Determination of Methionine in Certain Mixtures¹

A Precision Method

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THE ready oxidizability by hydrogen peroxide of the amino acid methionine to the sulfoxide level (4) suggests the possibility of analytical utilization of this reaction. It has been shown that it can serve for the determination of methionine in crude protein hydrolyzates but that the accuracy is impaired by the presence of interfering substances (3). The present communication has as its object the determination of suitable conditions for, and the precision attainable in, the application of peroxidimetry to the assay of methionine preparations, and the investigation of the oxidizability of other amino acids under comparable conditions.

Experimental

Since the time required for the completion of the methionine oxidation decreases with increasing acid concentration $(\mathcal{3}),$ and since among the mineral acids perchloric acid appears to be the one least likely to interfere in oxidations and reductions (4), the course of the reaction was carefully followed in perchloric acid solutions of 4 M, 2 M, and 1 M concentration. Table I shows the results obtained. The conclusions suggested by the data are: the freshly prepared solutions of hydrogen peroxide in aqueous perchloric acid are, apart from possible minor adjustments during a brief initial period, stable within the limits of analytical precision for 12 to 24 hours; the rate of the detectable decomposition decreases slightly with increasing concentrations of perchloric acid; the rate of the reaction of methionine with hydrogen peroxide increases with increasing acid concentration, in accordance with earlier findings (3); and the tendency of the oxidation to exceed the sulfoxide level (sulfone formation?) increases likewise with increasing acid concentration. Thus for the present analytical purpose the last factor militates against the use of a high acid concentration, while rapidity of reaction and stability of peroxide favor it.

Under the conditions used it seems that averages of several determinations obtained after 3 to 7 hours in 1 M perchloric acid, 1 to 3 hours in 2 M perchloric acid, or 0.5 to 2 hours in 4 M perchloric acid may be expected to yield rea-

¹ Methionine Studies V.

TABLE I. REACTION OF METHIONINE AND HYDROGEN PEROXIDE IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF PERCHLORIC ACID $-1 M \text{HClO}_{4}$ $H_2O_2 \qquad (+ \text{Methionine})$ Con^{-1} $\begin{array}{c} -2 \ M \ \text{HClO}_4 \\ \hline \text{H}_2\text{O}_2 \\ \hline \text{I}_2\text{O}_2 \\ (+ \ \text{Methionine}) \\ \hline \text{Opt} \end{array}$ -4 M HClO4- H_2O_2 H2O2 (blank) (+ Methionine) ${\rm H}_2{\rm O}_2$ H_2O_2 (blank) found Con-Con-(blank) Con-Found sumed Found sumed Time Time Found sumed found Time found mM./l.mM./l. mM./l.Min. Min.mM./l.mM./l.mM./l.Min. mM./l.mM./l.mM./l. $\begin{array}{c} 74.39\\74.54\\74.66\\74.68\\74.76\\74.86\\74.86\\75.98\\77.08\end{array}$ 83.05 (83.06)^a (83.10)^a $8.66 \\ 8.52 \\ 8.44 \\ 8.44 \\ 8.40 \\ 8.40 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 \\ 100 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Interpolated values. Methionine calculated according to weight used. Approximate amount of methionine (not weighed precisely).

sonably precise values for the peroxide consumed in the formation of sulfoxide from methionine. The reaction follows substantially a bimolecular course, and other determinations have shown that, in harmony with the bimolecular reaction formula, more time or a higher peroxide-methionine ratio is required for completion of the reaction when the absolute concentrations are lower.

The data of Table I give the following results for the purity of the methionine used: from the average of the figures obtained in 4 M perchloric acid between 25 and 120 minutes, 99.62 ± 0.12 per cent, and from that of the 2 M perchloric acid figures between 50 and 180 minutes, 99.60 ± 0.03 per cent. These figures were checked by a series of single titrations on individually weighed samples, as follows:

About 100 mg. of methionine are weighed into a 250-cc. glass-stoppered Erlenmeyer flask. After addition of 10 cc. of 0.08 Mhydrogen peroxide in 1 M perchloric acid the sample is dissolved by gentle swirling. After 4 or 5 hours 20 cc. of a solution of 5 millimoles of potassium iodide and 0.1 millimole of ammonium molybdate are added and the liberated iodine is at once titrated with thiosulfate. A control identical except for the omission of methionine is treated in the same manner.

