A Comparison of Some Biochemical Properties of the Beef Liver and Beef Kidney Rhodanases*

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Several reports have indicated that there may be differences among the proteins responsible for a given enzymatic activity in different tissues of the same animal (1-4). This paper reports the results of a comparative study of some biochemical properties of the enzyme rhodanese purified from the liver and the kidneys of the same steer. The two enzymes have been found to be identical by all of the tests applied. Some biochemical genetic implications of such studies are discussed.

EXPERIMENTAL

The liver and kidneys from the same steer were obtained fresh at the slaughter house and stored at -20° before use. Rhodanese was prepared from kilogram portions of the liver and from the pooled kidneys (700 gm.) after the method of Sörbo (5). The enzyme was assayed by estimating colorimetrically the thiocyanate produced from cyanide and thiosulfate. Either gelatin or glycine was used to prevent inactivation of the enzyme in dilute solution. Protein concentrations were determined by a biuret method except in highly purified preparations, where they were determined spectrophotometrically with use of the extinction coefficient for crystalline liver rhodanese at 280 mp (5). Rhodanese was obtained from the liver in crystalline form. Sörbo's observation that crystalline beef liver rhodanese sediments with a single boundary in the analytical ultracentrifugae (5) was confirmed. Kidney rhodanese could not be crystallized by the same fractionation procedure and the kidney preparation used in these studies was 40 per cent pure.1 Both the liver and kidney rhodanese preparations were colorless and free from proteases, dialyzable tissue components, and nucleotides (A260/A280 ≥ 1.7). For both enzyme preparations, the thiocyanate produced in the assay reaction was shown to be equivalent to the thiocyanate which disappeared, measured as a decrease in absorbance at 265 mp.

Sodium benzenethiosulfonate was synthesized by the method of Traeger and Linde (7). After several recrystallizations from ethanol, the compound yielded the theoretical quantity of thiocyanate when treated with alkali and cyanide (8, 9).

Variation of Activity with pH—Data representing initial rates of thiocyanate production in the presence of liver or kidney rhodanese at various pH values are shown in Fig. 1. The activity is an increasing function of pH over the entire range for which initial rate data can be obtained. Previous data showing decreases in activity on the alkaline side of a maximum (8, 10) are apparently due to alkaline inactivation of the enzyme during the incubation periods used. Neither in the experiment shown nor in any of several others performed with varying buffer conditions did the pH responses of the liver and kidney rhodanases differ.

Heats of Activation—The heat of activation of the reaction catalyzed by liver or kidney rhodanese was found to be 5800 calories, calculated from the Arhenius plots shown in Fig. 3. The slopes of the two lines and, therefore, the heats of activation of the reaction catalyzed by the two enzymes are identical within the limits of the experimental error. That this value for the heat of activation differs from the 7900 calories reported by Sörbo (8) can be attributed to differences in the conditions of the experiments.

Substrate Specificity—Sodium benzenethiosulfonate can replace sodium thiosulfate as a substrate for rhodanese (8). The liver and kidney enzymes were tested for activity in catalyzing thiocyanate formation from each of these substrates and cyanide. The results given in Table I show that the liver and kidney rhodanases do not differ in their relative activities on the two substrates.

Heat Inactivation—The inactivation of rhodanese by dilution can be prevented by including glycine and thiosulfate in the diluting medium. Liver and kidney rhodanese diluted under these conditions to a suitable level for assay were heated at specified temperatures for 30 or 60 minutes and assayed in the standard system for rhodanese activity. The results are shown in

1 Based on substrate concentration S equal to the sum of HCN and CN- concentrations. Although the significance of the calculated Michaelis constant is obscured by some properties of the rhodanese reaction system (8) the data are useful for comparative purposes.

1 Beef kidney rhodanese has subsequently been obtained in crystalline form by a fractionation procedure based on the use of the N,N-diethylaminoethyl-cellulose ion exchanger. The specific activity of the crystalline kidney enzyme is the same as that of crystalline beef liver rhodanese (J. Westley and J. Green, manuscript in preparation).
Liver and Kidney Rhodanese

The dashed line indicates apparent decrease in activity due to departure from initial rates during assay period (see text). Reaction systems were 0.04 M in Na$_2$S$_2$O$_3$, 0.04 M in KCN, 0.03 M in KH$_2$PO$_4$, 0.14 M in glycine, and 0.33 M in tris(hydroxymethyl)aminomethane as the free base and the hydrochloride. Incubations were conducted at 25° for 1 minute. Termination of reactions and analysis for SCN$^-$ were performed as in the rhodanese assay.

**FIG. 2.** Kinetics of rhodanese activity with cyanide as limiting substrate. O—O, crystalline beef liver rhodanese; •—•, beef kidney rhodanese. Reaction systems were 0.05 M in Na$_2$S$_2$O$_3$, 0.05 M in barbital, and 0.025 per cent in gelatin, pH 8.0. Incubations were conducted at 25° for 2 minutes, during which the reactions proceeded linearly. Termination of reactions and analysis for SCN$^-$ were performed as in the rhodanese assay.

