Enzymic Memory. A Consequence of Conformational Mobility†

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ABSTRACT: The sulfur-substituted rhodanese (thiosulfate:cyanide sulfurtransferase, EC 2.8.1.1) that is an obligatory intermediate in the catalytic cycle of this enzyme differs in reactivity depending on the nature of the sulfur-donor substrate. In other words, the enzyme appears to "remember" the structure of the substrate from which it received the transferable sulfur atom for a significant period after the first product, which contains that structure, has been discharged from the enzyme. This phenomenon is observed with both the human and bovine liver rhodaneses and is probably related to the high conformational mobility previously observed with these enzymes.

Results of a previous kinetic study (Leininger and Westley, 1968) indicated the probable occurrence of a significant conformational change in bovine liver rhodanese (thiosulfate:cyanide sulfurtransferase, EC 2.8.1.1) on formation of a complex with the sulfur-donor substrate thiosulfate. Recent optical rotatory work by Volini and Wang (1973) has shown that there is a large conformational difference between free rhodanese and the sulfur-substituted form that was previously shown to be an obligatory intermediate in the catalytic mechanism (Green and Westley, 1961; Westley and Nakamoto, 1962; Volini and Westley, 1966; Westley and Heyse, 1971). If these conformational changes occur in every catalytic cycle of the enzyme, as it appears they must, this high degree of conformational mobility should have some predictable kinetic consequences. For example, it might be anticipated that the sulfur-substituted rhodaneses obtained by use of different sulfur-donor substrates could have different reactivities. In particular, the rapid reaction of the sulfur-substituted intermediate with cyanide (Schlesinger and Westley, 1974) might show such an effect that would be readily measurable by steady-state techniques.

Experimental Procedures

Materials. Rhodanese was prepared from bovine liver by the method of Horowitz and DeToma (1970) and from human liver as described previously (Jarabak and Westley, 1974a). Sodium ethanethiosulfonate was synthesized as described previously (Westley and Heyse, 1971). Deionized water was used in all solutions. All other materials used were the best analytical grades commercially available.

Methods. The assay systems used for these kinetic studies have been described (Westley and Heyse, 1971; Jarabak and Westley, 1974a). Reactions were conducted at 0°. Measured initial velocities were proportional to enzyme concentration for all conditions used. Kinetic data were plotted in double reciprocal form and also processed statistically by use of a digital computer with a program providing an iterative fit to the best hyperbola (Jarabak and Westley, 1974a).

Results

Bovine Rhodanese. Extensive studies of the steady-state kinetic behavior of bovine rhodanese with thiosulfate and cyanide as substrates have been reported previously (Leininger and Westley, 1968; Westley and Heyse, 1971; Schlesinger and Westley, 1974; Westley, 1972; Mintel and Westley, 1966a,b; Wang and Volini, 1973). Two of these studies (Westley and Heyse, 1971; Westley, 1972) have included data for ethanethiosulfonate as donor substrate, utilizing the same kinetic conditions employed in the present work. Initial velocity patterns for both thiosulfate and ethanethiosulfonate have been redetermined in the present study to establish the concentration ranges in which donor substrate inhibition is insignificant. Figure 1 presents a direct comparison of cyanide double reciprocal plots determined at the same time under identical conditions for thiosulfate and ethanethiosulfonate as donor substrates for the bovine rhodanese. Care has been taken to avoid all concentration ranges in which donor substrate inhibition is detectable.

Human Rhodanese. Steady-state kinetic studies of human liver rhodanese with thiosulfate and cyanide as substrates were reported previously (Jarabak and Westley, 1974a). Comparable initial velocity patterns for this enzyme with ethanethiosulfonate as sulfur donor are presented in Figure 2. Figure 3 gives the formal mechanism corresponding to this reaction, along with the corresponding mechanism for the bovine enzyme. Figure 4 shows a direct comparison of the cyanide double reciprocal plots for these two sulfur-donor substrates, as determined under identical conditions over concentration ranges such that donor substrate inhibition was not significant.

Rate Constants. Numerical values for k_{+2} and K_m for ethanethiosulfonate, computed from the data in Figure 2, are 852 $\pm$ 36 sec^{-1} and (3.8 $\pm$ 0.8) $\times$ 10^{-3} M, respectively. The value of k_{+1} for bovine rhodanese is (3.0 $\pm$ 0.5) $\times$ 10^5 M^{-1} sec^{-1}. Values for the second-order rate constant (k_{+3}) for the reaction of cyanide anion with the sulfur-substituted rhodanese were obtained directly from the slopes of the cyanide double reciprocal plots shown in Figures 1 and 4. These numbers with their standard error values are presented in Table I along with comparable values for both the human and bovine enzymes obtained from secondary plot extrapolation of cyanide slopes against sulfur-donor concentration for a broader range of donor concentrations.

