# Release of Hydrogen Sulfide and Methyl Mercaptan from Sulfur-Containing Amino Acids

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The pyridoxal-catalyzed elimination of hydrogen sulfide or methyl mercaptan from α-amino acids such as L-cysteine, *S*-methyl-L-cysteine, and DL-methionine was studied quantitatively in an atmosphere of nitrogen at 100° C and at pH 5.8 or 6.2 in the presence and absence of Al(III), Fe(III), Fe(II), Sn(IV), and Sn(II). The catalytic activity of the metal ions decreases in the order Al(III) ≈ Fe(III) > Fe(II) ≫ Sn(IV) ≈ Sn(II) in the case of L-cysteine. With *S*-methyl-L-cysteine, the order is Fe(III) ≈ Fe(II) ≫ Sn(IV) ≈ Sn(II). Whereas methyl mercaptan is removed almost completely from *S*-methyl-L-cysteine within a 24-hr period in the presence of pyridoxal and Fe(III), the removal of hydrogen sulfide from L-cysteine amounts only to some 30% under the same experimental conditions. The pyridoxal–metal ion catalyzed elimination of a γ-substituent from an amino acid (*e.g.*, methionine) proceeds much more slowly than the removal of a β-substituent (*e.g.*, *S*-methylcysteine).

The sporadic occurrence of black deposits primarily consisting of iron sulfide in the headspace area of canned protein-rich foods is one of the technical problems in food processing that has received considerable attention during the past years. Although the discoloration has been observed in canned meats (Johnson and Frost, 1951; Obata *et al.*, 1957) and certain low-acid vegetable products (Davis, 1955), the blackening seems to be most severe in cases involving seafood products such as shrimp (Landgraf, 1956; Thompson, 1963), tuna (Pigott and Stansby, 1955, 1957; Pigott and Dollar, 1963; Pigott *et al.*, 1964), salmon (Tanikawa, 1958), crab (Arakawa, 1928), and clams (Tanikawa *et al.*, 1966).

It is generally believed that the discoloration results from the heat-induced decomposition of the sulfur-containing proteins and amino acids of the food products, which yields hydrogen sulfide and the subsequent formation of iron sulfide during the heating cycle of the canning process (Pigott and Dollar, 1963). Obviously, from a chemical point of view, the formation and precipitation of black FeS has to be expected whenever the product of the concentrations of $Fe^{2+}$ and $S^{2-}$ is larger than the solubility product of FeS. Ferrous ions, incidentally, are assumed to arise from the anodic dissolution of exposed parts of an otherwise protected steel base which, in turn, may have been created either by mechanical stress or by imperfections already present in the protective tin coating of the food container (Davis, 1955).

Although it seems to be quite certain that the hydrogen sulfide concentration and degree of blackening in the headspace area of the food container are closely interrelated with each other (Tanikawa *et al.*, 1966), it is nevertheless left open to conjecture whether the formation of hydrogen sulfide is simply the result of pyrolytic decomposition of the sulfur-containing proteins and amino acids or if it also involves the participation of other substances serving, for instance, as catalysts.

In an attempt to obtain more information with regard to possible catalytic effects upon the heat degradation of sulfur-containing amino acids, we decided to study quantitatively the release, respectively, of hydrogen sulfide and methylmercaptan from L-cysteine and *S*-methyl-L-cysteine as well as DL-methionine in the presence and absence of pyridoxal and several metal ions such as $Al^{3+}$, $Fe^{3+}$, $Fe^{2+}$, $Sn^{4+}$, and $Sn^{2+}$. The impetus for this approach was derived from the well known fact (Snell, 1963) that α-amino acids easily undergo cleavage in the presence of pyridoxal and polyvalent metal ions, resulting in transamination (Snell, 1945), racemization (Olivard *et al.*, 1952), dehydration (Metzler and Snell, 1952), desulfhydration (Metzler, *et al.*, 1954; Ratsisalovanina *et al.*, 1961), and a variety of other reactions involving, among others, the labilization of the carboxyl group (Snell, 1963). Interestingly enough, however, the desulfhydration reaction does not seem to have been studied quantitatively in detail. In part, this is undoubtedly

