Biochemical Pharmacology, Vol. 32, No. 5, pp. 947-950, 1983.
Printed in Great Britain.

0006-2952/83/050947-04$03.00/0

CONVERSION OF TRIFLUOROMETHIONINE TO A CROSS-LINKING AGENT BY γ-CYSTATHIONASE

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(Received 15 November 1982; accepted 13 January 1983)

S-Trifluoromethyl-L-homocysteine is a highly toxic fluorinated derivative of L-methionine (1-7). In vitro, the compound has been shown to competitively inhibit S-adenosylmethionine synthetase (EC 2.4.2.13) (2,8,9) and to be esterified by methionyl-tRNA synthetase (EC 6.1.1.10) (5,10), but the molecular basis for the biological activity of the compound is not known. It occurred to us that the compound may function as an enzyme-activated acylating agent. We report here that trifluoro-L-methionine is converted to 2-oxobutanoate and inorganic fluoride by γ-cystathionase (homoserine dehydratase) (EC 4.2.1.15) from rat liver. Carbonothioic difluoride is a highly reactive acylating agent (11,12) which must occur as an intermediate in this reaction (Fig. 1). We have reported previously that this enzyme is inactivated by the "suicide" substrates (13-15) 3-trifluoromethyl-L-alanine and 3-trifluorovinyl-L-alanine (16).

MATERIALS AND METHODS

Trifluoro-L-methionine was supplied by Bachem, Inc., Torrance, CA, and appeared free of impurities by ^{19}F-NMR spectroscopy and on ninhydrin-stained thin-layer chromatograms. γ-Cystathionase (homoserine dehydratase) was isolated from the livers of adult male Sprague-Dawley rats (17). One unit of cystathionase was taken to be that which generates 1.0 μmole of 2-oxobutanoate per min in a solution containing 100 mM DL-homoserine, 0.1 mM pyridoxal 5'-phosphate, 0.1 mM EDTA and 100 mM potassium phosphate at pH 7.2 and 25°. 2-Oxobutanoate was detected by the absorbance of its dinitrophenylhydrazone (17) and by the lactate dehydrogenase-coupled method (16,18) employing a Gilford spectrophotometer. Inorganic fluoride was detected by means of a Bruker WH 360/180 NMR spectrometer (18).

RESULTS

In 100 mM potassium phosphate buffer at pH 7.2 and 25°, trifluoro-L-methionine was converted to 2-oxobutanoate by rat liver cystathionase at maximal velocity which was 1.5% that of homoserine. The K_m values were 48 and 14 mM respectively. Methionine did not detectably serve as a substrate under these conditions and neither assay revealed any enzyme inactivation by trifluoro-L-methionine at concentrations between 0.2 x K_m and 2.0 x K_m for periods up to 1 hr. 2-Oxobutanoate was recovered from trifluoromethionine with a one-to-one stoichiometry upon exhaustive action of the enzyme when the product was detected either as the chromophoric dinitrophenylhydrazone or as an

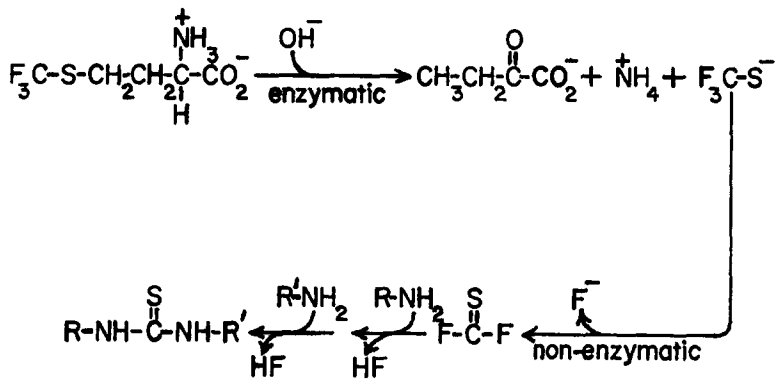

Fig. 1. Conversion of trifluoromethionine to a cross-linking agent by γ-cystathionase. Amines are depicted as reacting with carbonothioic difluoride, but thiols and other nucleophiles are also expected to react. Hydrolysis to COS and then to H_2S may also occur. γ-Difluoro thioethers may similarly yield monovalent acylating species.

NADH-bleaching substrate of lactate dehydrogenase (EC 1.1.1.27). In other experiments (Fig. 2), trifluoro-L-methionine was incubated in the presence and absence of cystathionase in phosphate buffer at 25°. No decomposition of the trifluoro compound occurred in the absence of the enzyme, whereas ^{19}F-NMR spectroscopy demonstrated only two fluorine-bearing species in the enzymatic reaction mixture. The more intense signal coincided with that of inorganic fluoride while the weaker signal coincided with that of the starting material.

