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Reactions of Thiosulphate with Proteins

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It has been shown that serum proteins corresponding electrophoretically to α_1-globulin bind the whole molecule of thiosulphate, whereas albumin, α_2- and β-globulins bind only the outer sulphur of thiosulphate. Oxytocine and papain bind the whole molecule of thiosulphate presumably to the disulphide group. In view of the possibility that thiosulphate reacts with the disulphide grouping, the rate of reduction of cytochrome *c* by thiosulphate in the presence of either cystine or cystamine was investigated. An acceleration of reduction rate was found in both cases.

Sulphur compounds are known to form a variety of mixed derivatives, but little information is available in the literature as to their possible role in biological systems, except for mixed disulphides. In the present paper experiments with thiosulphate as one of the intermediates of sulphur metabolism are reported. The studies involved some reactions of thiosulphate with proteins. Attention was also paid to the reduction of cytochrome *c* by thiosulphate in the presence of disulphide.

EXPERIMENTAL

Materials and methods

Radioactive thiosulphate labelled with ^{35}S in the inner or outer position (Sp. activity 10.6 mc/mM) were provided by the Radiochemical Centre, Amersham, England. Cytochrome *c*, pharmaceutical product of Biomed, Craców, was obtained from beef hearts by the adsorption method and proved to be spectrally pure according to Paléus[1]. Papain was purified from Light's preparation by the method of Kimmel and Smith[2]. The oxytocine used was a pharmaceutical product of Sandoz, Basel (10 IU/ml). All reagents used were either analytical grade or proved to be pure. Electrophoresis and chromatography were carried out on Whatman No. 1 filter paper. Reduction of cytochrome *c* was estimated spectrophotometrically with Hilger's "Uvispec". The estimation of radioactivity on paper strips was made with the automatic scanning device of Frieseke and Hoepfner.

RESULTS

1. Reactions of thiosulphate with serum proteins were investigated in fresh human sera. Thiosulphate labelled in the outer or in inner position was added in the proportion of 1 mg to 0.5 ml of serum. The added radioactivity was in the

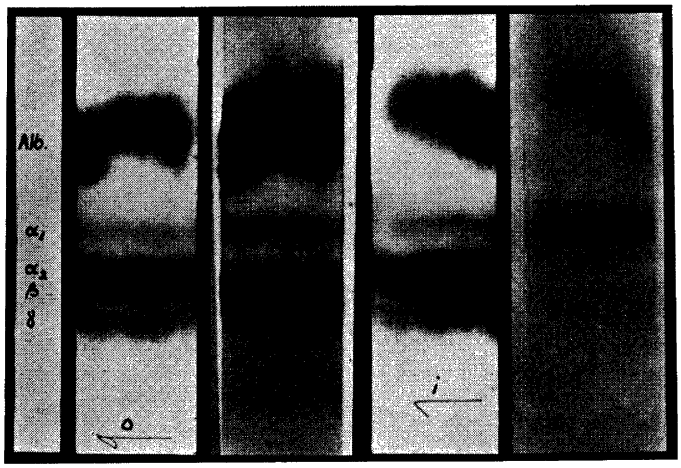

Fig. 1. Electrophorograms (o, i) and corresponding autoradiograms (O, I) of serum proteins incubated with thiosulphate labelled with ^{35}S : o, O — outer sulphur radioactive, i, I — inner sulphur radioactive.

range of 40–60 μC. After incubation for 30 min the serum was applied to paper strips and submitted to electrophoresis in veronal buffer, pH 8.6, ionic strength 0.05. Under experimental conditions free thiosulphate migrated beyond the paper strips. Autoradiograms were prepared from the electrophorograms and then the proteins were located with bromothymol blue. Typical results are represented in Fig. 1. It can be seen that radioactive sulphur has been bound to proteins in a different way depending on whether it occupied the inner or outer position in the thiosulphate. Radioactivity of the inner sulphur of thiosulphate occurred only in the fraction corresponding to α_1-globulin, whereas radioactivity of the outer sulphur atom was bound to albumin, α_1-, α_2- and β-globulins. This observation was supported by radioactivity measurements of the electrophorograms (before staining) and equal values were found for the α_1-globulin band regardless of the position of radioactive sulphur in the thiosulphate. These results are interpreted to mean that α_1-globulin combined with whole molecules of thiosulphate, whereas the other protein fractions were acceptors for outer sulphur only.