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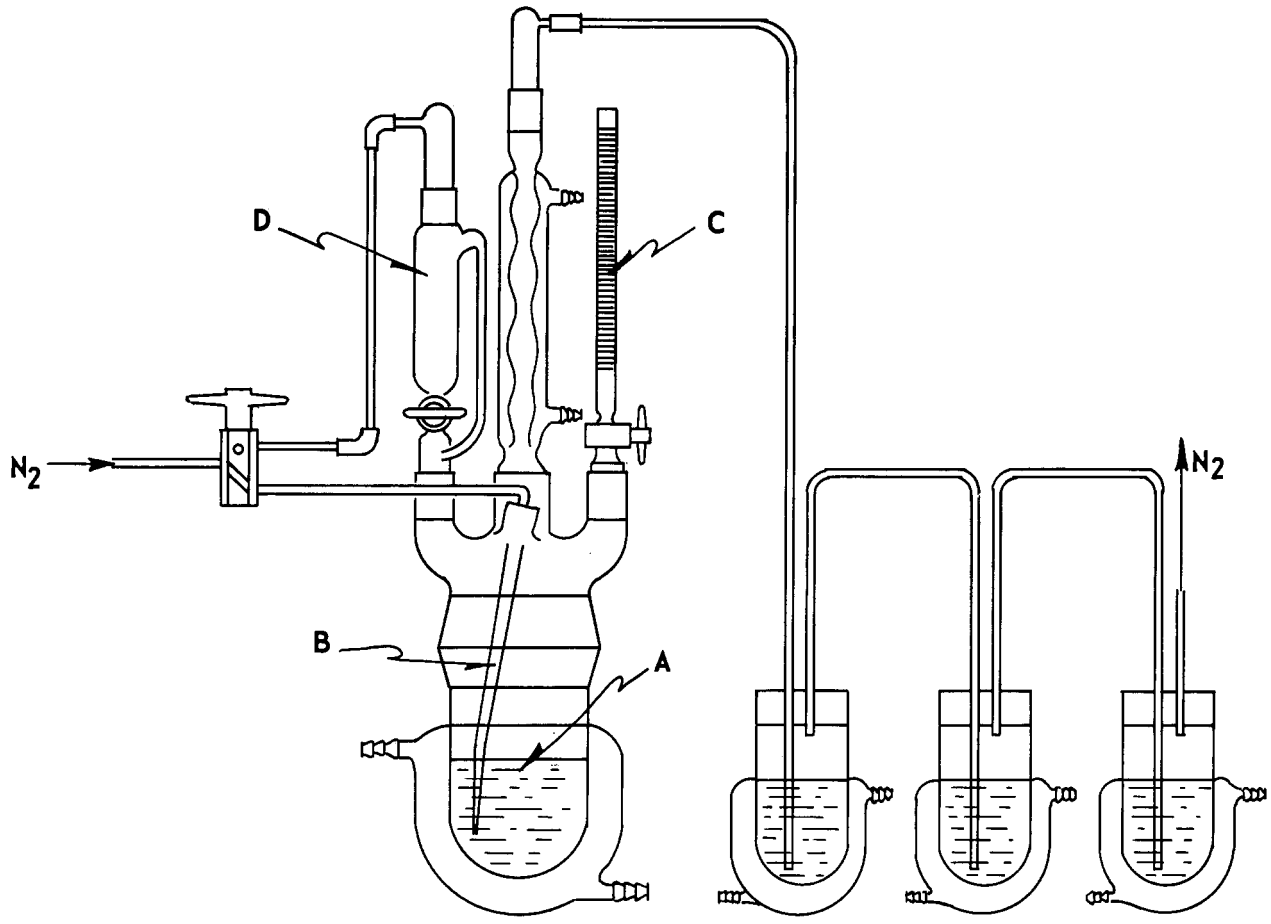


**Figure 1. Apparatus used for studying the release of $H_2S$ or $CH_3SH$ from L-cysteine, *S*-methyl-L-cysteine and DL-methionine. For details see text**

due to complications arising from the rapid formation of a thiazolidine compound between pyridoxal and cysteine (Heyl *et al.*, 1948). On the other hand, it may also have been due to the analytical difficulties that one encounters when attempting to determine accurately minute amounts of hydrogen sulfide (Fogo and Popowsky, 1949). Thus, the scarcity of quantitative data regarding the release of hydrogen sulfide and methylmercaptan from the respective amino acids made it seem worthwhile to embark upon this investigation. In this work we have used a sulfide ion specific electrode (Hseu and Rechnitz, 1968) that greatly facilitates the quantitative determination of hydrogen sulfide as well as methyl mercaptan.