Discussion

If the substituted enzyme intermediate in a double displacement mechanism[1] is the same for all donor substrates, as is generally assumed to be the case, kinetic parameters involving

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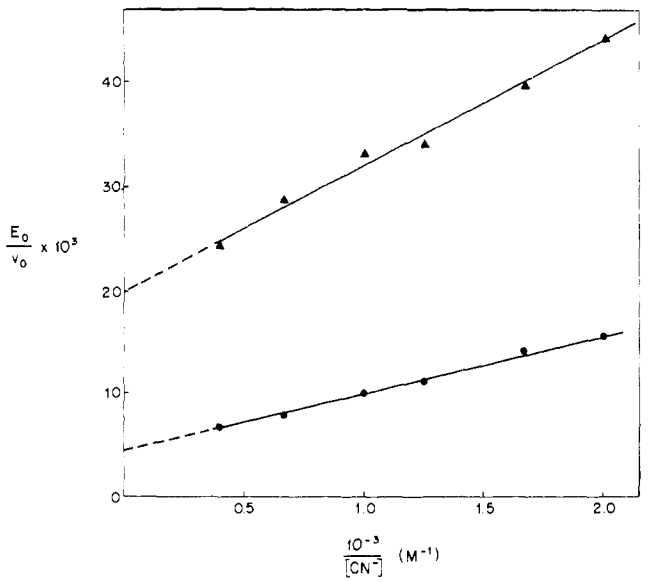


FIGURE 1: Double reciprocal cyanide plots for bovine liver rhodanese with 0.02 M thiosulfate (▲) and 0.001 M ethanethiosulfonate (●) as sulfur donors.

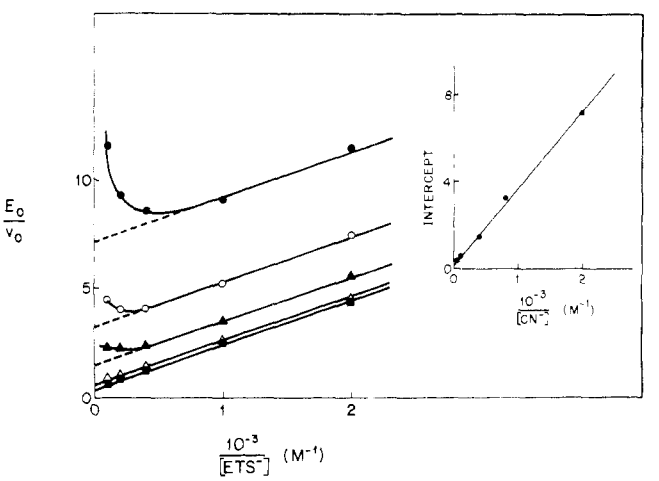
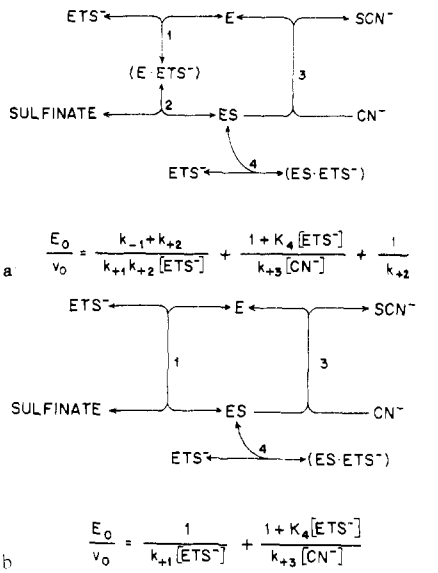


FIGURE 2: Kinetics of the ethanethiosulfonate–cyanide reaction catalyzed by human liver rhodanese. Cyanide concentrations: (●) 5×10^{-4} M; (○) 1.25×10^{-3} M; (▲) 2.5×10^{-3} M; (△) 1×10^{-2} M; (■) 2×10^{-2} M.