The following results were obtained on the same specimen of methionine as was used in the experiments of Table I: 99.49, 99.50, and 99.36 per cent; average 99.45 ± 0.06 per cent. Other specimens of synthetic methionine, some of which were the result of an attempted purification by way of the insoluble copper salt, gave the following values: 98.39, 98.45, average 98.42; 98.31, 98.38, 98.52, average 98.40 ± 0.08; 99.60, 99.78, average 99.69. The last-named sample gave by titration of amino groups with "aceteous" perchloric acid (2) values of 99.90 and 100.14 per cent.

In order to consider the possible interference of some other amino acids 4-cc. samples of a freshly prepared solution approximately 0.073~M in methionine, 0.090~M in hydrogen peroxide, and 2 M in perchloric acid were pipetted into flasks containing 1 millimole of another amino acid. After 2 hours the solutions were titrated as usual, with the following results, expressed as percentages of the values obtained by methionine alone: dl-alanine, centages of the values obtained by methorine alone: aranne, -0.10 per cent; dl-serine, +0.01 per cent; dl-threonine, +0.09 per cent; dl-phenylalanine, -0.02 per cent; l-tyrosine, -0.10 per cent; l-hydroxyproline, +0.02 per cent; l-hydroxyproline, +0.02 per cent; l-lysine dihydrochloride, +0.20 per cent; l-histidine monohydro-chloride. -0.10 per cent; l-arginine

per cent; *l*-institutie mononyuto-chloride, -0.10 per cent; *l*-arginine monohydrochloride, +0.09 per cent; *l*-aspartic acid, -0.02 per cent; *l*-glutamic acid, -0.06 per cent; *l*-tryptophane, +2.1 per cent; l-cystine, +1.8 per cent.

Apparently the ordinary amino acids, except to a small extent tryptophane and cystine, as well as cysteine (3), do not consume any hydrogen peroxide under the present conditions. This circumstance should recommend the present method for the detection of methionine present as a contaminant in preparations of leucine of natural origin (5).

The applicability of the present method to various other situations, and the accuracy possible, will have to be ascertained from case to case. Grace Medes of this institute has successfully adapted the method to the determination of the fate of methionine in tissue extracts; no difficulty was experienced in determining with an accuracy of 0.5 per cent amounts of the order of 0.25 millimole of methionine, dissolved in 5 cc, of buffer containing about 25 mg. of liver slices.

Summary

The purity of methionine can be determined with an accuracy of ± 0.1 per cent by oxidation with hydrogen peroxide under specified conditions. The principle of this method is applicable to the determination of methionine in certain other analytical situations, since other natural amino acids, except tryptophane, cysteine, and cystine to a small extent, do not seem to interfere. Data on the stability of hydrogen peroxide in 1 to 4 molar perchloric acid solutions are included.

A 0.21 M hydrogen peroxide solution in 1.25 M perchloric acid was prepared from Merck's Blue Label hydrogen peroxide which contains no preservative. Ten- or 20-cc. portions were trans-

ferred to 25- or 50-cc. volumetric flasks and the acidity was adjusted with perchloric acid so that the final acid concentrations would be 4.0, 2.0, and 1.0 M, respectively, when the solutions were diluted to the mark with water. In similar flasks weighed amounts of synthetic *dl*-methionine were dissolved in water and combined with hydrogen peroxide and perchloric acid in such a manner that the acid concentration and the initial amount of peroxide were identical with the contents of the corresponding blank solutions. The molar amount of methionine was 10 to 15 per cent less than that of the peroxide. At intervals 3- or 5cc. aliquots of the completed solutions were pipetted into an Erlenmeyer flask, 20 cc. of a solution containing 5 millimoles of potassium iodide and 0.1 millimole of ammonium molybdate were added (3), and the liberated iodine was immediately titrated with 0.025 N thiosulfate using starch indicator.

