

## Enzymatic Reduction of S-Sulfogluthathione in Rat Liver

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Since the discovery of S-sulfogluthathione (GSSO<sub>3</sub>H) in calf lens<sup>1</sup> little work on its biochemistry has been reported. Its presence in rat intestine has been demonstrated,<sup>2</sup> and an enzyme catalyzing its reduction by NADPH has been purified from pea tissues.<sup>3</sup> This communication describes the enzymatic reduction of GSSO<sub>3</sub>H by rat liver homogenates.

*Table 1.* Reduction of S-sulfogluthathione by rat liver homogenates.

The reaction mixture contained in a final volume of 2 ml: 0.5 ml of a 20 % rat liver homogenate in 0.14 M KCl; phosphate buffer 50 mM, pH 7.5; GSSO<sub>3</sub>H 2.9 mM; and (where indicated) 2.3 mM NADPH. After incubation at 30° for 60 min, the reaction was stopped by the addition of 1 ml of 10 % metaphosphoric acid. An aliquot of the centrifuged sample was passed through a Dowex 50 (H<sup>+</sup>) column and reduced by an electrolytical procedure.<sup>4</sup> After bubbling with nitrogen for 15 min, the formed GSH (equivalent to the GSSO<sub>3</sub>H in the sample) was determined with 5,5'-dithiobis-(2-nitrobenzoate).<sup>5</sup> Boiling completely abolished the activity.

Experiment	Remaining GSSO <sub>3</sub> H (mM)
1.	2.6
2. (not incubated)	2.5
3. (plus NADPH)	0.6
4. (minus GSSO <sub>3</sub> H)	0.04

The assay used was based on the determination of the GSSO<sub>3</sub>H consumption in the system. This was accomplished by removing formed GSH from the reaction mixture by ion-exchange chromatography, followed by reduction of the remaining GSSO<sub>3</sub>H to GSH, which was then determined. This procedure would not generally differentiate between an oxidative degradation (to give, e.g., the sulfonic acid, GSO<sub>3</sub>H)

and the proposed reduction of GSSO<sub>3</sub>H. However, it has been demonstrated that GSSO<sub>3</sub>H consumption is paralleled by a concomitant formation of GSH (to be published).

Since glutathione reductase from yeast or porcine erythrocytes does not catalyze the reduction of GSSO<sub>3</sub>H (B. Eriksson, unpublished experiments), it is probable that the reductive destruction of GSSO<sub>3</sub>H shown in Table 1 is due to an enzymatic activity distinct from glutathione reductase.

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## Preparation of Sodium Polysulfides by Solid and Molten State Reactions

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In connection with the recent interest in kraft pulping in the presence of sodium polysulfides, a number of different methods for the preparation of polysulfides have been considered. These are: dissolution of elemental sulfur in aqueous sodium sulfide,<sup>1</sup> oxidation of aqueous sodium sulfide either electrolytically,<sup>2</sup> or with air in the presence of alkaline lignin degradation products.<sup>3</sup> The possibility of making polysulfides by processing at elevated temperatures the sodium-sulfur compounds available in the recovery system of a kraft pulp mill (Na<sub>2</sub>SO<sub>4</sub>, Na<sub>2</sub>S, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) has not previously been considered. It is, however, known that polysulfides can be obtained

Table 1. The yields obtained in mole-% of charged sodium sulfide and sodium sulfate. Reaction temperature 900°C.

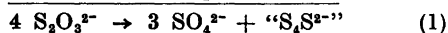
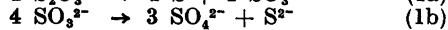
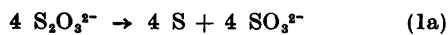
Reactants	Reaction time min	Yields		
		Polysulfide excess-S	Sulfide-S	Carbonate
Na <sub>2</sub> SO <sub>4</sub> + glucose	15	37	45	not detnd.
Na <sub>2</sub> SO <sub>4</sub> + CO	30	0	22	0
Na <sub>2</sub> SO <sub>4</sub> + CO	60	5	65	18
Na <sub>2</sub> S + CO <sub>2</sub>	15	24	64	29
Na <sub>2</sub> S * + Na <sub>2</sub> SO <sub>4</sub> * + C	15	10	47	9
—»—	30	26	45	25
—»—	60	24	56	18

\* Equimolar amounts.

by reacting sodium sulfide with elemental sulfur at elevated temperatures ( $\geq 400^\circ\text{C}$ ). Some of the polysulfides obtained were stable up to  $1300^\circ\text{C}$ .<sup>4</sup> Further, there are some indistinct claims that polysulfides can be formed when sodium sulfate and carbon are reacted in an electrode furnace,<sup>5</sup> or when thiosulfate is heated to  $300^\circ\text{C}$ .<sup>6</sup>

We have found that there are three possibilities for the formation of polysulfides from the above sodium-sulfur compounds, namely, thermal decomposition of thiosulfate, partial oxidation of sulfide, and partial reduction of sulfate. The reactions are performed at elevated temperatures in the absence of water, at atmospheric pressure and at restricted air contact. The reaction product should be cooled before dissolution in water, as the polysulfides otherwise partly decompose into thiosulfate and sulfide.

*Decomposition of thiosulfate.* When thiosulfate was heated to temperatures above  $450^\circ\text{C}$  in a pyrex vessel, it decomposed to polysulfide and sulfate probably according to:



The "S<sub>4</sub>S<sup>2-</sup>" here means a mixture of polysulfides with the stoichiometric composition Na<sub>2</sub>S<sub>5</sub>. In separate experiments it was shown that sulfite decomposed according to (1b) upon heating. On heating thiosulfate at  $550^\circ\text{C}$  for 15 min the yields obtained of sulfide and polysulfide excess sulfur were

11.3 % and 47.2 %, respectively. This is close to the theoretical values of 12.5 % sulfide and 50 % excess sulfur.

*Partial reduction of sulfate and partial oxidation of sulfide.* On partial reduction of sulfate or partial oxidation of sulfide, in the presence of carbonaceous material, and at reaction temperatures above  $800^\circ\text{C}$  polysulfide and carbonate were obtained (Table 1). Both reactions were carried out in zirconium dioxide or carbon crucibles.

The products obtained were analyzed acidimetrically.<sup>7</sup> The simultaneous formation of carbonate seems to be a prerequisite, as reduction of sulfate with pure hydrogen results in mixtures of sulfide and sulfate.

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