## EXPERIMENTAL

**Materials.** *S*-Methyl-L-cysteine, L-cysteine hydrochloride monohydrate, and DL-methionine were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. Potentiometric standardization showed that they were of excellent quality, and they were therefore used without further purification. Aluminum ammonium sulfate, $Al(NH_4)(SO_4)_2 \cdot 12H_2O$, ferric ammonium sulfate, $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$, ferrous ammonium sulfate, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, silver nitrate, lead carbonate, and sodium cacodylate, $(CH_3)_2AsO_2Na$, were reagent grade materials that had been purchased from Matheson, Coleman & Bell. Reagent grade $SnCl_4 \cdot 5H_2O$, $SnCl_2 \cdot 2H_2O$, and 60% perchloric acid were obtained from Mallinckrodt. Pyridoxal hydrochloride was purchased from Sigma Chemical Co., St. Louis. All other chemicals were of reagent grade. Distilled and subsequently doubly deionized water was used throughout the investigation.

The various amino acids, together with the respective metal salts, were combined with pyridoxal hydrochloride in such a way that each final reaction mixture (20 ml) was 0.01 *M* in amino acid, 0.001 *M* in metal salt, 0.001 *M* in pyridoxal hydrochloride, and 0.05 *M* in sodium cacodylate buffer, adjusted with dilute HCl to pH 6.2. In reaction mixtures containing cysteine hydrochloride, the final pH was 5.8.

**Methods.** The reaction mixtures were heated in an atmosphere of nitrogen at 100° C for varying periods of time in the apparatus shown in Figure 1. The apparatus consists of a 100-ml jacketed cylindrical reaction flask (A) (Bantam-ware, Kontes Glass Co., Vineland, N.J.) containing, at the top, one ⅋ 19/22 center joint, two ⅋ 14/20 side joints, and one additional ⅋ 10/18 joint. The top and bottom parts of the reaction flask can be easily disassembled; they are joined together by a flat ground desiccator-type flange. To avoid evaporation of the reaction mixture during heating, an Allihn-type reflux condenser was used. A constant stream of nitrogen (High-Pure, Liquid Carbonic Co.) was passed through the reaction mixture *via* a ⅋ 10/18 gas-inlet capillary (B). Trace amounts of oxygen had been removed from the nitrogen by utilizing the photoreduction of riboflavin in the presence of ethylenediaminetetraacetate (EDTA) and methylene blue as described by Strauss and Nickerson (1960). After introducing the reaction mixture containing all reactants with exception of the respective amino acids, into flask A, the mixture was heated to boiling by circulating glycerol of 110–115° C through the jacket of the reaction

Table I. Molar Ratios of Released Hydrogen Sulfide to L-Cysteine Hydrochloride after Heating at 100° C for Given Periods of Time

| Reaction Mixture[a] (20 ml; pH 5.8) | Molar Ratios $(H_2S)/(Cys)$ | | | |
|---|---|---|---|---|
| Reaction Time[b] (hr) | 1 | 6 | 12 | 24 |
| Cysteine + pyridoxal + Fe(III) | 0.211 | 0.244 | 0.291 | 0.320 |
| Cysteine + pyridoxal + Al(III) | 0.200 | 0.244 | 0.282 | 0.300 |
| Cysteine + pyridoxal + Fe(II) | 0.119 | 0.141 | 0.170 | 0.172 |
| Cysteine + pyridoxal + Sn(IV) | 0.034 | 0.079 | 0.090 | 0.185 |
| Cysteine + pyridoxal + Sn(II) | 0.033 | 0.078 | 0.090 | 0.165 |
| Cysteine + pyridoxal | 0.018 | 0.058 | 0.073 | 0.093 |
| Cysteine + Fe(III) | 0.009 | 0.032 | 0.035 | 0.088 |
| Cysteine | 0.009 | 0.031 | 0.035 | 0.059 |

