ENHANCEMENT OF RHODANESE ACTIVITY DURING CONTROLLED DIGESTION WITH TRYPSIN

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Summary When the enzyme rhodanese (EC 2.8.1.1) is digested with trypsin under controlled conditions, the parent protein is converted from a polypeptide of molecular weight 32,600 to a polypeptide of molecular weight 28,800. This proteolytic conversion occurs with no loss of rhodanese activity. In fact, preliminary results indicate that the polypeptide produced by proteolysis has higher sulfur transferase activity than the native rhodanese.

Introduction

Rhodanese (EC 2.8.1.1) is a sulfur transferase which transfers the outer sulfur atom from thiosulfate to a variety of nucleophilic acceptors including cyanide (1). The enzyme has been purified from several sources including bovine liver (2) and is present in a wide variety of mammalian and bacterial species (3). Various laboratories are engaged in physical-chemical studies directed to elucidating the structural basis for rhodanese catalysis, including crystallographic analysis and determination of amino acid sequence. An extensive report has recently appeared on the role of protein conformational changes in rhodanese catalysis (4-6).

Rhodanese possesses several unusual features. First, and of perhaps foremost importance, is that the bona fide substrate and product of rhodanese may not yet be identified. This possibility stems from the fact that this mitochondrial protein accounts for as much as one-tenth percent of the total soluble protein in liver, yet the known catalytic properties of this enzyme have not been demonstrated as fulfilling an obligatory role in intermediary

metabolism. In addition, there has been an ongoing controversy, albeit not apparent in the literature, as to whether rhodanese is a dimeric protein or a "monomer" of molecular weight approximately 18,500 (cf 7). And finally, maximum amounts of rhodanese activity in liver are present only after aging or freezing and thawing of the tissue (cf 2). These considerations prompted us to undertake the experiments reported below.

Materials and Methods

Rhodanese was purified from bovine liver and crystallized as described by Horowitz and DeToma (2). Activity of the enzyme was measured with cyanide as acceptor (8). Bovine pancreatic trypsin (2X crystallized) and soybean trypsin inhibitor were obtained from Sigma Chemical Co.

Rhodanese was treated with trypsin by incubating 1.33 mg per ml of rhodanese with 100 µg and 200 µg of trypsin per mg of enzyme at 37°C in 0.1 M sodium phosphate, pH 7.4, for various periods of time as indicated in the figure legends. A control was included in which rhodanese was similarly incubated without addition of trypsin. Treatment with trypsin was terminated by adding trypsin inhibitor to the mixture in the amount of 2 mg per mg of trypsin. Duplicate 10 µg aliquots of rhodanese were withdrawn for measurement of activity and the remainder of the trypsin-treated rhodanese was denatured for electrophoresis by adding sodium dodecyl sulfate and β-mercaptoethanol to obtain 1 mg per ml of rhodanese in 5 percent dodecyl sulfate and 2 percent β-mercaptoethanol. The rhodanese in denaturants was immediately transferred to a boiling water bath for 10 minutes.

Acrylamide gel electrophoresis in dodecyl sulfate was performed according to Weber and Osborn (9) employing standards of known molecular weight as listed in Figure 6. After staining with

