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Short Communication

Sulphur compounds

CLVII.☆ Determination of cysteine-*S*-sulphonate by ion-pair chromatography and its formation by autoxidation of cysteine persulphide

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ABSTRACT

Chromatographic analyses and UV spectra show that alkaline aqueous solutions of cystine (or homocystine) and sulphide contain the anions HS^-, RS^-, RSS^-, and $RSSO_3^-$ (R = alanyl). The latter species originates from the autoxidation of the persulphide RSS^- by air. Under special conditions, $S_2O_3^{2-}$ and R_2S_3 are observed in addition.

INTRODUCTION

Cystine is an important constituent of most proteins. Its disulphide bond links neighbouring parts of the amino acid chains and it is therefore responsible for the conformation of the protein. Reductive or nucleophilic cleavage of the disulphide bridge changes the chemical and physical properties of the protein considerably, as is used, for instance, in the treatment of hair and wool. The two most important nucleophiles in this context are probably sulphide (S^{2-} and HS^-) and sulphite, which react with organic disulphides according to eqns. 1 and 2:

$$RSSR + HS^- \rightleftharpoons RSS^- + RS^- + H^+ \quad (1)$$

$$RSSR + SO_3^{2-} \rightleftharpoons RSSO_3^- + RS^- \quad (2)$$

In the case of cystine (bis-alanyldisulphane), the "persulphide" RSS^- (alanyldisulphide) formed in reaction 1 has been detected by its UV absorption spectrum, which is characterized by a maximum at 335 nm in 0.2 *M* sodium hydroxide solution [2]. According to Rao and Gorin [2], reaction 1 is relatively slow ("half-life" of *ca.* 12 min at concentrations of 0.0185 *M* for cystine and 0.069 *M* for sulphide at 25°C) and does not go to completion. Lipoic acid also reacts with sulphide in 0.1 *M* NaOH to form a persulphide absorbing near 335 nm [3,4]. Reaction 2 is also relatively slow in the case of cystine at pH 7 and 20°C, taking *ca.* 15 min for completion [5]. Because of its importance for the chemistry of proteins this reaction has been studied extensive-

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☆ For Part CLVI, see ref. 1.

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ly; at pH > 9 reaction 2 is reversible [5–10].

Cysteine-*S*-sulphonate is also formed on treatment of cysteine with thiosulphate at pH 7–7.4 (eqn. 3) [11]:

$$RSH + S_2O_3^{2-} \rightleftharpoons RSSO_3^- + HS^- \quad (3)$$

A convenient preparation of the sodium salt of cysteine-*S*-sulphonate from cysteine and sodium tetrathionate has been reported [12]; the product obtained was shown by X-ray crystallography to have the composition $C_3H_6O_5NS_2Na \cdot 3/2\ H_2O$ [13].

Inorganic sulphide (HS^-), polysulphide (S_x^{2-}), sulphite (SO_3^{2-}), thiosulphate ($S_2O_3^{2-}$) and polythionate ($S_nO_6^{2-}$) anions can be separated and determined by ion-pair chromatography [14–18]. This method uses an inert organic stationary phase and a water–acetonitrile mixture containing tetrabutylammonium ions and a buffer as a mobile phase. We have now applied this analytical technique to study reactions 1 and 2.

EXPERIMENTAL

The experimental conditions were similar to those described in ref. 18, and the same chromatographic and spectroscopic equipment was used. The UV detector (variable wavelength) was operated at 210 or 215 nm. A PLRP-S column (Polymer Laboratories, 120 mm × 4 mm I.D., particle size 8 μm) was used. The eluent compositions used for the results shown in Figs. 1, 3 and 4 were in the following range: 91.5–93.0% water (doubly distilled), 7.0–8.5% (v/v) acetonitrile, 2 mmol/l tetra-*n*-butylammonium hydroxide, 1 mmol/l Na_2CO_3. The eluent was degassed, and in some cases oxygen-free helium was bubbled through the eluent; but even in this case it was not possible to detect RSS^- chromatographically.

Cysteine, cystine (both from Merck; biochemical standards), homocysteine and homocystine (both from Fluka, purum) were used as delivered. $Na_2S \cdot 7H_2O$ (Merck) was recrystallized weekly from deoxygenated water to remove thiosulphate, sulphite and polysulphide [18]. Sodium cysteine-*S*-sulphonate (Bunte salt of cysteine) prepared after Inglis and Liu [12] gave excellent analytical data (C, H, N, S).

The sodium salt of alanylthiosulphate, $RSSO_3Na \cdot 3/2\ H_2O$, prepared by the published procedure [12], was used to determine the calibration function shown in Fig. 1; this function is linear up to at least 3 mmol/l provided the peak height is used.

