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Micro Determination of Cysteine plus Cystine in Proteins

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A method is described for microdetermination of the total amount of cysteine and cystine in proteins. By means of hydrazinolysis the cysteine and cystine sulphur is transformed to hydrogen sulphide which is subsequently measured colorimetrically as bismuth sulphide. Quantities of 20–200 μg cysteine plus cystine can be determined. Neither methionine nor ammonium sulphate interfere with the reaction.

The method of Kuratomi, Ohno and Akabori [1] for the determination of cysteine plus cystine in proteins is very convenient. The procedure involves reduction of cysteine- and cystine-sulphur by hydrazine hydrate to hydrogen sulphide which is determined by Caro's reaction (dimethyl-*p*-phenylendiamine + H_2S → methylene blue) followed by colorimetry.

The method works with amounts of 10 to 50 μmole of cystine or 2 to 10 mg. With smaller amounts the Caro reaction does not give quantitative results. Thus with proteins containing 2 % cysteine plus cystine at least 100 mg are required for a single determination. In protein work such amounts are not always available and it is necessary to analyse much smaller quantities.

The aim of this work was therefore to find a method 50 to 100 times more sensitive. This was obtained by leading the hydrogen sulphide formed by hydrazinolysis into a solution of bismuth tartrate whereby bismuth sulphide was formed. When held in colloidal form the bismuth sulphide could be measured colorimetrically [2].

REAGENTS

Hydrazine hydrate, 99–100 per cent (British Drug Houses LTD).

Bismuth reagent. Dissolve 2.2 g $Bi(NO_3)_3 \cdot 5H_2O$ in 250 ml of a 3.2 per cent solution of mannitol in water. Add 80 ml redistilled glycerol (Merck) and 360 ml of a 2.5 per cent solution of gum arabic in water. Dilute to 1,000 ml with 0.2 N acetate buffer pH = 5.2 (11 vol. 0.2 N NaAc + 3 vol. 0.2 N HAc) and filter. The reagent is stable for at least one month.

Cystine standard. Dissolve 40 mg recrystallized cystine in a small amount of 0.1 N hydrochloric acid in a 100 ml volumetric flask and dilute to the mark with distilled water.

6 N sulphuric acid.

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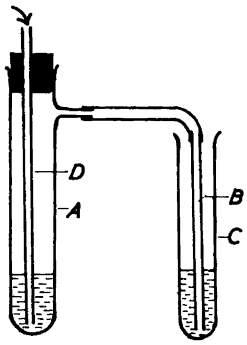

Fig. 1. Arrangement for transferring the hydrogen sulphide into the bismuth reagent. Explanation is given in the text.

PROCEDURE

From the standard cystine solution amounts of 50 to 500 μl (= 0.02 to 0.20 mg cystine) are pipetted into small ampullas or tubes and an equal volume of hydrazine hydrate is added. Into another series of tubes the protein to be analysed are placed in amounts equal to 0.02 to 0.2 mg cystine, *i.e.* at least 1 mg of a protein containing 2 % cysteine plus cystine. The protein can be placed in the tubes either in dry form or in solution. Hydrazine hydrate is then added in such amounts that the final concentration will be 50 to 100 %. The tubes are sealed and heated for 18 h at 120°C. After cooling the tubes are opened and the contents quantitatively transferred by means of 1—2 ml water to a test tube fitted with a side arm (tube A in fig. 1). The side arm of tube A is connected to a glass tube B by polyethylene tubing. B is placed into another test tube C containing 4 ml of bismuth reagent. The top of tube A is closed by a rubber stopper through which a glass tube D is inserted. Through D is added 2 ml 6 N sulphuric acid and nitrogen gas is immediately passed through the system at a rate of about three bubbles per second. The nitrogen gas is first washed by passing it through a 10 % solution of sodium hydroxide. Tube A is placed in a water bath at 60°C. The liberated hydrogen sulphide is under these conditions quantitatively transferred to the bismuth reagent within 15 min. The brown colour of the bismuth sulphide formed is read in a spectrophotometer at 400 mμ. Bismuth reagent serves as blank.