a $$\frac{E_0}{v_0} = \frac{k_{-1}+k_{+2}}{k_{+1}k_{+2}[ETS^-]} + \frac{1+K_4[ETS^-]}{k_{+3}[CN^-]} + \frac{1}{k_{+2}}$$

b $$\frac{E_0}{v_0} = \frac{1}{k_{+1}[ETS^-]} + \frac{1+K_4[ETS^-]}{k_{+3}[CN^-]}$$

FIGURE 3: (a) Formal mechanism and corresponding rate equation for the ethanethiosulfonate–cyanide reaction catalyzed by human liver rhodanese: (E) enzyme; (ES) sulfur-substituted enzyme; (ETS^-) ethanethiosulfonate; (E_0) total enzyme concentration; (v_0) initial velocity. (b) Formal mechanism for the ethanethiosulfonate–cyanide reaction catalyzed by bovine liver rhodanese. Symbols as in Figure 3a.

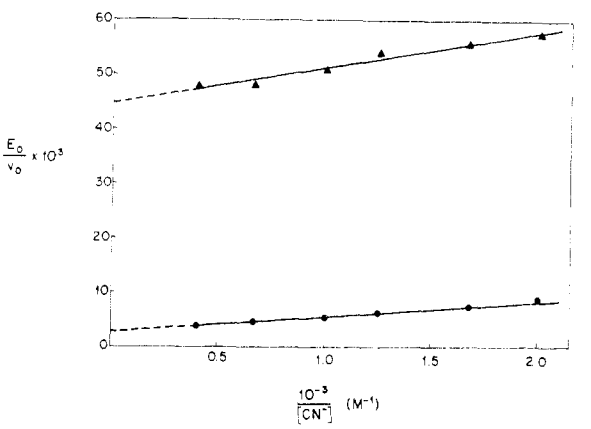


FIGURE 4: Double reciprocal cyanide plots for human liver rhodanese with 0.02 M $S_2O_3^{2-}$ (▲) and 0.001 M ethanethiosulfonate (●) as sulfur donors.

only reaction with the acceptor substrate should be independent of the nature of the donor. Conversely, if any aspect of the structure of the substituted enzyme retains a response relating to the structure of the donor substrate, the substituted enzymes obtained with different donor substrates, not being identical chemical species, may yield quantitatively different kinetic behavior in reaction with the same acceptor substrate. The substituted enzyme may, so to speak, remember the donor from which it received its transferable group.

The evidence presented here indicates that rhodanese displays such an enzymic "memory." The second-order rate constants for the reaction of the sulfur-substituted rhodanese with cyanide anion, evaluated from the slopes of double reciprocal plots of initial velocity data, clearly differ for different sulfur-donor substrates.

There are two critical features of this analysis: (a) the validity of the evaluation of the rate constants from the steady-state data and (b) the uniqueness of the assignment of cause for the difference found.

All kinetic data published for rhodanese from either bovine or human liver are in accord with a double displacement formal mechanism for this enzyme. Further, the sulfur-substituted enzyme intermediate form of the bovine rhodanese has been isolated and its identity established by electrochemical (Green and Westley, 1961) and tracer isotope (Westley and Nakamoto, 1962) techniques. The established formal mechanism with thiosulfate as substrate includes an enzyme–thiosulfate complex, but no complex of the sulfur-substituted enzyme with cyanide has been established definitely as kinetically significant under initial velocity conditions. With thiosulfate as sulfur donor, $K_m^{SSO_3^{2-}}$ reduces to k_{-1}/k_{+1} since k_{+2} is much smaller than k_{-1}.

The initial velocity rate equation for the bovine rhodanese catalyzed thiosulfate–cyanide reaction in the absence of significant substrate inhibition is as follows

$$\frac{E_0}{v_0} = \frac{k_{-1}}{k_{+1}k_{+2}[SSO_3^{2-}]} + \frac{1}{k_{+3}[CN^-]} + \frac{1}{k_{+2}}$$

where k_{+3} is the second-order rate constant for reaction of the sulfur-substituted enzyme with cyanide. Accordingly, the value

[1] Also called a "ping-pong" mechanism.

TABLE I: Rate Constants for the Reaction of Cyanide with Sulfur-Substituted Rhodanese.