[a] For details of composition see Experimental, Materials. [b] For definition of reaction time see Experimental, Methods.

flask. Then the decomposition reaction was "started" by introducing the required amount of amino acid from buret C (⅌ 14/20 drip joint). Any released hydrogen sulfide or methyl mercaptan that did not remain in the reaction mixture was carried automatically by the nitrogen through the reflux condenser into three thermostated (25° C) titration vessels, interconnected in series, that served as absorption bottles. Hydrogen sulfide was absorbed exclusively in the so-called sulfide antioxidant buffer (50% SAOB; Orion, 1969) consisting of 2 *M* KOH, 1 *M* salicylic acid, and 0.22 *M* ascorbic acid, pH 12.8. Methyl mercaptan, on the other hand, was absorbed exclusively in 1 *M* NaOH. The decomposition reaction was "stopped" by adding 25 ml of 3 *M* $H_2SO_4$ under $N_2$ from funnel D, equipped with pressure equalizer and ⅌ 14/20 drip joint. At this point the pH in the reaction mixture dropped to a value around 0.5. Thus, the reaction time is defined as the elapsed time from the addition of an amino acid to the addition of sulfuric acid. $H_2S$ or $CH_3SH$ were removed quantitatively from the acidified reaction mixture by passing $N_2$ at 100° C for an additional 2 hr through the solution.

Sulfide ions were determined quantitatively by titrating the 50% SAOB, containing the absorbed hydrogen sulfide, with a 0.01 *M* $Pb(ClO_4)_2$ solution which had been prepared from $PbCO_3$ and $HClO_4$. The sulfide ion activity was monitored with the help of the Model 94-16 solid-state membrane silver/sulfide electrode using a Model 90-02 (double junction) calomel-type reference electrode (Orion Research, Inc., Cambridge, Mass.). 10% $KNO_3$ served as supporting electrolyte in the outer liquid junction chamber. Due to the high reduction potential of ascorbate in SAOB, silver ions become immediately reduced to elementary silver and thus cannot serve as titrant for sulfide ions. On the other hand, since the solid-state silver/sulfide electrode senses only $S^{2-}$ and/or $Ag^+$, $CH_3S^-$ has to be determined by using $Ag^+$ as a titrant. Consequently, methyl mercaptan was absorbed in 1 *M* NaOH instead of in 50% SAOB. It was then titrated with 0.1 *M* $AgNO_3$ containing 0.1 *M* $NH_4OH$.

Just prior to titration the 1 *M* NaOH was also made 0.1 *M* with respect to $NH_4OH$ to avoid precipitation of silver oxide (Tamele *et al.*, 1941). The silver ion activity was monitored with the same electrodes as described above. All titrations were performed under a cover of nitrogen at 25° C. A Model 801 digital pH meter (Orion Research, Inc., Cambridge, Mass.) was used to measure the potentials developed between indicator and reference electrode. Equivalence points were calculated as described by Hahn and Weiler (1926).

## RESULTS AND DISCUSSION

When heating mixtures of cysteine and pyridoxal in the presence of Fe(III), Fe(II), and Al(III) at pH 5.8 and 100° C for extended periods of time, considerable amounts of hydrogen sulfide are released. This is shown in Table I where the ratios of the concentrations of released hydrogen sulfide to cysteine originally present in the reaction mixtures are listed as a function of time. From the data given, the amounts of the reactants involved can be easily calculated. For instance, from Table I we see that 20 ml of 0.01 *M* cysteine, corresponding to 0.2 mmol cysteine, produce 0.2 × 0.2 mmol = 0.04 mmol $H_2S$ after heating for 1 hr at 100° C in the presence of pyridoxal and Al(III), and that this amount increases to 0.2 × 0.30 mmol = 0.060 mmol $H_2S$ after a 24-hr heating period.

