OXIDATION OF CYSTINE TO CYSTEIC ACID BY BROMINE IN ^{18}O-LABELLED WATER, EVIDENCE FOR A CYCLIC CARBOXYLIC-SULFENIC ANHYDRIDE

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SUMMARY

^{18}O-enriched water (95 atom % ^{18}O) Oxidation by bromine of L-cystine dissolved in acidified led to the production of cysteic acid which contained two ^{18}O atoms in the carboxylic acid portion and two ^{18}O atoms in the sulfonic acid portion of the molecule. It is postulated that the incorporation of a single ^{16}O into the sulfonic acid results from an intramolecular transfer of a carboxyl oxygen via a cyclic carboxylic-sulfenic anhydride intermediate which is formed during the oxidation. The remaining carboxyl oxygen is postulated to exchange from either the cyclic anhydride or the free carboxylic acid during the reaction.

Key Words: Cystine, [sulfonic acid-$^{18}O_2$]Cysteic acid, Cyclic Carboxylic-Sulfenic Acid Anhydride

INTRODUCTION

The inability of the oxygens of sulfate to exchange with water has been known for a long time.[1] If the oxygens of the chemically similar sulfonic acid group do not readily exchange with water as well, then ^{18}O-labelled sulfonic acids could be used as stable isotope tracers in mapping biosynthetic pathways which involve sulfonic acids. Cysteic acid, a central sulfonic acid involved in these pathways, has been shown to be the biochemical precursor for the naturally occurring sulfonic acids taurine[3] and 6-sulfoquinovose[3] and for the sulfonolipid capnine[4]. Since cysteic acid is easily prepared by the oxidation of cystine with bromine in aqueous acid[5], this synthesis, performed in ^{18}O-enriched water, should readily lead to ^{18}O-labelled cysteic acid. Since

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the labelled water is the most likely source of the sulfonic acid oxygen, one would expect the cysteic acid which is produced in the reaction to contain three ^{18}O in the sulfonic acid. However, as shown in this paper, only two oxygens are incorporated into the newly formed sulfonate group. This can only be explained by the incorporation of one of the ^{16}O-carboxylic acid oxygens into the sulfonic acid during the oxidation. A possible mechanism for this is discussed.

RESULTS AND DISCUSSION

The FAB mass spectrum of the labelled cysteic acid showed an M^+-H ion at m/z 176. Since this is 8 m/z higher than that observed for unlabelled cysteic acid, one must conclude that four ^{18}O have been incorporated into the molecule. Since cysteic acid has only five oxygen atoms, there are only two possible arrangements for these four ^{18}O in the molecule. In the first arrangement, two ^{18}O would be in the carboxylic acid and two ^{18}O would be in the sulfonic acid. In the second arrangement, one ^{18}O would be in the carboxylic acid and three ^{18}O would be in the sulfonic acid. The first arrangement was determined to be the correct one after comparing the mass spectrum of the ditrimethylsilyl derivative of labelled cysteic acid with that of unlabelled cysteic acid. As outlined in Fig. 1, the mass spectrum of the labelled molecule shows an M^+-15-30 ion (15 = CH_3, 30 = C ^{18}O) at m/z 276 with three ^{18}O and a base peak at M^+-121 (121 = TMSC $^{18}O_2$) with two ^{18}O. Considering the mass and origin of these fragments, they could only result from cysteic acid which contains two ^{18}O in the carboxylic acid and two ^{18}O in the sulfonic acid portion of the molecule.

Additional support for this assignment of the ^{18}O label in the cysteic acid comes from comparing the mass spectra of the dimethyl and ditrimethylsilyl derivatives of the ^{18}O-labelled cysteic acid with the carboxylic acid ^{18}O removed by exchange with aqueous acid, with those of the derivatives prepared from unlabelled cysteic acid. Although the molecular ion is not observed in either case, the labelled compound shows an M^+-15-28 and M^+-117 (117 = $TMSCO_2$) for the ditrimethylsilyl derivative and an M^+-31 for the dimethyl derivative which is four masses higher than the unlabelled compound (Fig. 2). This