DISCUSSION

Trifluoro-L-methionine is a remarkably stable compound which substantially survives refluxing in aqueous potassium hydroxide for 24 hr (1). However, the trifluoromethanethiolate ion which leaves in the cystathionase-catalyzed reaction decomposes under mild conditions to afford the highly reactive acylating agent carbonothioic difluoride (11,12,19), a chemical analog of phosgene. Cross-linking reactions of this species may impart toxicity to trifluoromethionine. Although trifluoromethionine is a poor substrate when compared to homoserine, cystathionase is widely distributed in high activity (17). Furthermore, other enzymes are also expected to liberate carbonothioic difluoride from trifluromethionine. For instance, certain microorganisms possess methionine γ-lyase (EC 4.4.1.11) activity (20). Also, elimination of the trifluoromethanethiolate ion may occur nonenzymatically in a reverse-Michael reaction (21,22) upon enzymatic oxidation of the α-amino acid to the α-oxo acid as is catalyzed, for instance, by aspartate aminotransferase (EC 2.6.1.1) from porcine heart (23).

The scheme outlined in Fig. 1 bears analogy to that thought to explain the toxicity of S-(1,2-dichlorovinyl)-L-cysteine, a contaminant of cattle feed treated with trichloroethylene (24-27). In that case, enzymes catalyze β-elimination, rather than γ-elimination, of a product capable of cross-linking macromolecules.

In some cases, enzyme-activated acylating agents have proven to be highly selective "suicide" inactivators (13-15) of enzymes. Examples include inactivation of γ-cystathionase by 3-trifluoro-L-alanine (13) and by 3-trifluoromethyl-L-alanine (16), inactivation of alanine aminotransferase (EC 2.6.1.2) by 3-cyano-L-alanine (28), inactivation

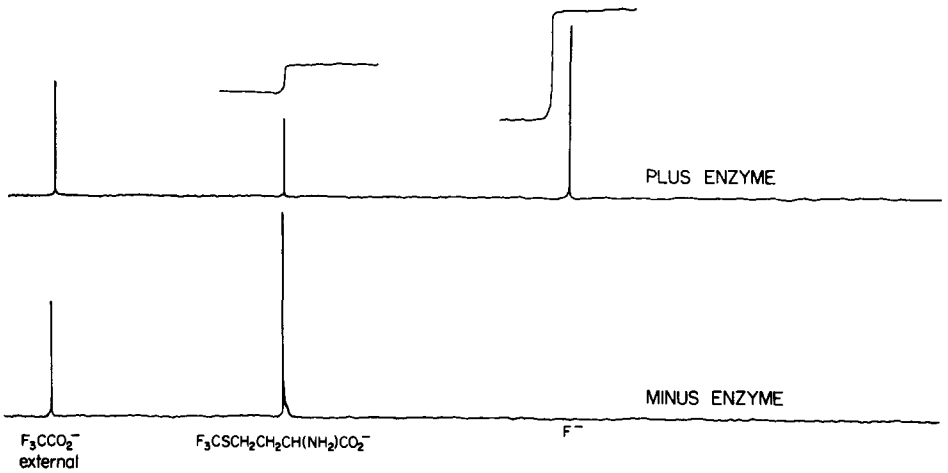

Fig. 2. Appearance of inorganic fluoride in reaction solutions of trifluoromethionine with cystathionase. Trifluoro-L-methionine (10 mM) was incubated for 24 hr in the presence and absence of cystathionase (13 units/ml) in a solution also containing 0.01mM pyridoxal 5'-phosphate, 0.1 mM EDTA and 200 mM potassium phosphate at pH 7.4 and 25°. Deuterium oxide (10% by volume) was then added, and the fluorine NMR spectra shown above were obtained with sodium trifluoroacetate present as an external standard. As shown, no decomposition of the trifluoromethionine was apparent in the absence of the enzyme. The only apparent fluorine-bearing product under these conditions was inorganic fluoride, the expected decomposition product of the unstable trifluoromethanethiolate ion. The signal ascribed to enzymatically generated fluoride was coincident with that of added potassium fluoride.

of aromatase by 10β-difluoromethyl-estr-4-ene-3,17-dione (29), and inactivation of a nitroethane-oxidizing flavoenzyme by 1-chloro-1-nitroethane (30). The adrenocorticolytic action of the chlorinated drug mitotane (o,p'-DDD) may depend upon its enzymatic oxidation to an acyl halide (31) whereas adverse reactions to chloramphenicol (32) and to polyhalogenated inhalation anesthetics (33,34) may involve metabolism to reactive acyl halides.

In view of the conversion of trifluoromethionine to carbonothioic difluoride by γ-cystathionase, other fluorinated thioethers and ethers may be deliberately designed as enzyme-activated acylating agents. For instance, S-(1,1-difluoroalkyl)-L-homocysteine and O-(1,1-difluoroalkyl)-L-homoserine are expected to yield monovalent acylating agents when processed by amino acid γ-lyases. Though chemically accessible (1,12,35, 36), α-polyfluoro thioethers and ethers have thus far been little investigated as pharmaceutical agents.

Acknowledgments - This study was supported by Research Grant GM 11040 from the National Institutes of Health. T.A.A. has received support from the Medical Scientist Training Program, GM 07170, NIH. We thank Dr. June S. Taylor for assistance with the NMR experiments.

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