Of the metal ions tested, Al(III) and Fe(III) are the most effective ones, followed by Fe(II). Sn(IV) and Sn(II) are quite ineffective in promoting the release of hydrogen sulfide, at least during the shorter heating periods. Thus, since iron and tin ions are the metal ions most likely to be found in the "tin can" food container due to the corrosive action of the food material, any marked accumulation of $H_2S$ in the headspace area during or after retorting (*i.e.*, heating the food container to 100° C) has to be attributed primarily to the catalytic effect of Fe(III) and, to a lesser extent, Fe(II), provided that the pyridoxal catalyzed heat decomposition of sulfur-containing amino acids is the sole source of the hydrogen sulfide. To the extent that the food product contains Al(III), *e.g.*, introduced in the form of $Al(NH_4)(SO_4)_2 \cdot 12H_2O$ as a neutralizing agent (Food Chemicals Codex, 1966), this ion will also contribute considerably to the release of $H_2S$. In this investigation, a pH of 5.8 or 6.2 was used in order to simulate somewhat the slightly acid medium of most canned seafood products.

From Table I it is seen that the release of $H_2S$ according to

$$\text{cysteine} \xrightarrow[\text{pyridoxal}]{M^{3+},H_2O} \text{pyruvate} + NH_3 + H_2S \qquad (1)$$

(Metzler and Snell, 1952) is incomplete, amounting, in the case of Al(III) and Fe(III), only to some 30% of the $H_2S$ to be expected theoretically. It has been suggested that the rapid formation of a thiazolidine compound from pyridoxal and cysteine is the primary cause of the incompleteness of the desulfhydration reaction since it removes the catalyst from the system (Buell and Hansen, 1960; Heyl *et al.*, 1948; Metzler and Snell, 1952). Consequently, by preventing the addition reaction between the –SH group and the –N=CH– group of the pyridoxylidenecysteinate Schiff base,

Table II. Molar Ratios of Released Methyl Mercaptan to *S*-Methyl-L-cysteine or DL-Methionine after Heating at 100° C for Given Periods of Time

| Reaction Mixture[a] (20 ml; pH 6.2) | Molar Ratios ($CH_3SH$)/(amino acid) | | | |
|---|---|---|---|---|
| Reaction Time[b] (hr) | 1 | 6 | 12 | 24 |
| *S*-Methylcysteine + pyridoxal + Fe(III) | 0.480 | 0.673 | 0.780 | 0.907 |
| *S*-Methylcysteine + pyridoxal + Fe(II) | 0.435 | 0.577 | 0.664 | 0.809 |
| *S*-Methylcysteine + pyridoxal + Sn(IV) | 0.018 | ... | 0.029 | 0.033 |
| *S*-Methylcysteine + pyridoxal + Sn(II) | 0.018 | ... | 0.029 | ... |
| *S*-Methylcysteine + pyridoxal | 0.008 | ... | 0.012 | ... |
| *S*-Methylcysteine + Fe(III) | 0.006 | ... | 0.007 | ... |
| *S*-Methylcysteine + Fe(II) | ... | ... | 0.007 | ... |
| *S*-Methylcysteine | ... | 0.005 | 0.006 | ... |
| Methionine + pyridoxal + Fe(III) | 0.023 | ... | 0.055 | 0.090 |
| Methionine + pyridoxal + Fe(II) | ... | ... | 0.048 | ... |
| Methionine | ... | ... | 0.005 | ... |

[a] For details of composition see Experimental, Materials. [b] For definition of reaction time see Experimental, Methods.

the desulfhydration reaction should proceed to completion. That this is indeed the case is shown in Table II, where *S*-methyl-L-cysteine was used instead of L-cysteine. Thus, keeping 20 ml of 0.01 *M* *S*-methyl-L-cysteine in the presence of 0.001 *M* Fe(III) and 0.001 *M* pyridoxal hydrochloride for 24 hr at pH 6.2 and at 100° C leads to an almost complete release of $CH_3SH$. This result is to be expected since the methyl group is incapable of participating in a ring closure reaction between pyridoxal and *S*-methyl-L-cysteine, although the two functional groups, $-NH_2$ and $-SCH_3$, are in suitable proximity. Incidentally, *S*-methyl-L-cysteine has been isolated from kidney beans (*Phaseolus vulgaris*) which may thus also account for the discoloration observed in canned vegetable products (Meister, 1965).