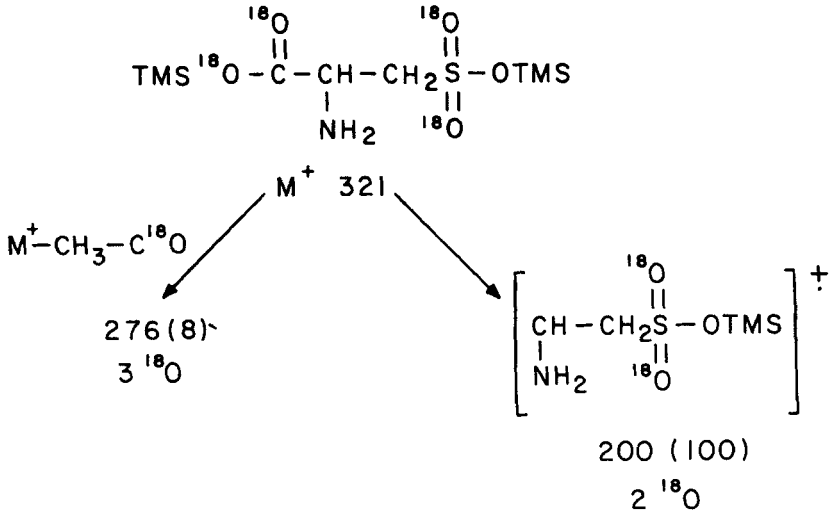

Fig. 1. Fragmentation of the ditrimethylsilyl derivative of the ^{18}O-labelled cysteic acid produced by the oxidation of cystine in ^{18}O-labelled water. The numbers in parentheses are the relative intensities of the indicated ions.

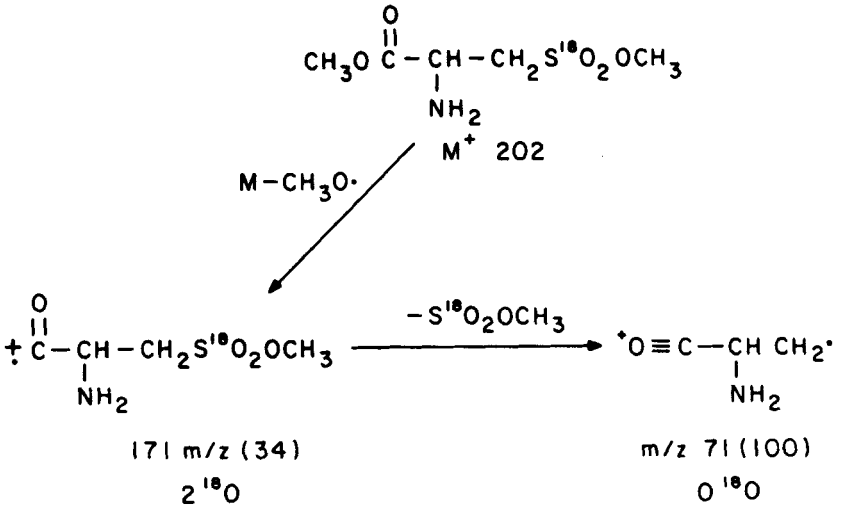

Fig. 2. Fragmentation of the dimethyl ester derivative of the ^{18}O-labelled cysteic acid after exchange of the carboxylic acid oxygens.