Enzyme	Slope of $1/[CN^-]$ Plot[a]	Corresponding Rate Constant k_{+3} (M^{-1} sec^{-1})	k_{+3} (M^{-1} sec^{-1}) (Extrap. Value)
Bovine rhodanese			
$S_2O_3^{2-}$	$(0.653 \pm 0.01) \times 10^{-5}$	$(1.53 \pm 0.03) \times 10^5$	$(1.7 \pm 0.1) \times 10^5$
ETS^-	$(0.274 \pm 0.02) \times 10^{-5}$	$(3.65 \pm 0.3) \times 10^5$	$(4.5 \pm 0.2) \times 10^5$
Human rhodanese			
$S_2O_3^{2-}$	$(1.22 \pm 0.12) \times 10^{-5}$	$(0.82 \pm 0.09) \times 10^5$	$(0.95 \pm 0.10) \times 10^5$
ETS^-	$(0.55 \pm 0.05) \times 10^{-5}$	$(1.84 \pm 0.17) \times 10^5$	$(2.4 \pm 0.25) \times 10^5$

[a] Computer value with standard error limits.

of k_{+3} can be calculated directly from the slope of the cyanide double reciprocal plots. Interference by donor substrate inhibition, which would alter the cyanide slope, is easily avoided either by extrapolation of secondary slope plots to zero donor concentration or by carrying out all experimental work at donor concentrations below the level yielding significant inhibition. Both of these tactics have been used in the present work, with the same results. The cyanide double reciprocal slope values obtained with the different sulfur-donor substrates thiosulfate and ethanethiosulfonate differ consistently in this reaction system for both the bovine and human enzymes (Table I). In each case the significance of the difference is far beyond the 99% level of statistical probability.

There are some other differences between thiosulfate and ethanethiosulfonate as sulfur-donor substrates. In the case of bovine rhodanese, it has not been possible to establish the occurrence of kinetically significant complexes with either ethanethiosulfonate (ETS) or cyanide (CN) (Westley and Heyse, 1971). The rate equation for initial velocity then reduces to the following form

$$\frac{E_0}{v_0} = \frac{1}{k_{+1}[ETS^-]} + \frac{1}{k_{+3}[CN^-]}$$

Secondary plots of intercepts appear to pass through the origin. Clearly, however, this alteration cannot distort the cyanide slopes, which remain equal to inverse k_{+3}. The bovine liver rhodanese thus provides a particularly clear and simple case in which the substituted enzyme obtained with different donor substrates must be different chemically.

Such a chemical difference could be envisioned in either of two simple ways. Either the different donor substrates utilize different active sites or the substituted enzymes obtained for the two substrates differ because of different responses to the original substrate structure. For rhodanese the first of these two possibilities has been eliminated conclusively by kinetic experiments with mixed donor substrates (Jarabak and Westley, 1974b). The remaining possibility warrants serious consideration.

Following the suggestion of Laidler (1951) that enzymes may respond conformationally to their substrates and the further development of the induced fit hypothesis by Koshland (1958), it was shown that the formation of the rhodanese–thiosulfate complex includes a major nonelectrostatic entropy change. This suggested a conformational response of the enzyme to the presence of the donor substrate (Leininger and Westley, 1968). It has recently been shown (Volini and Wang, 1973) by direct observation of the various enzymic forms, coupled with an extensive kinetic analysis (Wang and Volini, 1973), that the entropy loss on thiosulfate complex formation is indeed attributable to a peptide backbone conformational alteration and that the altered form is maintained in the sulfur-substituted enzyme.

If conformational changes of this kind occur every catalytic cycle, as the correlation of direct physical observations with the thermodynamic and activation parameters indicates that they do, it might then be anticipated that different donor substrates would yield significantly different sulfur-substituted enzymes. The present observations on the bovine liver rhodanese confirm this expectation.

The situation with the rhodanese from human liver is more complicated. Here, in contrast to the case of the bovine liver enzyme, there may be a kinetically significant unimolecular step with the donor-substrate ethanethiosulfonate (nonzero intercept in secondary intercept plot of Figure 2). Further, cyanide-substrate activation behavior is seen when thiosulfate is the donor substrate (Jarabak and Westley, 1974a). However, these complications do not alter the conclusion that the sulfur-substituted intermediate form of the human rhodanese, like that of the bovine enzyme, differs depending on the donor substrate used.

The occurrence of a kinetically significant binary complex with the donor substrate could not alter the slope of the acceptor double reciprocal plots. The formation of an isomerizing substituted enzyme, as suggested previously (Jarabak and Westley, 1974a) to explain the substrate activation behavior with cyanide, would of course alter the cyanide slopes, but this cannot be regarded as contradicting the enzymic memory analysis of the phenomenon seen with the bovine enzyme. Rather it is a particular case of the same phenomenon, a case in which the rate constants are so balanced that it is possible to observe the mobile substituted enzyme in the process of change.

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