Fe(III) and Fe(II) exhibit about the same effectiveness in releasing $CH_3SH$ whereas Sn(II) and Sn(IV) still do not promote the decomposition reaction. The high reactivity of both the Fe(II) and Fe(III) chelate of pyridoxylidene-(*S*-methyl)cysteinate suggests that the effective charge distribution within the two coordination compounds does not differ too greatly from one another. Although it is generally recognized that tervalent metal ions are much more suitable for providing the additional electron attracting group in the coordinated Schiff base than divalent metal ions (Metzler *et al.*, 1954), the recent finding that, for instance, pyridoxylidenealaninatoaluminum(III) can exist in two isomeric forms, namely in the tridentate 1:1:1 complex in which the carboxyl group of the amino acid is bound to the central atom and the "dangling carboxyl" complex in which bonding to the central ion occurs only *via* the aldimino group and phenol group (Gansow and Holm, 1969), may very well account for the fact that Fe(III) as well as Fe(II) show about the same catalytic activity (*cf.* Table II). In other words, since both iron(II) and iron(III) form complexes mostly of identical symmetry, the participation, for instance, of the carboxyl group in the pyridoxylidene(*S*-methyl)cysteinate Fe(III) complex in bonding but not in the Fe(II) complex could conceivably lead to the same overall charge distribution. By contrast, in the cysteine-pyridoxal system, the HS-group has to be expected to participate quite strongly in chelation in both complexes, thereby reducing the effective positive charge much more drastically in the Fe(II) chelate than in the Fe(III) chelate, which obviously impairs the $\beta$-elimination of the $H_2S$ group. Nuclear magnetic resonance studies of the various iron complexes of pyridoxylidenecysteinate and pyridoxylidene(*S*-methyl)cysteinate would certainly be helpful in clarifying several of the aspects discussed above.

At present, we are at a loss to explain unambiguously the "failure" of Sn(II) and Sn(IV) to promote the release of methyl mercaptan as well as to understand their quite reduced effectiveness in releasing hydrogen sulfide. On one hand this behavior might be due to the fact that $Sn^{II}$ solutions and $Sn^{IV}$ solutions are extensively hydrolyzed at pH values above 2, yielding hydrous oxides of zero ionic charge which may not coordinate with the Schiff bases. Indeed, *S*-methyl-L-cysteine and DL-methionine do not chelate with Sn(II) and Sn(IV) as evidenced from potentiometric pH titration experiments (Gruenwedel and Hao, 1971). On the other hand, L-cysteine as well as the cysteine analog DL-penicillamine complex Sn(II) quite strongly already at low pH values (Gruenwedel and Hao, 1971) which may also impair the elimination of a $\beta$-substituent from the coordinated Schiff base in case the ternary complex should prove to be too stable. Clearly, more experiments have to be performed in order to arrive at an accurate understanding of the possible reactions involved.

Our finding that the elimination of a $\gamma$-substituent from an amino acid proceeds much more slowly than the release of a $\beta$-substituent (*cf.*, Table II, methionine *vs.* *S*-methylcysteine) is in agreement with the data obtained by other investigators (Metzler *et al.*, 1954). It has been suggested that the Schiff base catalyzed $\gamma$-elimination involves an imine–enamine tautomerization step in which the electronegative $\gamma$-substituent is removed by donation of electrons from the $\beta$ carbon to the $\beta$–$\gamma$ carbon–carbon bond (Abbott and Martell, 1969). Consequently, in solvents of high proton activity and mobility, *i.e.*, at low pH values, this reaction may be blocked by protonating the $\beta$ group. That the $\beta$ carbon atom of an amino acid can indeed participate in proton exchange reactions was shown by deuteration experiments at pH 5 (Junk and Svec, 1964; Abbott and Martell, 1969). This would then imply that high pH values facilitate the $\gamma$-elimination in a pyridoxal–metal ion-model system.

Finally, the experimental data obtained so far suggest that the accumulation of substantial amounts of hydrogen sulfide or methyl mercaptan in the headspace area of canned protein-rich foods during retorting is the result primarily of a pyridoxal–metal ion-catalyzed decomposition of the sulfur-containing amino acids. By contrast, noncatalyzed degradation reactions seem to be of lesser importance.

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