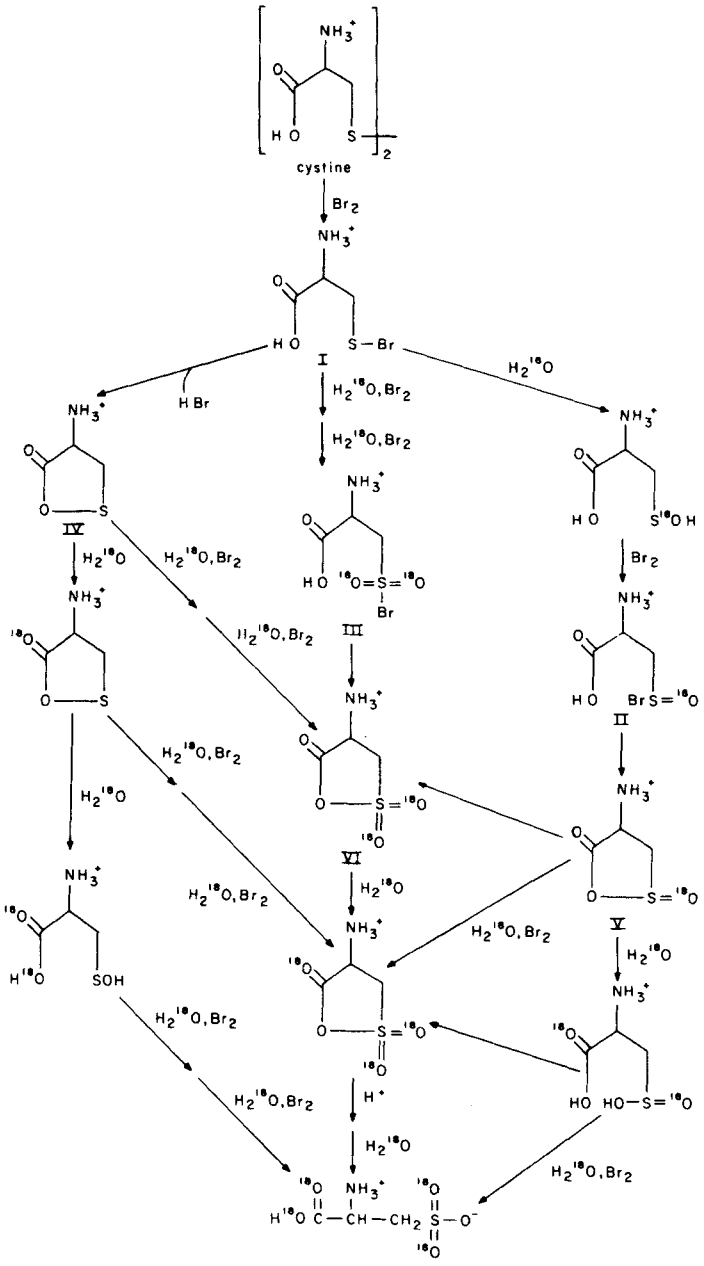

Fig. 3. Sequence of reactions which may be involved in the oxidation of cystine to cysteic acid by bromine in ^{18}O-labelled water.

indicates that the molecule has two ^{18}O in the sulfonic acid and that the M^+-31 fragment from the dimethyl derivative originates solely from cleavage of the carboxylic acid methyl ester.

Since the only source for the single ^{16}O incorporated into the sulfonic acid is one of the carboxylic acid oxygens of the original cystine, a single cyclic intermediate must be involved in the transfer of the carboxylic oxygen to the sulfonic acid at some stage in the oxidation. Several pathways which might explain this observed labelling pattern are outlined in Fig. 3. Each pathway begins with the oxidative cleavage of the disulfide bond of cystine to produce the sulfenyl bromide (I). This reaction is analogous to the well studied oxidation with iodine of cystine[7] and of amine disulfides[8] in which sulfenyl iodides are intermediates. The resulting sulfenyl bromide can either be cyclized or further oxidized by one or more of the several possible pathways shown in Fig. 3. Common to all these pathways is the cyclization of either the sulfenyl bromide (I), the sulfinyl bromide (II) or the sulfonyl bromide (III) to form a cyclic mixed anhydride between the carboxylic acid and either a sulfenic acid (IV), a sulfinic acid (V) or a sulfonic acid (VI), respectively. Mixed noncyclic carboxylic-sulfenic acid[9] and cyclic carboxylic-sulfonic acid[10] anhydrides have been prepared by this method. Carboxylic-sulfinic anhydrides have been reported[11] but their existence has been questioned.[12]

Exchange of the carboxylic acid oxygens with the labelled water could occur from any of the indicated intermediates but would be expected to be much more likely from the anhydride-containing structures due to their increased rate of exchange.[13] The resulting anhydrides in any case would undergo hydrolysis and exchange with the labelled water to produce either the final product in the case of compound VI or a sulfenic or sulfinic acid in the case of compounds IV and V, respectively. If a sulfenic or sulfinic acid were produced they would be subsequently oxidized to the sulfonic acid with the aqueous bromine. Alternatively, the sulfenic acid mixed anhydride or the sulfinic acid mixed anhydride could be oxidized to the sulfonic acid anhydride prior to hydrolysis. What is important, however, is that the hydrolysis of one of the anhydride intermediates must proceed by a nucleophilic attack of the labelled water at

the carboxyl group of the anhydride with either the sulfenic, sulfinic or sulfonic acid acting as a leaving group. Only in this way could an ^{16}O from the carboxylic acid be incorporated into the final sulfonic acid.