The thiosulfate was standardized against copper (1). Pipets and flasks used in the experiments with 4 M and 2 M perchloric acid were carefully calibrated.

Literature Cited

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Determination of Organic Sulfur in Combustible Gas

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Purified air is used to burn the gas in a special burner, the sulfur dioxide resulting from the combustion of the gas is absorbed in sodium hypobromite solution, and the sulfate in this solution is determined turbidimetrically. The method is adapted to the determination of less than 0.2 grain (12.96 mg.) of sulfur per 100 cu. feet (283.2 imes 10^4 cc.) of gas.

NUMBER of methods for the determination of organic A sulfur in gas have been described (1-4, 6), but none is applicable to the analysis of hydrocarbon gas containing sulfur to the extent of 0 to 0.2 grain (12.96 mg.) per 100 cu. feet $(283.2 \times 10^4 \text{ cc.})$. Lieber and Rosen (3) have obtained excellent results by their method on gas containing as little as 0.6 grain of sulfur per 100 cu. feet. However, this laboratory has found that the time involved in burning a sufficient quantity of gas to give a weighable amount of barium sulfate prohibits the use of this method for routine analyses when quick results are desired.

The method developed by this laboratory has filled the demand for a thoroughly reliable, relatively rapid routine analysis of combustible gases for organic sulfur. It is to some extent a modification of existing methods and has been adapted to the determination of sulfur of the order of 0 to 0.2 grain per 100 cu. feet.

The method uses purified air (7) to burn the gas in a specially designed burner. The sulfur dioxide resulting from the combustion of the sulfur compounds is absorbed in sodium hypobromite solution. The sulfate in this solution is subsequently determined by the turbidimetric method (5) according to a modification previously described (7).

Analysis is made on gas from which all hydrogen sulfide has been removed by a suitable scrubbing procedure, such as passing the gas several times through a 20 per cent solution of lead acetate containing 5 per cent acetic acid or neutral cadmium sulfate solution.

Purification of Air

The air used for combustion is first filtered and dried by passing it through a tube containing a section of activated char-coal and then a section of soda lime. It is next passed through to a and then a section of solar line. To is next passed billough a copper spiral made from copper tubing 0.234 cm. $(\frac{3}{322}$ inch) in outside diameter with 0.117-cm. $(\frac{3}{54}$ inch) wall, maintained at a temperature of 540° C. (1000° F.) by means of a muffle furnace. The spiral is made up of 15 to 20 coils approximately 3.75 cm. (1.5 inches) in diameter. This arrangement permits very good heat transfer, and can be used at this temperature for many months without replacement. Higher furnace tem-peratures, however, cause the copper tubing to deteriorate fairly rapidly. It is important to subject the copper tubing to a pre-liminary ignition period at 1000° F, while a stream of purified air is passed through it to remove any sulfur compounds in the tubing.

After leaving the furnace, the copper tubing is spiraled and submerged in a water bath. The air is scrubbed first with 2 per cent sodium hydroxide-2 per cent bromine solution and then with 5 per cent sodium hydroxide solution. It is advisable to select for this purpose gas washing bottles which do not produce a fine mist of sodium hydroxide, as this will carry through to the burner and produce a yellow sodium flame. From the gas scrubbing bottles the air is passed into a pressure reservoir from which a number of air take-offs can be made. This

reservoir permits burning the gas with a steady, nonflickering flame. One of the take-offs from the reservoir leads to a manifold, from which in turn a number of leads can be taken. These are used for maintaining a sulfur-free atmosphere over the evaporating acidified sodium hypobromite solutions.

All rubber tubing and rubber stoppers used must first be heated for some time in strong caustic solution.