2. In view of the possibility that thiosulphate reacts with the disulphide grouping, the products formed with oxytocine were investigated. To a neutralized oxytocine solution of 0.5 ml volume was added 0.1 mg of differently labelled thiosulphate, and the mixture incubated for 30 min. The aliquots were then chromatographed on filter paper using butanol-acetone-water (2:2:1) as solvent, or submitted to electrophoresis. The corresponding autoradiograms revealed minor spots perhaps due to some impurities but the main pictures were similar for the two kinds of thiosulphate. Thus it may be concluded that the whole thiosulphate molecule is bound by the oxytocine moiety.

3. Similar experiments with papain and thiosulphate labelled in the outer or

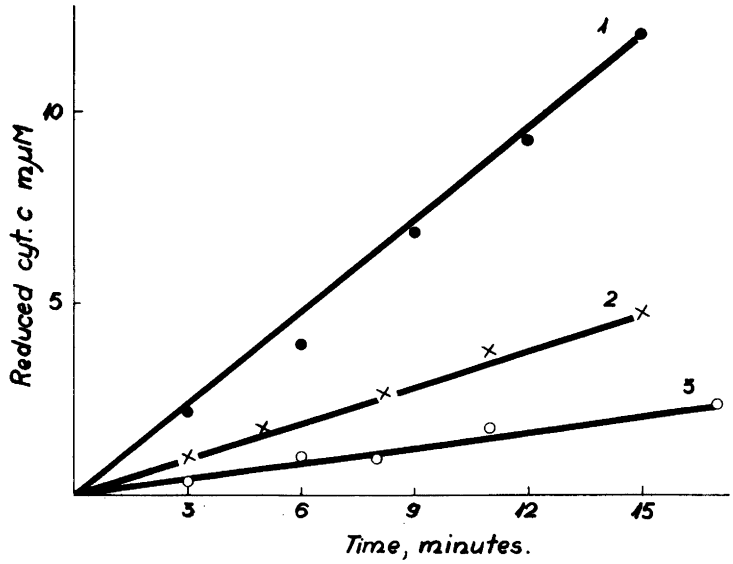

Fig. 2. The rate of reduction of 0.1 μmoles of cytochrome *c* by 10 μmoles of thiosulphate in the presence of: (1) 1 μmole cystamine; (2) 0.1 μmoles cystine; (3) 0.2 ml serum. Medium: 0.1 M phosphate buffer, pH 7. Final volume 2.5 ml. Blank values for the reduction due to thiosulphate alone was about 0.4 mμmoles and that for serum alone about 0.8 mμmoles of cytochrome *c* after 15 min. Temp. 14°C.

inner position showed that in either case radioactivity was bound to the protein, as estimated on the basis of electrophorograms and autoradiograms. It appears then that papain as well as oxytocine is capable of binding the whole molecule of thiosulphate.

4. As shown by the experiments with oxytocine and by earlier work on the reaction of thiosulphate with cystine[3], thiosulphate can react with disulphide linkage. Thus it seemed probable that thiol groups formed in the course of reaction could alter the reductive properties of the solution. In order to prove such an assumption, the rate of reduction of cytochrome *c* by solutions of thiosulphate in a mixture with disulphide, *e. g.* cystine or cystamine, was studied. Neutral and dilute solutions of thiosulphate were found to reduce cytochrome *c* only very slowly. However, as can be seen in Fig. 2, the rate of reduction increased in the presence of disulphides. Serum also stimulated the reduction, though very slightly, and this effect might perhaps be related to the results of a preceding experiment.

DISCUSSION

Thiosulphate is known to be formed in sulphur metabolism as an intermediate[4,5], quickly undergoing oxidation in the animal organism[6]. It therefore seemed desirable to examine the possibility that thiosulphate reacts in biological systems. Two atoms of sulphur may be distinguished in the thiosulphate mole-

cule, and so two kinds of labelled thiosulphate are available, one labelled with ^{35}S in the outer position ($^*S \cdot SO_3^=$) and the other labelled in the inner position ($S \cdot {}^*SO_3^=$). The experiments described in this paper have shown that thiosulphate is not an inert compound and can in fact react with proteins as well as with disulphides. It also appears to be involved in the reductive processes. The experiments with serum proteins indicate that in its reactions with different electrophoretic fractions, thiosulphate can be bound in the form of the whole molecule or give off outer sulphur only. The combination of outer sulphur with the protein moiety has so far only been reported in the case of rhodanese[7,8]. The present experiments show that this kind of binding is more common.

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