That a cyclic anhydride of structure VI can be formed and hydrolyzed with the transfer of a carboxylic acid oxygen to the sulfonic acid was demonstrated by the oxidation of cysteine sulfinic acid in ^{18}O water to cysteic acid. In this case the isolated cysteic acid contained no ^{18}O in the sulfonic acid portion of the molecule which, supports the involvement of a cyclic anhydride as an intermediate in the reaction. Additional support of this mechanism has been reported for the cleavage of acyclic sulfenyl carboxylates[9] and the cyclic anhydrides 3-sulphopropionic acid[14] and 3-sulfenylpropionic acid.[11]

If it is assumed that both the oxidation and cyclization reactions that form the anhydrides are faster than the rate of hydrolysis, then only the reaction sequence I→IV→VI→ final product, with exchange of the carboxyl oxygen occurring at each step, can explain the results observed here. These assumptions are certainly valid since the oxidation of cystine to cysteic acid by bromine is observed to be essentially instantaneous and the rate of cyclization of the sulfenyl bromide (I) to form a five-member ring would be expected to proceed faster than its hydrolysis. In addition, if the oxygen transfer didn't occur during the formation of the first anhydride (IV), then some of the cysteic acid formed would be expected to contain up to three ^{18}O in the sulfonic acid due to ^{18}O introduction into the carboxyl oxygens. Since this was not observed, the simplest explanation is that the critical step in the ^{16}O transfer is the formation of IV.

EXPERIMENTAL

^{18}O-enriched water (2.8% ^{16}O, 1.8% ^{17}O and 95.4% ^{18}O) was obtained from the Monsanto Research Corporation, Mound Facility, Miamisburg, Ohio. Electron impact mass spectra of the derivatives reported in this paper were obtained by direct insertion, after the sample solvents were evaporated in the probe, using a Varian MAT 112 mass spectrometer. The fast atom bombardment (FAB) spectrum of free cysteic acid was obtained on a VG70-70 instrument using glycerol as the supporting matrix and xenon as the fast atoms.

Preparation of [sulfonic acid-$^{18}O_2$]cysteic acid. L-Cystine was oxidized while dissolved in the ^{18}O-enriched water by a modification of the method described by Clarke and Inouye.[5] To 2.3 mL of the labelled water was added 300 mg of anhydrous HCl gas followed by 480 mg of L-cystine. Upon solution, bromine (~1.6 g) was added with stirring until the orange color of Br_2 was visible for 30 min. Volatile components were then removed under vacuum at 60°C, and the resulting L-cysteic acid was crystallized from hot water. The first set of crystals yielded 630 mg (84%) of L-cysteic acid after vacuum drying.

Oxidation of cysteine sulfinic acid in $H_2{}^{18}O$. Cysteine sulfinic acid was oxidized to cysteic acid in the ^{18}O enriched water by the following procedure. Five mg of the acid was dissolved in 60 µL of $H_2{}^{18}O$ (95.4 atom % ^{18}O) and 30 µL of 3 M HCl was added at 0°C. The solution was then titrated by the addition of bromine until the colour of free bromine persisted. Evaporation in vacuo gave crystals of cysteic acid. The ^{18}O was exchanged from the carboxylic acid position of cysteic acid by heating with 1 M HCl at 100°C for 3 hrs.

Preparation of derivatives. Cysteic acid was converted into its O,O-ditrimethylsilyl derivative with 1-(trimethylsilyl)imidazole as described by Eagles and Knowles.[6] The dimethyl ester was prepared by passing a solution of cysteic acid down a small column of Dowex-50 H^+, evaporating the water and treating the residue, which was dissolved in methanol, with excess diazomethane in ether.

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