Cystine and cysteine peaks in amino acid analysis

Aminograms obtained using semi-automated amino acid analyzers universally lack a peak labeled cysteine[1–3]. Indeed, it has not been specified whether there are separate peaks for cysteine and cystine; it is probably assumed that any cysteine present in original samples is rapidly oxidized to cystine unless immediately converted to some stable derivative[4]. It seems at least theoretically possible that the "cystine" labeled peak is actually cysteine due to the large excess of the antioxidant thiodiglycol (2,2'-thiodiethanol) in the eluant buffers, which could potentially react with and reduce cystine by disulfide–sulfhydryl interchange reaction[5]. A serious objection to this idea is the fact that the interchange reaction occurs only to a very limited extent at the low pH (pH 3.2 in our system) at which the "cystine" peak emerges. Also, other analogous sulfhydryl–disulfide pairs such as glutathione, pencillamine, homocysteine–homocystine, etc. and mixed disulfides have individual peaks, indicating that the dithioglycol does not reduce the disulfide form to thiols.

The present work was performed to ascertain the identity of the "cystine" peak and to determine the elution position of both cystine and cysteine.

Methods

The version of the Amino Acid Auto Analyzer available April, 1965, was used for this study. The components have been described in detail elsewhere[6], but the following should be mentioned here specifically. The nine-chamber Autograd device was used for simultaneously producing a smooth pH gradient between 2.875 and 5.000; five and two ml of methanol were added to the first and second chambers, respectively, in place of an equal volume of buffer. Chromobead resin Type A, 8 % cross-linked, spherical particles having a specific diameter of 18 to 25 μ. A cyanide ninhydrin reagent was used for analysis[7]. Norleucine and cysteic acid were used as internal standards[8]. Cysteine HCl solutions were freshly prepared before applying to the column.

^{35}S cystine was purchased from Schwarz Bioresearch. Cystine and cysteine were separated by paper chromatography in the system butanol–propanol–0.1 *N* HCl (3:1:1). Samples were applied in the hydrochloride form. Chromatogram scanner No. RSC 363, available from Atomic Accessories, was used to examine radioactive strips.

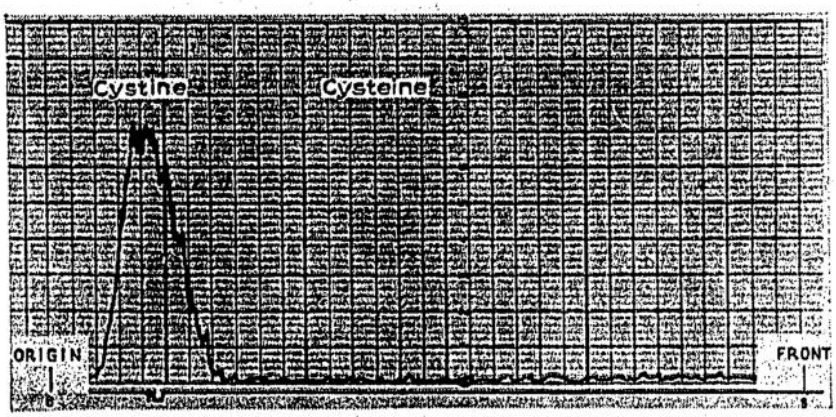

Fig. 1. Radiogram of the "cystine" fraction collected directly from the column. Radioactivity is only present in the location of cystine. The expected position of cysteine is indicated.

Results

To determine the identity of the "cystine" peak, 2.0 μmoles of ^{35}S cystine were applied to a column and the effluent stream diverted from the analyzing portion of the system at the time the peak was to emerge. An aliquot was chromatographed on Whatman No. 1 paper and the chromatogram was radioassayed (Fig. 1). Only the radioactive and ninhydrin-positive peak for cystine appears (R_F about 0.15). Cysteine, if present, would have an R_F of about 0.44.

The pattern obtained with 0.5 μmole of cysteine HCl is shown in Fig. 2. A very small peak is present at the cystine location, equivalent to 0.015 μmole of half cystine or 3 % of the cysteine added. Another peak emerges at about 5 h (150 ml) in this system. This peak is identified as cysteine and has a 570 mμ/440 mμ ratio of 0.590.