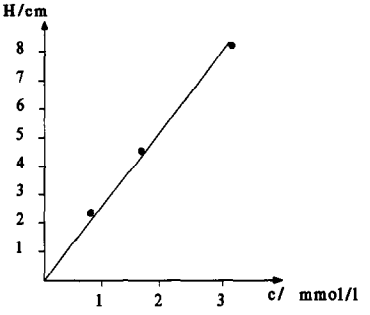

Fig. 1. Calibration function (peak height *H versus* concentration of sodium cysteine-*S*-sulphonate (detected at 210 nm, flow 1 ml/min, sample loop 50 μl).

All solutions were prepared with exclusion of oxygen unless stated otherwise. $Na_2S \cdot 7H_2O$ was dissolved in oxygen-free water and RSH or R_2S_2 in dilute NaOH of pH 11.0–13.0. The clear solutions were then mixed in appropriate ratios.

RESULTS

When D,L-cystine, dissolved in aqueous NaOH at pH 13.0, was treated with aqueous Na_2S in a molar ratio of 1:1 at 20°C, the above-mentioned UV absorption maximum at 335 nm appeared (Fig. 2a). A similar spectrum was obtained when homocystine was used at the same molar concentration (peak maximum at 335 nm). To check the air sensitivity of the alanyldisulphide (RSS^-) oxygen was bubbled into a solution of cystine and Na_2S at pH 13.0 (molar ratio 19:1): Fig. 2b demonstrates that the persulphide absorption disappeared almost completely within 45 min.

Both the unoxidized and the oxygenated persulphide solutions were analysed by ion-pair chromatography. Fig. 3a shows the chromatogram of the same solution as used to record the UV spectrum of Fig. 2a (curve 3). The expected components HS^-, RS^- and R_2S_2 (R = alanyl) are well separated (peaks 1, 2 and 3). In addition, two minor peaks were observed which could definitely be assigned to the trisulphane R_2S_3 [19] and the Bunte salt anion $RSSO_3^-$. All peaks were assigned on the basis of

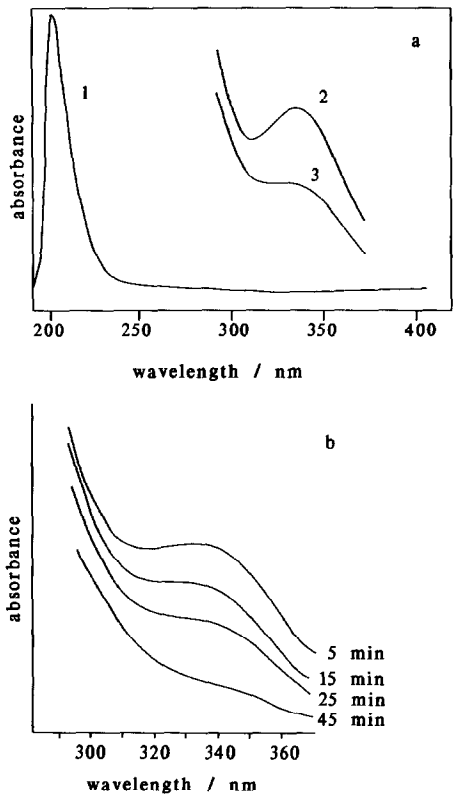

Fig. 2. (a) UV absorption spectra of cysteine persulphide (curve 3), homocysteine persulphide (curve 2) and cysteine-*S*-sulphonate (curve 1). Curves 2 and 3 were recorded by a conventional spectrometer (solvent: water–sodiumhydroxide). Curve 1 applies to the solvent acetonitrile–water (8.5/91.5, v/v) recorded by a diode-array detector after chromatographic separation; peak maximum at 200 nm. (b) Spectral changes when oxygen was bubbled into a solution of cysteine persulphide for the time indicated (0.038 mol/l cystine, 0.002 mol/l Na_2S in water–sodiumhydroxide of pH 13.0 at 20°C; solution prepared with exclusion of oxygen).

their retention times and UV spectra (recorded on-line using a diode-array detector) by comparison with chromatograms and spectra obtained with authentic samples. No peak for the persulphide RSS^- was observed and the UV spectra of the substances causing the large peaks 2 and 3 did not show any UV absorption near 335 nm, which would indicate that RSS^- was obscured by either RS^- or R_2S_2. Obviously, the persulphide concentration was too small for chromatographic detection owing to the secondary reactions 4 and 5 which remove persulphide:

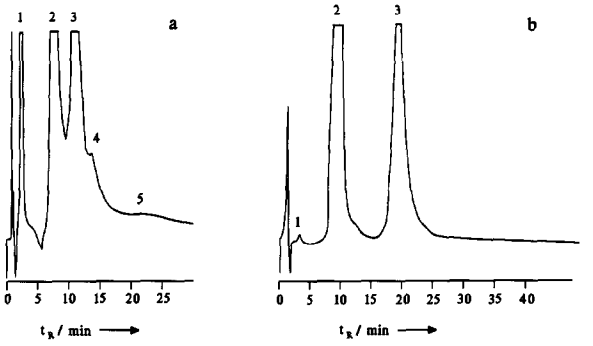

Fig. 3. (a) Ion-pair chromatogram of a solution of 1.00 mmol of cystine and 1.04 mmol of Na_2S in dilute NaOH (pH 13.0) with exclusion of oxygen; peaks: 1 = HS^-; 2 = RS^-; 3 = R_2S_2; 4 = R_2S_3; 5 = $RSSO_3^-$; R = alanyl. (b) Chromatogram of a solution of 0.20 mmol of cystine and 0.25 mmol of Na_2S at pH 11.0 after exposure to air for 48 h at 20°C; peaks: 1 = HS^-; 2 = R_2S_2; 3 = $RSSO_3^-$.

$$RSS^- + 3/2\ O_2 \rightarrow RSSO_3^- \quad (4)$$

$$RSS^- + R_2S_2 \rightleftharpoons RS^- + R_2S_3 \quad (5)$$

Reaction 4 is analogous to the well-known autoxidation of inorganic polysulphide yielding thiosulphate, eqn. 6 [20]:

$$S_2^{2-} + 3/2\ O_2 \rightarrow S_2O_3^{2-} \quad (6)$$

When an alkaline cystine solution was treated with Na_2S in a molar ratio of 1:1.25 (pH 11.0) and kept in air for 48 h, the chromatogram shown in Fig. 3b was obtained. It reveals cystine and the Bunte salt as the main constituents (peaks 2 and 3); the UV spectrum of peak 3 (Fig. 2a, curve 1) is identical to the UV spectrum of cysteine-*S*-sulphonate prepared from cysteine and tetrathionate [12].

The peak for the cysteine-*S*-sulphonate was also observed when cystine was treated with sulphite at pH 9.5 and 20°C (0.025 mmol of cystine and 0.05 mmol of sulphite in 10 ml of water, reaction time 1–24 h).

When aqueous cystine was treated with Na_2S at 20°C in a molar ratio of 1:5 (pH 11.0), the products found were dependent on whether or not air had access to the solution. In Fig. 4a the chromatogram of the mixture which was kept under nitrogen for 150 min is shown. Besides HS^-, the presence of RS^- and R_2S_2 is evident. Fig. 4b, on the other hand, demonstrates that the same mixture stored in

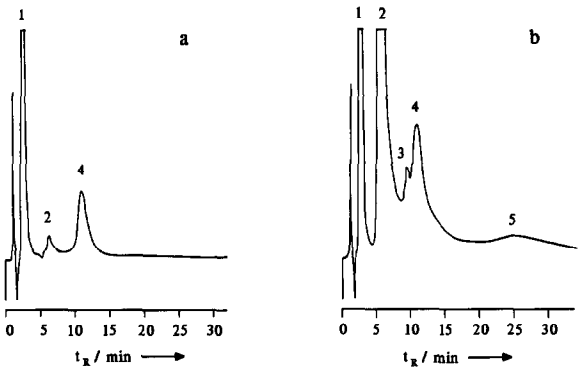

Fig. 4. Chromatograms of aqueous solutions prepared from 0.025 mmol of cystine and 0.125 mmol of Na_2S at pH 11.0. (a) Exclusion of oxygen; (b) exposure to air for 150 min. Peaks: 1 = HS^-; 2 = RS^-; 3 = $S_2O_3^{2-}$; 4 = R_2S_2; 5 = $RSSO_3^-$.

air for 150 min was composed of HS^-, R_2S_2, $S_2O_3^{2-}$ and $RSSO_3^-$, originating from reactions 1, 4, 6 and 7:

$$RSS^- + HS^- \rightleftharpoons RS^- + S_2^{2-} + H^+ \quad (7)$$

Another explanation for the presence of thiosulphate may be the oxidation of HS^- by oxygen, which is known [18] to produce $S_2O_3^{2-}$, S_x^{2-}, SO_3^{2-} and, finally, SO_4^{2-}.

CONCLUSION

Cysteine, homocysteine, cystine, homocystine and cysteine-*S*-sulphonate can be separated by ion-pair chromatography. Reaction of sulphide with cystine produces the persulphide, which reacts with oxygen to give the *S*-sulphonate and with cystine to give the trisulphide.

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