From a standard curve the cysteine + cystine content of the analysed protein sample is read. In our laboratory the equation of the standard curve was found to be:

$$\text{mg cysteine + cystine in the sample} = A^{1\,\text{cm}}_{400\,\text{m}\mu} \times 0.32 + 0.008.$$

EXPERIMENTAL

Hydrazinolysis. The final concentration of hydrazine hydrate during hydrolysis was varied between 40 and 99 % and time of hydrolysis was varied between 12 and 24 h without change in the results. A concentration of about 50 % and hydrolysis for 18 h was chosen for convenience.

Aeration of hydrogen sulphide. Nitrogen gas was allowed to pass the system for varying times. An aeration time of 5 min gave a yield of 80 %, after 10 min the yield was close to 100 % and after 15 min or longer the yield was constant 100 %. It was observed that when new polyethylene tubings were used there was a considerable loss of hydrogen

Table 1. Analyses with the given method on different combinations of sulphur containing substances.

Analysed	$A^{1\,cm}_{400\,m\mu}$	mg found mg = A × 0.32 + 0.008	Per cent of theoretical
0.1 mg methionine	0.002	0.0	
0.2 mg »	0.000	0.0	
1 mg ammonium sulphate	0.000	0.0	
2 mg » »	0.000	0.0	
0.051 mg cystine	0.135	0.051	100
0.077 mg »	0.215	0.077	100
0.077 mg » + 0.1 mg methionine	0.225	0.080	104
0.077 mg » + 0.2 mg »	0.211	0.075	98
0.077 mg » + 1 mg ammonium sulphate	0.210	0.075	98
0.077 mg » + 2 mg » »	0.220	0.079	103
1.87 mg β-lactoglobuline	0.170	0.063	
1.87 mg »	0.176	0.065	
1.87 mg » + 0.1 mg methionine	0.178	0.065	102
1.87 mg » + 0.2 mg »	0.178	0.065	102
1.87 mg » + 1 mg amm. sulphate	0.178	0.065	102
1.87 mg » + 2 mg » »	0.180	0.066	103
1.87 mg » + 0.051 mg cystine	0.330	0.114	99
1.87 mg » + 0.077 mg »	0.425	0.146	104

sulphide and small amounts of sulphur could be seen inside the tubing. However, if a surplus amount of hydrogen sulphide was allowed to pass the tubing before use, no further loss could be measured.

Colorimetry. The brown colour of bismuth sulphide has no absorption maximum, the absorption increases with decreasing wavelength. Thus at 400 mμ the absorption is twice that read at 475 mμ and five times that read at 575 mμ. In this work a Beckman spectrophotometer model B was used with 1 cm cells and readings at 400 mμ.

Specificity of the method. This was investigated by analysing different combinations of cystine, methionine, ammonium sulphate and protein in the following way: (see also Table 1).

1. Methionine or ammonium sulphate gave no absorbancy after hydrolysis.
2. Addition of methionine or ammonium sulphate to cystine standards gave no increase in absorbancy.
3. Addition of methionine or ammonium sulphate to protein samples gave no increase in absorbancy.
4. Addition of cystine to protein samples gave full recovery.

With the described method the content of cysteine plus cystine in crystalline β-lactoglobuline was found to 3.4 % and in crystalline insulin to 11.7 %. Tristam [3] gives the respective values to 3.39 and 12.0—12.5. The cysteine plus cystine content in pea albumin was found to be 5.5 %, in vicilin 0.0 % and in legumin 0.9 % [4]. In orosomucoid the value 1.7 % was found.

The method works with an accuracy within ± 5 %.

REFERENCES

1. Kuratomi, K., Ohno, K. and Akabori, S. *J. Biochem. (Tokio)* **44** (1957) 183.
2. Koren, H. and Gierlinger, W. *Mikrochim. Acta* **1953** 220.
3. Tristram, G. R. *Advances in Protein Chem.* **5** (1949) 83.
4. Goa, J. and Strid, L. *Arch. Mikrobiol.* **33** (1959) 253.

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