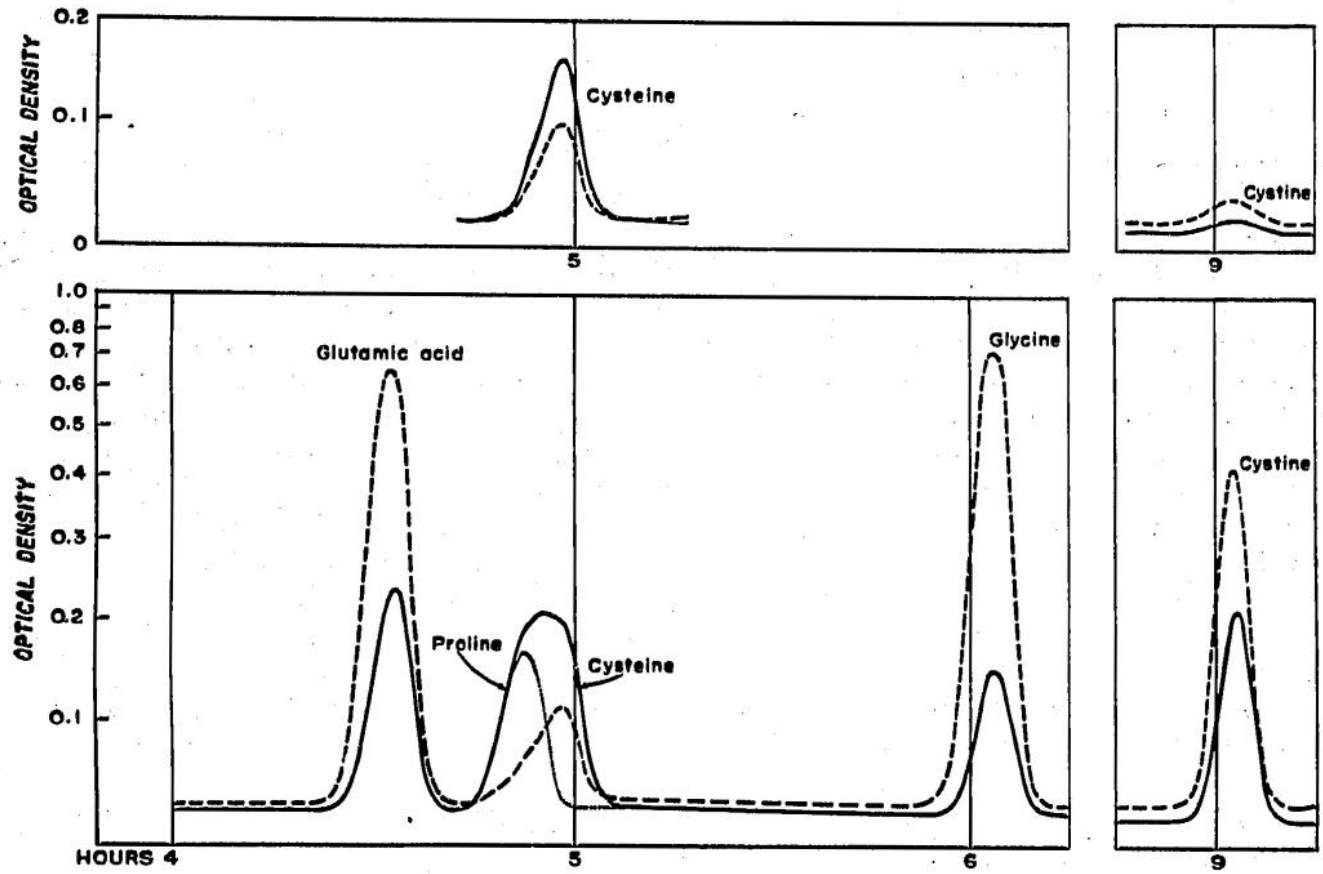

Fig. 2. Aminograms of 0.5 μmoles cysteine (top) and 0.5 μmoles cysteine plus 0.25 μmoles each of a standard amino acid mixture (bottom). Only a small fraction of the cysteine added appears as cystine. The cysteine peak is to the right and merges with proline. The location of proline with no cysteine added is indicated. Symbols: – – – = absorption at 570 mμ; ——— = absorption at 440 mμ; · · · = location of proline in absence of cysteine.

The color yield is about 6 % that of norleucine at 570 mμ. Also shown in Fig. 2 is the pattern obtained when cysteine is added with a mixture of standard amino acids. The cysteine peak emerges almost simultaneously with the proline peak.

The identity of the cysteine peak was confirmed by comparing it to the absorption spectrum of cysteine HCl treated with the same ninhydrin reagent used in the automated analysis. Peaks were found at about 565 mμ, 460 mμ, and 410 mμ (Fig. 3).

The cysteine peak could also be found with virtually complete recovery when cysteine was added to a urine sample and immediately applied to the column.

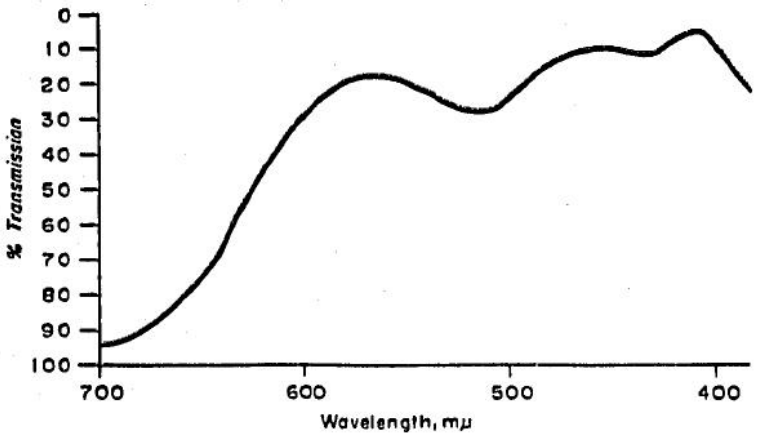

Fig. 3. The absorption spectrum of cysteine treated with the ninhydrin reagent used in the amino acid analysis. The peaks obtained and their relative intensities are consistent with the peak labeled as cysteine in Fig. 2.

Discussion

The results indicate that the peak labeled "cystine" in automated amino acid analysis is indeed cystine. Cysteine emerges as a well separated peak with a color yield much less than any other of the common α-amino acids. No doubt it could be obscured by other amino acids or remain unnoticed when present in amounts normally placed on the column. Because of its low color yield, the direct analysis of solutions for cysteine is probably not practicable in most cases; however, the fact that cysteine can retain its identity separate from cystine should be used in routine analysis. Thus, if it is the practice to use the "cystine" peak to represent cystine plus cysteine, the conversion of cysteine to cystine should be complete before adding the sample to the column.

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