Rhodanese-catalyzed Reduction of Thiosulfate by Reduced Lipoic Acid*

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Rhodanese (thiosulfate : cyanidesulfurtransferase, EC 2.8.1.1) catalyzes the reaction

$$SSO_3^- + CN^- \rightarrow SCN^- + SO_4^{2-}$$

by a double displacement mechanism (1, 2). Thiosulfate and organic thiosulfonates will act as sulfur donors for the enzyme (3). Cyanide ion, $SO_3^{2-}$, and the organic sulfonates will accept sulfur from the enzyme-sulfur compound (1, 4, 5).

In this study, crystalline beef liver rhodanese has been found to catalyze the reduction of $SSO_3^-$ to $HS^-$ and $SO_4^{2-}$. Several powerful reducing agents such as NaBH₄ and Na₂S₂O₄ could be utilized as substrates for the enzymatic reaction. In the range of physiologically accessible oxidation-reduction potentials, however, only reduced lipoate and lipoamide were reactive. Rhodanese did not utilize DPNH, TPNH, cysteine, GSH, or formaldehyde for $SSO_3^-$ reduction although these reactions would be exergonic. Evidence was obtained indicating that the enzymic reaction with lipoate or lipoamide is stereospecific.

Crystalline beef liver rhodanese, prepared by the method previously described (6), was used in all enzymic studies. DL-Lipoic acid was purchased from the California Corporation for Biochemical Research. Reduced DL-lipoate was prepared by NaBH₄ reduction in a buffer containing sodium phosphate and sodium tetraborate, both at 0.05 M, pH 8.5. DL-Lipoamide was purchased from the Aldrich Chemical Company. The use of a Heyrovsky recording polarograph with a dropping mercury electrode permitted measurement of the concentrations of reduced lipoic acid, $HS^-$, and $SSO_3^-$, which display well defined anodic diffusion currents, and oxidized lipoate, which has a cathodic diffusion current at the same half-wave potential as the anodic wave of reduced lipoate ($-0.66$ volt versus the saturated calomel electrode). The $HS^- E_h (0.66$ volt) is too close to that of the lipoate to be resolved but quantitation of all the components of the wave is possible by algebraic addition. The $SSO_3^- E_h (-0.12$ volt) is far enough from the composite lipoate-sulfide wave to permit good measurement of $SSO_3^-$ concentration. When the enzymic reaction was run in the polarographic cell, the disappearance of reduced lipoate and $SSO_3^-$ and the appearance of oxidized lipoate and $HS^-$ could be followed throughout the course of the reaction (Fig. 1). In the absence of rhodanese, no reaction occurred. Lipoamide could be used in place of lipoate in the enzymic-catalyzed reaction.

The data presented in Fig. 1 establish the stoichiometry of the following reaction:

$$\text{Reduced lipoate} + SSO_3^- \rightarrow \text{oxidized lipoate} + HS^- + H^+ + SO_4^{2-} \ (1)$$

The utilization of reduced DL-lipoate never exceeded 50%, regardless of initial concentrations of reactant or repeated additions of enzyme, indicating that rhodanese is specific for one optical isomer of lipoic acid. A thiosulfate analogue, benzene thiosulfonate, which has a more reactive sulfur atom than $SSO_3^-$ (3, 5, 7), reacts spontaneously with reduced DL-lipoate. This spontaneous reaction proceeds far beyond 50% oxidation of added reduced DL-lipoate, under the conditions used for the enzyme-catalyzed reduction of $SSO_3^-$. Reaction 1 proceeds far to the right. Under the conditions illustrated in Fig. 1, in which the initial $SSO_3^-$ concentration was approximately equimolar to the initial reduced DL-lipoate concentration, the reaction proceeded to within 95% of the maximal yield, taking into account the stereospecificity for reduced lipoate. The reverse reaction, starting with equimolar amounts of $SO_4^{2-}$, $HS^-$, and oxidized DL-lipoate, could be detected, but the changes (5% or less) could not be measured accurately in this polarographic system.

When $CN^-$ was substituted for $SO_4^{2-}$ in the reverse reaction, nearly half of the DL-lipoate was reduced and $SCN^-$ was formed:

$$\text{Oxidized lipoate} + HS^- + CN^- + H^+ \rightarrow \text{reduced lipoate} + SCN^- \ (2)$$

Under the conditions of the enzymatic assay (25°C, pH 8.5), no reaction with $CN^-$ occurred in the absence of enzyme. Reduction of lipoate and the disappearance of $CN^-$ were followed polarographically ($E_h$ for $CN^- = -0.27$ volt). Thiocyanate formation was demonstrated by withdrawing aliquots for colorimetric analysis (8).

![Fig. 1. Stoichiometry of rhodanese-catalyzed reduction of $SSO_3^-$ as measured polarographically. ▲, $SSO_3^-$ disappearance; △, $HS^-$ appearance; ●, oxidized lipoate appearance. The reaction was carried out at room temperature in a 20-ml polarographic H-cell with a buffer containing sodium phosphate and sodium tetraborate, both at 0.05 M, pH 8.5, as supporting electrolyte. The reaction system was 1.25 X 10⁻⁴ M in reduced DL-lipoate, 1.5 X 10⁻⁴ M in Na₂S₂O₃, and less than 5 X 10⁻⁴ M in enzyme.](image-url)
It was of interest to determine whether crystalline rhodanese contains bound lipoate. Microcell polarography (1) of a solution containing a high concentration of native rhodanese (7 x 10^{-4} M) showed no wave in the vicinity of the lipoate E_{1/2} nor did such a wave appear on reduction of the enzyme with NaBH_{4}. Crystalline rhodanese, 50 mmoles, was hydrolyzed in 6 N HCl at 110° for 16 hours. The HCl was evaporated, and the amino acid hydrochlorides were dissolved in water. The acid solution (pH ~ 1) was extracted three times with volumes of benzene each time. Thorough emulsification of phases was achieved by sonication. The pooled benzene extracts were evaporated with a stream of N_{2}, and the residue was dissolved in phosphate-borate buffer and polarographed. No lipoic acid was found. When lipoic acid was added to such a rhodanese hydrolysate in the molar ratio 2:1 (assuming two active sites per rhodanese molecule), it could be recovered in 90% yield by the benzene extraction technique. Since Reed et al. (9) have shown that lipoic acid is not destroyed during acid hydrolysis of proteins, it was concluded that rhodanese contains no bound lipoic acid.

**REFERENCES**


**Isolation of a Naturally Occurring Polyadenylate from Calf Thymus Nuclei**

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The synthesis of polyadenylate from adenosine triphosphate by an adenosine triphosphate polymerase purified from calf thymus nuclei has been shown to depend upon a polyadenylate present in the enzyme (1). The adenylate content of the polyadenylate primer isolated from a boiled extract of the enzyme was raised from 26 to 87% by the removal of inactive polynucleotides after their degradation with pancreatic and Taka-Diastase (T1 RNase) ribonucleases. The high adenine content of the primer was recovered in an identical column from an alkaline hydrolysate of Fraction B (Fig. 1).

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*The abbreviation used is: poly 'T, polythymidylic acid.