Anomalous titrations with cysteine

It has already been reported by SLUYTERMAN[1] and by STAIB AND TURBA[2] that cysteine titrated amperometrically against Ag^+ shows 1.3–1.6 times its theoretical –SH content. Similar findings have now been made in the case of potentiometric titrations of cysteine hydrochloride against *p*-chloromercuribenzoic acid for –SH determinations by the method of CALCUTT AND DOXEY[3].

The cysteine hydrochloride used in this work has been obtained in small lots from British Drug Houses Ltd., London. This was supplied as containing not more than 1 % water, but on drying over P_2O_5 no weight loss was found. Samples as received from the suppliers have usually been titrated to the theoretical –SH content. After being held in the laboratory for periods of 1–6 weeks the apparent –SH content by potentiometric titration against *p*-chloromercuribenzoate has been found to increase. Further titrations at later intervals showed that the increase—once it had appeared—was progressive. In one case a sample rose from its theoretical –SH content to 2.25 times this figure during a period of 4 weeks. Titration curves for samples giving a theoretical and an anomalous end point are shown in Fig. 1.

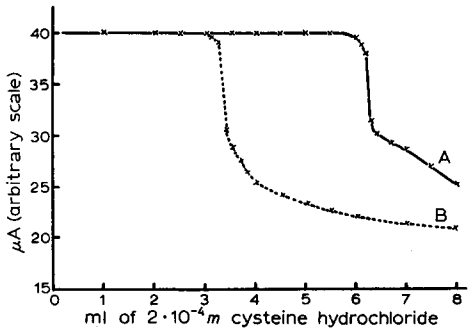

Fig. 1. The potentiometric titration of 25 ml $5 \cdot 10^{-5}$ *M* *p*-chloromercuribenzoate against $2 \cdot 10^{-4}$ *M* cysteine hydrochloride. Curve A, Sample of cysteine hydrochloride showing theoretical end point; curve B, Sample of cysteine hydrochloride showing an apparent 187% of theoretical –SH.

It has been suggested that such anomalous findings are due, either to reaction of the –SH group with more than one Hg, or to reaction of the mercury with the amino or carboxyl groups of the cysteine. In the latter event this would presuppose some internal rearrangement of the cysteine molecule to allow such reaction. It is widely recognised that internal rearrangements of molecules can be brought about by heat. Considerations of conditions in the laboratory showed that cysteine samples were being submitted to temperatures up to 35° during storage.

This possibility of effects due to heating was tested experimentally. A sample of cysteine which titrated to its correct –SH content was divided into two lots. One lot was maintained in the dark at a temperature below 20° whilst the other lot was incubated at 37° in the dark. At successive daily intervals samples were taken from the two lots and titrated against *p*-chloromercuribenzoate. Over a period of 4 days the lot left at 20° showed no deviations from its theoretical –SH content but the incubated lot titrated as 1.03; 1.07; 1.09 and 1.15 times the theoretical content over the same period. The possibility of similar effects due to heat in commercial samples is

obvious from the fact that a common method of drying cysteine (and its hydrochloride) is by warming in a stream of nitrogen.

If internal rearrangement does occur in cysteine then solution and recrystallisation might be expected to restore the original state. A sample of cysteine hydrochloride which had changed to give 2.1 times its theoretical –SH content was dissolved in distilled water, concentrated *in vacuo* and precipitated with conc. HCl. The precipitate was filtered off and divided into two lots. One was dried in a stream of warm nitrogen and on titration gave an –SH content of 1.3 times the theoretical. The second lot was dried by successive washes of acetone and finally ether. This lot on titration gave a theoretical –SH content. Left in the dark at a normal room temperature (not over 22°) this particular sample has not varied from its theoretical –SH content during 8 weeks.

The evidence given above is strongly suggestive that anomalous cysteine titrations are the result of internal rearrangements of the molecule leading to availability of amino or carboxyl group for reaction. Certainly, the evidence indicates the necessity for the avoidance of heat during the final stages of preparation or storage of cysteine or its hydrochloride.

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Reduction of protein disulfide bonds by sodium borohydride

Although a number of substances have been used for the reduction of disulfide bonds in proteins there still exists a need for more satisfactory means of reducing such disulfides quantitatively[1]. MOORE *et al.*[2] described the use of $NaBH_4$ to reduce quantitatively disulfides in a number of proteins in 8 *M* urea. This paper describes the use of $NaBH_4$ for the quantitative reduction of disulfide bonds in proteins in aqueous solution in the presence and absence of the denaturant, sodium dodecyl sulfate.

Reagents and proteins were of commercial origin except whale myoglobin which was isolated from muscle tissues by ammonium sulfate precipitation as described previously[3]. The extent of the reduction was determined by measuring sulfhydryl concentration by amperometric titration[3,4]. All titrations were conducted in a nitrogen atmosphere; reduced glutathione served as a standard. Titration values were corrected by a blank value (10 % or less of total) obtained by titrating a solution containing $NaBH_4$, buffer and other additives, but no disulfide-containing material. The titrations were readily reproducible and the standard titration value was always within 5 % of theory.

Reduction of the proteins and other materials was achieved by combining solutions of the reactants (0.10–2.00 μmoles protein + 1 ml of a 25 mg/ml freshly

Abbreviation: SDS, sodium dodecyl sulfate.