**FIG. 3.** Arrhenius apparent heat of activation for the rhodanese reaction. O and □, crystalline beef liver rhodanese; • and ●, beef kidney rhodanese. Since only the slopes are significant, the data for the two enzymes are shown arbitrarily displaced from each other in the vertical dimension to avoid confusion. Reaction mixtures contained Na$_2$S$_2$O$_3$ and KCN at 0.05 M, KH$_2$PO$_4$ at 0.04 M, and either tris(hydroxymethyl)aminomethane at 0.25 M and glycine at 0.12 M (■ and ■) or glycine at 1 M (○ and ○). Incubations were conducted for 2 minutes at pH 8.8. Above 40° inactivation occurred during the incubation period. Termination of reactions and analysis for SCN$^-$ were performed as in the rhodanese assay.

**TABLE I**

**Substrate specificity**

<table>
<thead>
<tr>
<th>Rhodanese source</th>
<th>Substrate*</th>
<th>Experiment 1†</th>
<th>Experiment 2†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef liver</td>
<td>Na$_2$S$_2$O$_3$§</td>
<td>2.63</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>Na$_2$S$_2$O$_3$</td>
<td>0.283</td>
<td>0.661</td>
</tr>
<tr>
<td>Beef kidney</td>
<td>Na$_2$S$_2$O$_3$</td>
<td>2.06</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>Na$_2$S$_2$O$_3$</td>
<td>0.327</td>
<td>0.776</td>
</tr>
</tbody>
</table>

* Complete systems were 1 M in glycine, pH 8.8, with other conditions as in the rhodanese assay.
† All reactions were conducted at 25° for 2 minutes.
‡ The thiosulfate reactions were conducted at 25° for 5 minutes, the thiosulfonate reactions at 0° for 2 minutes to decrease the extent of the spontaneous reaction.
§ Sodium benzenethiosulfonate.

**TABLE II**

**Heat inactivation**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Duration of treatment*</th>
<th>Inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min.</td>
<td>Beef liver rhodanese</td>
</tr>
<tr>
<td>45°</td>
<td>60</td>
<td>30.5</td>
</tr>
<tr>
<td>50°</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>55°</td>
<td>30</td>
<td>52.5</td>
</tr>
</tbody>
</table>

* The enzyme solutions were 1 M in glycine and 0.0125 M in Na$_2$S$_2$O$_3$, pH 6.3.

Table II as per cent inactivation during a specified time at each temperature. The differences between the data for the liver and kidney enzymes are smaller than the experimental error. In an effort to improve the precision of these observations, data for several time intervals at 50° were plotted and half times were calculated from the slopes of the statistically best fitting lines for the liver and kidney enzymes (Fig. 4). The values 73 and 75 minutes, respectively, differ by an amount smaller than the experimental error.

**Guanidine Inactivation**—Equal amounts of liver and kidney rhodanese in glycine-thiosulfate solution were incubated in the presence and absence of 3 M guanidine. After dilution, aliquots were placed in a 50° water bath for 60 or 120 minutes. After dilution, aliquots of each sample were assayed for SCN$^-$. Table II presents the results of these experiments.
were identical within the limits of the experimental methods used. It is concluded that there were no structural differences which appreciably affected the catalytic properties of the two enzymes.

The work of Sanger and his associates on the species differences in insulins (11) and Ingram’s recent studies on the mutant hemoglobins (12) together with the data of Pauling et al. (13) may be interpreted as evidence that the amino acid sequence in proteins is genetically specified. On the other hand, it is not known whether the entire primary structure is so determined (14) and there are no indications in the literature concerning the determination of the biologically active three-dimensional configurations of proteins.

One approach to this problem is an effort to distinguish those aspects of protein structure which are specified by the genetic material from those which can be significantly influenced by extragenetic factors. Experimentally, this approach involves the detailed examination of a protein which is synthesized under the influence of the same genetic determinants acting in different biochemical environments, a situation which presumably exists in different tissues of the same individual. After a number of proteins from several different tissues have been examined, those properties which are never found to differ should represent aspects of protein structure which are specified genetically. Similarly, any differences which are found should indicate aspects of structure which can be significantly influenced by extragenetic factors.

To obtain data useful for such an interpretation, this study has satisfied the following criteria: (a) The enzyme studied catalyzes with high specificity a reaction which is completely defined chemically. Thus there could be reasonable certainty that it was the same enzymatic activity which was being examined in different sources. (b) The tissues used as enzyme sources were taken from the same individual animal to avoid uncertainties attributable to possible genetic variation. (c) The tests by which the purified enzymes were compared are intelligible in terms of enzymology and protein chemistry.

This study of the liver and kidney rhodaneses from the same steer has provided data on some properties of the two enzymes which are indistinguishable. The immunological differences between the phosphorylases from two tissues of the same animal, which are indistinguishable. The immunological differences between the phosphorylases from two tissues of the same animal, recently demonstrated by Henion and Sutherland (4), reflect some structural differences which can occur.

**SUMMARY**

The enzyme rhodanese was purified from the liver and the kidneys of the same steer. The two enzymes were compared with respect to activity as a function of pH, kinetics of cyanide binding, heats of activation of the catalyzed reactions, substrate specificities, and extent of inactivation by heating or guanidine treatment. The enzymes were found to be identical by all the criteria applied. Some biochemical genetic implications of such studies have been discussed.

**REFERENCES**


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7. TRAEGER, J., and LINDE, O., Arch. Pharm., 239, 121 (1901).


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