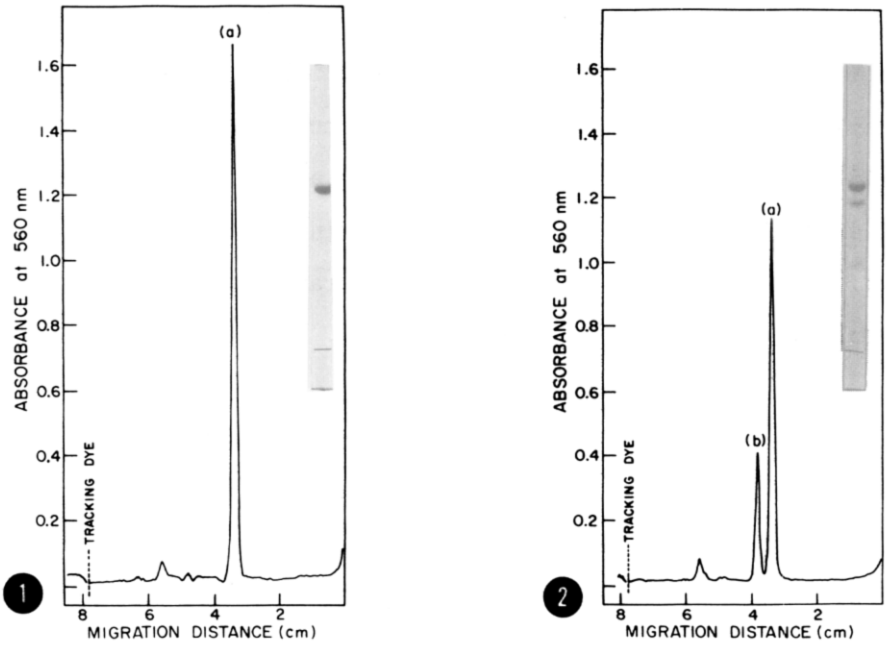


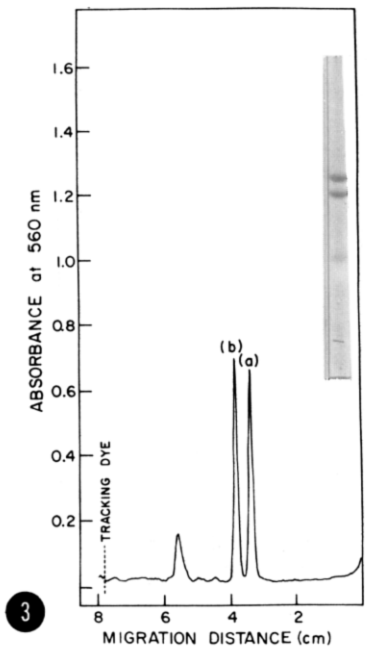
Figure 1 Electrophoresis of native rhodanese, not treated with trypsin. Rhodanese was added to a mixture of trypsin and trypsin inhibitor in the amounts employed when rhodanese was treated with 100 μg of trypsin per mg of enzyme, after which the mixture was denatured for electrophoresis. Electrophoresis of appropriate amounts of trypsin and trypsin inhibitor in the absence of rhodanese established that the species migrating at 4.8 cm and 5.5 cm correspond to trypsin and trypsin inhibitor, respectively. Native rhodanese in the absence of trypsin and trypsin inhibitor revealed a profile identical to that shown except lacking the species corres ponding to trypsin and trypsin inhibitor. In this and subsequent electrophoresis profiles (a) designates native rhodanese.

Figure 2 Rhodanese after treatment with 100 μg of trypsin per mg of enzyme for 5 minutes. The 28,800 polypeptide produced by treatment with trypsin is designated (b).

Coomasie brilliant blue, the gels were scanned with the Linear Transport Accessory of a Gilford Model 2400S spectrophotometer. Relative amounts of native and digested rhodanese were calculated from tracings of the type shown below.

Results and Discussion

When rhodanese is treated with trypsin the native protein can be hydrolyzed in a controlled manner as shown in Figures 1 through 5. We treated rhodanese with 100 and 200 μg of trypsin per mg of



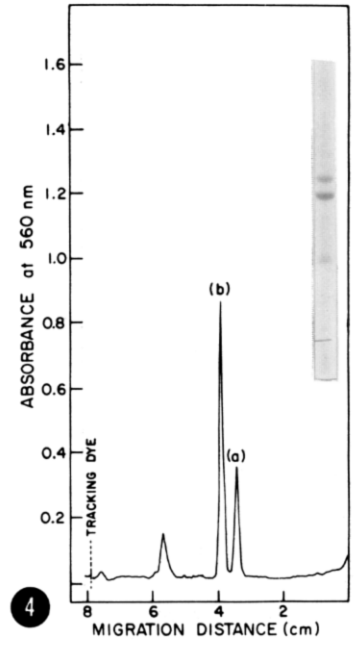


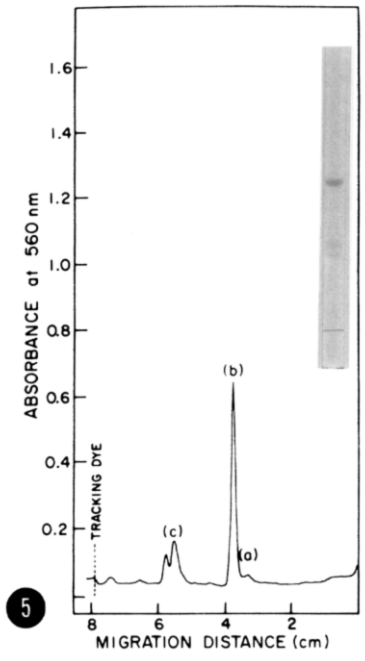
Figure 3 Rhodanese after treatment with 200 μg of trypsin per mg of enzyme for 5 minutes.

Figure 4 Rhodanese after treatment with 200 μg of trypsin per mg of enzyme for 10 minutes.

enzyme for 5, 10, 30, 60, and 90 minutes. The hydrolysis is proportional to the amount of trypsin, as seen by comparing Figures 2 and 3, and to the time of incubation, as seen from Figures 4 and 5.

As shown in Figure 6, acrylamide gel electrophoresis in dodecyl sulfate indicated molecular weights of 32,600 for native rhodanese and 28,800 for the polypeptide produced by treatment with trypsin, implying that a peptide of approximately 34 amino acid residues is removed by the cleavage with trypsin. The exact size and composition of this peptide are currently under investigation.

