# On the Active Group in Rhodanese

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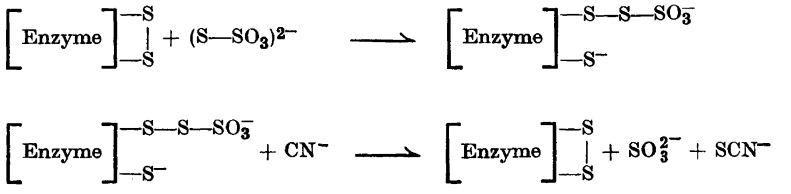
Saunders and Himwich [1] recently studied the effect of some inhibitors on rhodanese and found the enzyme to be inhibited by cysteine and other sulfhydryl compounds and in agreement with Lang [2] also by cyanide if added before the thiosulfate. They therefore suggested that the enzyme might contain a heavy metal as prosthetic group. On the other hand we [3] found no inhibition with cysteine, on the contrary it could protect the enzyme from the inhibition with cyanide. No inhibition was observed with other metal enzymes inhibitors, but sulfite in the absence of thiosulfate and strong oxidants were found to be potent inhibitors. Further investigations [4] showed that the cyanide inhibition was a comparatively slow reaction, 15 minutes being required for reaching 50 % inhibition in 0.001 *M* cyanide at pH 7.4. With purified preparations or aged homogenates the reaction was of the first order, but with fresh rat liver homogenates a much weaker inhibition was obtained and the reaction did not follow the first order curve. This was shown to be due to the presence of a sulfhydryl compound in the fresh homogenates, presumeably glutathione. Glutathione had a stronger protecting effect against cyanide inhibition than cysteine. The cyanide inhibition was not abolished by dialysis. These results seem to be incompatible with the assumption that rhodanese is a heavy metal enzyme.

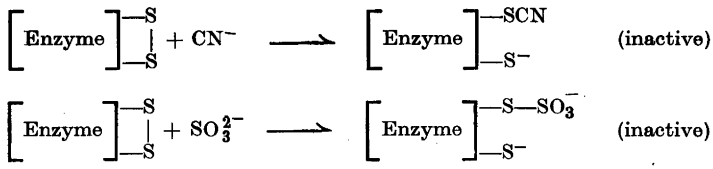
Sulfite was found to react more rapidly, 50 % inhibition being obtained in less than one minute in 0.001 *M* sulfite at pH 7.4.

The protecting effect of cysteine (and other sulfhydryl compounds) against cyanide can be explained if cysteine combines with the enzyme, giving a compound not attacked by cyanide. As no inhibition is obtained with cysteine alone, the cysteine must be easily displaced by thiosulfate. We thus have a case where thiosulfate, other sulfhydryl compounds, cyanide and sulfite react with the active group in the enzyme. This is easily explained if the active group is a disulfide linkage or a carbonyl group. The presence of an active carbonyl group in rhodanese has already been excluded [3] but all our findings are consistent with a disulfide linkage as the active group in the enzyme. The reaction mechanism could then be pictured as follows.

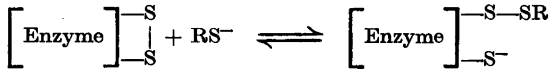
In the reaction scheme proposed, the enzymatic formation of thiocyanate from cyanide and thiosulfate thus consists of a hydrocyanolysis of one disulfide bond in the enzyme-substrate compound first formed from rhodanese and thiosulfate. Presumably sulfite is first split off, in analogy with similar reactions [5, 6] whereupon the labile enzyme-SCN compound breaks down, giving thiocyanate and regenerating the free enzyme. The inhibitions with cyanide or sulfite may be explained by the formulae:

The splitting of disulfide bonds with cyanide and sulfite has been studied by different authors, and the velocity, with






which the bond is attacked, is strongly influenced by other substituents in the molecule. Cystine and cyanide thus give thiocyanoalanine and cysteine [8], and cystine plus sulfite give a sulfinic acid and cysteine [9]. It is easy to understand that the corresponding reactions with the enzyme may destroy the activity of the latter. The protecting effect of sulfhydryl compounds ($RS^-$) may depend on the formation of a reversible compound.




The weak inhibition caused by *p*-chloromercuribenzoate, iodoacetate and iodosobenzoate [2,3] are also understandable, as sulfhydryl groups are released in the enzyme, when it combines with thiosulfate (eq. 1.).

Finally we found that rhodanese could not catalyse the reaction.

$$CNO^- + S_2O_3^{2-} \longrightarrow CNS^- + SO_4^{2-}$$

which could be predicted from the theory described above. No enzyme with a disulfide linkage as the active group has as yet to our knowledge been described, but their importance for the activity has been established in insulin and oxytocin. Gulland and Randall [10] studied the inactivation of the latter hormone with cyanide and sulfite, and obtained results similar with ours for the inactivation of rhodanese.

1. Saunders, J. P., and Himwich, W. A. *Am. J. Physiol.* **163** (1950) 404.
2. Lang, K. *Z. Vitamin. Hormon. Fermentforsch.* **2** (1949) 288.
3. Sörbo, B. H. *Acta Chem. Scand.* **5** (1951) 724.
4. Sörbo, B. H. To be published.
5. Foss, O. *Kgl. Norske Vid. Selsk. Skrifter* (1945) nr. **2**.
6. Foss, O. *Acta Chem. Scand.* **4** (1950) 404.
7. Schöberl, A., and Ludwig, E. *Ber.* **70** (1937) 1422.
8. Mauthner, J. *Z. Physiol. Chem.* **78** (1912) 28.
9. Clarke, H. T. *J. Biol. Chem.* **97** (1932) 235.
10. Gulland, J. M., and Randall, S. S. *Biochem. J.* **29** (1935) 391.

Received October 24, 1951.