Some Problems Related to Detection of Methionine Sulphoxide in Protein Hydrolysates

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Methionine and methionine sulphoxide are completely oxidized to the sulphone stage by H_2O_2 treatment on filter paper if the pH of the solutions applied is less than 1.4. Above this pH a mixture of methionine sulphoxide and methionine sulphone is obtained. At pH about 6.0 practically no sulphone is detected.

Under the conditions used when protein hydrolysates are prepared by heating with 6 N HCl under reflux, methionine sulphoxide is partly reduced to methionine.

It has been suggested that methionine sulphoxide may be formed in fish meal when this contains peroxidized fat¹. During preliminary work on the possible presence of methionine sulphoxide in hydrolysates of herring-meals, the method of Dent^{2,3} for distinguishing between methionine and its sulphoxide was studied. At the same time, the effect of heating methionine sulphoxide under reflux with HCl was examined. It was found that the suggested method^{2,3} does not distinguish between methionine and its sulphoxide, and that heating of methionine sulphoxide with 6 N HCl under reflux results in partial reduction of the sulphoxide to methionine.

OXIDATION OF METHIONINE AND METHIONINE SULPHOXIDE WITH H_2O_2 ON FILTER PAPER

The method of Dent^{2,3} is based on the observation that methionine and methionine sulphoxide react differently when treated with 30 % H_2O_2 on the filter paper prior to chromatography. It is claimed that methionine is completely oxidized to the sulphone stage by this treatment while the sulphoxide is unaffected.

Portions of aqueous solutions (5 μl , 0.02 M) of DL methionine and of sulphoxides prepared from DL, D and L methionine by methods given in literature^{4,5} were applied to sheets of Whatman paper No. 3 and treated with H_2O_2 as described by Dent^{2,3}. Ascending chromatograms were run overnight using acetone containing 40 % water and 1 % acetic acid as solvent. Ninhydrine (0.3 % in methanol) was used as spraying reagent. Methionine was oxi-

dized almost completely to the sulfoxide stage by the H_2O_2 treatment while methionine sulfoxide was unaffected.

A clue to the reason for the discrepancy between this result and those reported by Dent^{2,3} may lie in his statement that a small amount of HCl was added to dissolve the less soluble of the amino acids³, which were used as 0.01 M solutions in 75 % (v/v) ethanol. Methionine requires a long time to dissolve in this solvent while the sulfoxide dissolves instantly. It is probable, therefore, that Dent³ classified methionine among the less soluble amino acids.

Methionine and methionine sulfoxide were therefore prepared in 0.02 M aqueous solutions to which were added 2, 1, 0.5 and 0.25 % concentrated HCl. The corresponding solutions of both substances had the same pH: 1.05, 1.45, 1.85 and 2.30, respectively. Treatment with H_2O_2 prior to chromatography resulted in complete oxidation of both substances to the sulphone stage in the two more acid solutions; in the two less acid solutions both methionine sulfoxide and sulphone were detected on the chromatograms. Thus, complete oxidation to the sulphone stage took place when the pH of the original solution was 1.4 or less. Solutions (0.02 M) of the hydrochlorides were found to have pH's 2.15 and 2.00 for methionine and methionine sulfoxide, respectively; the pH's of the bases were 5.65 and 6.00. Treatment of portions of these solutions with H_2O_2 on the filter paper resulted in more sulphone than sulfoxide for the hydrochlorides while the opposite was true for the bases. By the criteria of Dent³ the results would have indicated the presence of methionine and methionine sulfoxide in these solutions. Similar treatment of 0.01 M solutions of both substances in 75 % ethanol resulted in complete oxidation of both substances to the sulphone stage when 1 % concentrated HCl was present; without added HCl methionine was oxidized only to the sulfoxide stage while the sulfoxide was unaffected by the treatment. It may be mentioned that HCl was used as a preservative in the urines in which Dent² claimed to have detected methionine sulfoxide.

Methionine and methionine sulfoxide did not oxidize when solutions with pH's ranging from 1.0 to 9.0 were applied to the filter paper and left exposed to the air for 23 h prior to chromatography. No sulphone was detected with either substance, and with methionine no sulfoxide was detected. This is rather surprising since methionine when left exposed to the air is often partly oxidized to the sulfoxide³. The results taken together seem to indicate that the effect of HCl in the H_2O_2 oxidation of methionine and methionine sulfoxide is through an activating effect on the oxidizing agent and not on the substrate.

