or homogenate of intestinal mucosa, 0.02 ml. The reaction mixture was incubated at 37° for 20 min under anaerobic conditions. The incubation was terminated by addition of 0.4 ml of acetonitrile containing p-fluorothiophenol as an internal standard (60 μg) and the mixture was cooled to 0°. After centrifugation at 500 x g for 10 min, 5-50 μl of the supernatent was injected into a high pressure liquid chromatograph (Waters, Model 6000A) equipped with a μBondapak C_{18} column (3.0 mm x 30 cm). Detection was performed at 254 nm with a variable-wavelength-u.v. detector (Shimadzu, Model SPD-2A). p-Bromothiophenol and p-fluorothiophenol were eluted with methanol/H_2O/acetic acid (65:35:1, v/v) at a flow rate 1.5 ml/min with retention times of 5.8 and 3.8 min, respectively. Around positions at these retention times, no interference was observed in a control sample (from which the substrate and internal standard were absent). The calibration curve obtained by plotting the ratios of peak height of p-bromothiophenol over that of p-fluorothiophenol against varying concentrations of p-bromothiophenol was linear over the range 0.03 μg to 3.3 μg. Under the stated conditions the lower detection limit was 0.5 μg per 0.4 ml of the incubation mixture.

GC-mass analysis of p-bromothioanisole: The mass spectra were obtained with JEOL DX-300 high resolution mass spectrometer coupled with a Hewlett Packard M5710A gas chromatograph. The gas chromatograph was equipped with a 5 mm x 1 m glass column packed with 1% OV-1 (100-120 mesh). The oven temperature was programmed to rise to 180° at a rate of 30°/min with initial setting at 100°. A temperature of injection port and chamber heater was kept at 210° and 200°, respectively. Helium was used as the carrier gas at a flow-rate of 40 ml/min. The ionizing energy was 20 eV. Under these conditions, p-bromothioanisole appeared at 5 min.

RESULTS AND DISCUSSION

Identification of p-bromothiophenol: S-(p-Bromophenyl)-L-cysteine, 2 μmol was incubated with the homogenate of the large intestinal contents

under the incubation conditions described in MATERIALS AND METHODS except that the volume of the mixture was 5 times as large. p-Bromothiophenol was extracted from the incubation mixture with 4 ml of ether and then derivatized to p-bromothioanisole with diazomethane. After condensation, the solution was injected into the GC-mass spectrometer.

The mass spectrum of the methylated derivative of the thiophenol was identical with that of the authentic sample: molecular peak at m/e 202 with its isotope peak at m/e 204 (base peak), m/e 187 ($M^+ - CH_3$, relative intensity 20%, isotope peak; 189), m/e 169 ($M^+ - SH$, 7%, isotope peak; m/e 171), m/e 156 ($M^+ - SCH_2$, 7%, isotope peak; m/e 158), m/e 123 ($M^+ - Br$, 10%) and m/e 108 ($M^+ - Br - CH_3$, 8%).

From an incubation mixture without the homogenate of the intestinal contents the thioanisole could not be detected.

C-S cleavage enzyme activity in the gastrointestinal contents: High C-S bond cleavage-activity was found only in the large intestinal contents while virtually no activity in the contents of stomach and small intestine as well as in the intestinal mucosa. S-(p-Bromophenyl)-L-cysteine was converted to 4.1 ± 0.5 μmol of thiophenol (n=5) in the whole large intestinal contents per min under the present incubation condition. The activity was lost by heat treatment of the contents (100° for 10 min), indicating the presence of an enzyme(s) responsible for C-S cleavage of cysteine conjugates in the contents.

Pretreatment of rats with neomycin, bacitracin and tetracycline for 2 days resulted in the loss of 90% or more of the whole activity in the large intestinal contents. This result, together with the finding that the intestinal mucosa showed no C-S cleavage activity, indicated microorganisms populating in the large intestinal tract possessed C-S bond cleavage enzyme(s).

Among the three substrates (i.e. S-(p-bromophenyl)-L-cysteine, N-acetyl-S-(p-bromophenyl)-L-cysteine and S-p-bromophenylglutathione) used in the present study, the cysteine conjugate of p-bromobenzene showed the highest substrate activity, whereas the mercapturic acid or the glutathione conjugate was not cleaved.

The results presented in this communication are the first direct demonstration of a contribution of the intestinal microorganisms to the formation of a thiol compound from its corresponding cysteine conjugate.

REFERENCES

1. M. Tateishi and H. Shimizu, Xenobiotica 6, 431 (1976).
2. T. Ou, K. Tatsumi and H. Yoshimura, Biochem. Biophys. Res. Comm. 75, 401 (1977).
3. W.G. Stillwell, O.J. Bouwsma, J.P. Thenot, M.G. Horning, G.W. Griffin, K. Ishikawa and M.Takaku, Res. Commun, Chem. Pathol. Pharmacol. 20, 509 (1978).
4. T. Mio, K. Sumino and T. Mizutani, Chem. Pharm. Bull. 24, 1598 (1976).
5. M. Tateishi, S. Suzuki and H. Shimizu, J. Biol. Chem. 253, 8854 (1978).
6. S. Suzuki and M. Tateishi, Drug Metab. Dispos. 9, 573 (1981).
7. K. Ohigashi, A. Tsunetoshi and K. Ichihara, Med. J. Osaka Univ. 2, 111

(1951)

8. H. Tanaka, N. Esaki and K. Soda, Biochemistry 16, 100 (1977).
9. K. Willi and H. Catheterine, Cancer Res. 33, 1862 (1973).
10. J.E. Bakke, J. Rafter, G.L. Larsen, J.A. Gustafsson and B.E. Gustafsson, Drug Metab. Dispos. 9, 525 (1981).
11. B.C. Saunders, Biochem. J. 28, 1977 (1934).