Measurements of rhodanese activity revealed that the conversion from a polypeptide of molecular weight 32,600 to one of 28,800 occurred with no loss of enzyme activity. In Figure 7 the results



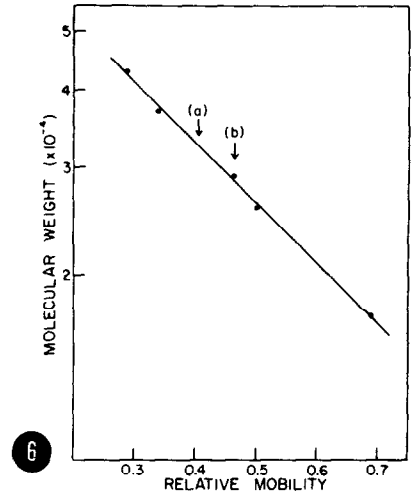


Figure 5 Rhodanese after treatment with 200 µg of trypsin per mg of enzyme for 90 minutes. As discussed in the text, the species designated (c) appears to be a secondary product of the trypsin digestion arising from hydrolysis of the 28,800 polypeptide.

Figure 6 Molecular weight by polyacrylamide gel electrophoresis in dodecyl sulfate of rhodanese before and after controlled digestion with trypsin. Standard proteins, whose relative mobilities are indicated by the open circles, were ovalbumin, 43,000; yeast alcohol dehydrogenase, 37,000; carbonic anhydrase, 29,000; chymotrypsinogen, 25,700; and myoglobin, 17,200. The relative mobility of native rhodanese is indicated by the arrow (a) and that of the 28,800 polypeptide by the arrow (b).

of an experiment are shown in which rhodanese was treated with 200 µg of trypsin per mg of enzyme. During the first 60 minutes of incubation with trypsin there was no loss of rhodanese activity, although 95 percent of the native protein was hydrolyzed. In fact, there was a 25 percent increase in activity, expressed as total enzyme units, as a result of the trypsin treatment. Since the activity is uncorrected for the decrease in molecular weight and destruction of the 28,800 polypeptide by further hydrolysis, this represents a minimum value for the increase in activity.

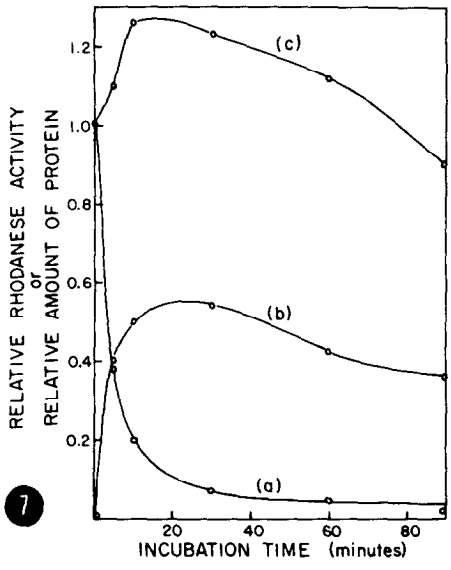


Figure 7 Relative amounts of native rhodanese and its tryptic product and rhodanese activity during controlled digestion with trypsin. Rhodanese was incubated with 200 μg of trypsin per mg of enzyme for various times as indicated. Curve (a) shows the relative amount of native rhodanese, curve (b) is the amount of 28,800 polypeptide relative to native rhodanese at zero time, and curve (c) is rhodanese activity relative to that at zero time which was 530 units per mg.

This increase was consistently observed in other experiments not reported here, and as seen in Figure 7, appears to correlate with the formation of 28,800 polypeptide. Only after 90 minutes of incubation was there a decline in rhodanese activity, and this coincides with destruction of the 28,800 polypeptide.

As seen in Figure 5, the 28,800 polypeptide is hydrolyzed to yield, among other possible products, a lower molecular weight species which migrates slightly ahead of trypsin inhibitor. This low molecular weight species only appeared as a result of the trypsin treatment.

Control experiments established that rhodanese incubated for these periods of time in the absence of trypsin showed a constant activity of 530 units per mg, which agrees with standard values of 540 units per mg in the literature (cf 1). Hydrolysis by trypsin

did not occur after addition of denaturing agents as evidenced by the "zero time" sample shown in Figure 1. And finally, trypsin and trypsin inhibitor exhibited no rhodanese activity.

We conclude that rhodanese activity can be demonstrated with enzyme of at least 2 different molecular weights, although we find no evidence for an active rhodanese of molecular weight 18,500. This does not exclude the possibility that such an active polypeptide might not be produced by action of an appropriate protease on native rhodanese. In addition, it seems possible that the sulfur transferase activity of rhodanese itself may be induced as a result of prior proteolysis of some other, as yet unidentified enzyme. This possibility is currently under investigation.

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