EFFECT OF HEATING METHIONINE SULFOXIDE WITH HYDROCHLORIC ACID

Synge and Wood⁶ observed that S-methylcysteine was formed when the corresponding sulfoxide was heated for 24 h with 6 N HCl in a sealed tube at 105°C. Since protein hydrolysates are often made by heating with 6 N HCl under reflux⁷, the effect of this treatment on methionine sulfoxide was studied. 0.01 M solutions in 6 N HCl were heated under reflux for 24 h, and then taken to dryness and redissolved in water. The aqueous solutions were then chromatographed by a circular filter paper technique⁸ using acetone

Fig. 1. Circular chromatography on Whatman filter paper No. 3 of 0.01 M solutions in 6 N HCl heated for 24 hours under reflux of methionine sulfoxide (a); a treated on the starting line with H_2O_2 and ammonium molybdate (b); methionine sulfoxide plus cysteine (c); methionine sulfoxide plus cysteine (d); cystine (e); d treated as b (f). (Schematic presentation). (M = methionine; MSO = methionine sulfoxide; MSO_2 = methionine sulphone; Cy = cystine; CySO_3H = cysteic acid).

containing 15 % water and 1 % concentrated ammonia. When the chromatograms were run overnight the solvent front moved off the paper but good separation of methionine, methionine sulfoxide and methionine sulphone was obtained. None of the common amino acids moved so fast on the paper that they would have escaped notice.

When methionine sulfoxide alone was heated with 6 N HCl the chromatograms indicated the presence of about equal amounts of methionine and methionine sulfoxide as judged by the intensity of the ninhydrine colour (Fig. 1 a). Two weaker zones were observed between the sulfoxide and the starting line. Treatment on the starting line with H_2O_2 and ammonium molybdate³ reduced the four zones to two. The stronger one corresponded to methionine sulphone formed from methionine and methionine sulfoxide (Fig. 1 b).

It has been demonstrated that methionine sulfoxide oxidizes cysteine to cystine⁴. It was therefore tested whether the presence of an equivalent amount of cysteine together with methionine sulfoxide resulted in a more complete reduction of the latter to methionine during the heating with HCl. As a control the sulfoxide was also heated with cystine. Neither cysteine, nor cystine

appreciably increased the amount of methionine formed (Fig. 1 c, d). With both substances two zones were observed between the starting line and the methionine sulfoxide zone. The innermost one coincided with cystine (Fig. 1 e) and with the innermost of the zones observed on heating methionine sulfoxide alone (Fig. 1 a). For the mixture methionine sulfoxide and cystine an additional weak zone was observed between methionine sulfoxide and methionine (Fig. 1 d). Treatment of the heated mixture with H_2O_2 and ammonium molybdate on the starting line reduced the number of zones to two. The outermost one corresponded to methionine sulphone, the innermost to cysteic acid (Fig. 1 f). The latter zone also corresponded to the second zone from the starting line formed when methionine sulfoxide was heated alone (Fig. 1 a). The results indicate that when methionine sulfoxide is heated under reflux with 6 N HCl methionine and small amounts of two substances occupying the same zones on the chromatograms as cystine and cysteic acid are formed. It seems likely that the latter substances are homocystine and homocysteic acid. The former was not available, but H_2O_2 treatment of DL homocysteine S lactone gave one zone at the same place on the chromatograms as cysteic acid. It was not attempted to identify more definitely the substances formed. The extra zone formed in the mixture of methionine sulfoxide and cystine is suggested to be cystine disulphoxide.

The extra zones observed beside those corresponding to the substances present in the starting material could not be detected when the substances were dissolved in 6 N HCl and then, without heating under reflux, taken to dryness before redissolving them in water. The reactions responsible for the substances formed seem therefore to require some time of heating. It has not been investigated how long time of heating is required for the reactions to take place.

CONCLUSION

It seems a vain task to search for methionine sulfoxide in protein HCl hydrolysates. If present in the proteins it may be partly reduced during the heating with HCl, and if detected on paper chromatograms it may have been formed by oxidation on the filter paper³. By the qualitative means used in the present investigation it cannot be decided whether the presence of cysteine in the proteins may increase the amount of methionine formed from hypothetically present methionine sulfoxide. The method suggested by Dent³ for the purpose of distinguishing between methionine and methionine sulfoxide in solution is not reliable.

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