Crystalline Beef Kidney Rhodanese*

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Although rhodanese activity occurs in many tissues (1–3), the enzyme had been obtained in crystalline form from beef liver only (4). Beef kidney rhodanese could not be crystallized by the same procedure (5). To extend the biochemical genetic comparison reported previously (5), however, it was desired to obtain the kidney enzyme in a very high state of purity. This paper reports the purification and crystallization of beef kidney rhodanese by a method which combines an ion exchange fractionation with steps based on Sörlöf’s procedure for the crystalline liver enzyme (4). The same method has also been used for preparing crystalline beef liver rhodanese. Some further comparative data are presented.

EXPERIMENTAL

Enzymatic activity and protein content were determined as previously reported (3). The tissues used as enzyme sources were frozen and thawed before use. Glycine and thiosulfate were included in rhodanese solutions to prevent denaturation. All fractionation procedures were conducted at 0–5°C unless otherwise specified.

Preparation of Crystalline Beef Kidney Rhodanese—The following method differs from Sörlöf’s procedure for preparing the beef liver enzyme in the addition here of an ion exchange fractionation, the elimination of a lead acetate precipitation and an ammonium sulfate fractionation, and with respect to the conditions required in several other steps.

1. One beef kidney (600 gm.) was homogenized in a Waring Blender with 800 ml. of a buffer consisting of acetate and glycine, both at 0.1 M, pH 5. The homogenate was centrifuged for 30 minutes at 3000 × g and the residue was extracted again with 400 ml. of buffer.

2. To the combined extracts solid ammonium sulfate was added to 0.4 M and the pH was then adjusted to 3.8 with cold 1 N HCl. After removal of the precipitate by centrifugation, the ammonium sulfate concentration was raised to 1.4 M, and the solution was allowed to stand for 3 hours to allow complete precipitation. After removal of the precipitate by centrifugation, the ammonium sulfate concentration was raised to 2.0 M. The precipitated rhodanese was collected by centrifugation after 3 hours and dissolved in 100 ml. of a buffer consisting of 10⁻³ M Tris,¹ 10⁻³ M Na₂S₂O₃, and 0.2 M glycine, pH 7.6, and designated Buffer A.

3. To remove ammonium sulfate carried over from the previous step, the enzyme solution was dialyzed against a buffer containing 10⁻³ M Tris and 10⁻³ M Na₂S₂O₃, pH 7.6, designated Buffer B, until the ionic strength, measured conductimetrically, was less than that of Buffer A. After centrifugation the dialyzed enzyme solution was diluted with Buffer A to a protein concentration of 4 mg. per ml.

DEAE-cellulose² was equilibrated against a buffer containing Tris, thiosulfate and glycine, each at 0.1 M, pH 7.7, and then washed free of unbound ions. The equilibrated ion exchanger was suspended in 1:200 diluted equilibration buffer at a density of 50 mg., dry weight, of exchanger per ml.

The diluted enzyme preparation (500 ml.) was added to 750 ml. of the DEAE-cellulose suspension. After being stirred for 5 minutes, the mixture was filtered through Whatman No. 1 filter paper on a 32-cm. Büchner funnel. The loaded exchanger was washed once on the filter with the 1:200 diluted equilibration buffer.

Rhodanese was eluted by treatment of the exchanger on the filter with 10 successive 150 ml. washes with a solution made up by adding to Buffer A ammonium sulfate to 3 × 10⁻³ M. The filter flask which received the eluted enzyme contained 300 ml. of a stabilizing solution consisting of 1 M glycine and 0.1 M Na₂S₂O₃, pH 4.5. After elution, the rhodanese was precipitated from solution by adding solid ammonium sulfate to 2.5 M; this was followed by adjustment of the pH to 4.5 with 1 M acetic acid. After 3 hours the precipitated enzyme was collected by centrifugation, dissolved in 20 ml. of Buffer A and dialyzed against Buffer B.

4. The pH of the dialyzed solution was adjusted to 4.8 with 0.1 M acetic acid, and the preparation was fractionated with acetone at −10°C. The protein fraction which precipitated when the volume percentage of acetone was increased from 30 to 45 was taken up in 5 ml. of Buffer A and dialyzed against Buffer B to remove residual acetone.

5. After centrifugation to remove a small precipitate of inactive material, the pH of the dialyzed solution was adjusted to 4.8, and the rhodanese was precipitated by adding an equal volume of 3.78 M ammonium sulfate.

The precipitate was collected by centrifugation and extracted with 1.5 ml. of solution containing 1.8 M ammonium sulfate and 0.01 M Na₂S₂O₃, pH 7.9. After removal of the insoluble residue by centrifugation, the extract was treated with 1.5 ml. of 3.25 M ammonium sulfate, pH 7.9. The precipitated amorphous rhodanese was collected by centrifugation and crystallized by resuspension in 0.1 ml. of 1 M ammonium sulfate, pH 7.9. The

* This investigation was aided by grants from the National Science Foundation and the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

¹ The abbreviation used is: Tris, tris(hydroxymethyl)aminomethane.

² N,N-diethylaminomethyl cellulose. Purchased from Distillation Products Industries. For properties of DEAE-cellulose see references (6) and (7).
**Preparation of crystalline rhodanese from beef kidney and beef liver**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total activity*</th>
<th>Protein*</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kidney</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>1. Extract</td>
<td>25,000</td>
<td>81,000</td>
<td>64,000</td>
</tr>
<tr>
<td>2. 1.4 to 2 M Ammonium sulfate precipitate</td>
<td>15,500</td>
<td>60,000</td>
<td>3,700</td>
</tr>
<tr>
<td>3. 2.5 M Ammonium sulfate precipitate after ion exchange fractionation</td>
<td>6,000</td>
<td>17,000</td>
<td>240</td>
</tr>
<tr>
<td>4. 30 to 45% Acetone precipitate</td>
<td>2,500</td>
<td>10,000</td>
<td>43</td>
</tr>
<tr>
<td>5. Crystals</td>
<td>234</td>
<td>5,080</td>
<td>0.93</td>
</tr>
<tr>
<td>Recrystallization</td>
<td>260 ± 6 ± 265 ± 8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*From 1 kg of tissue. The numbers given are mean values for 2 or 3 preparations.
† As defined by Sörbo (4).

**Sedimentation coefficients of beef kidney and beef liver rhodaneses**

<table>
<thead>
<tr>
<th>Rhodanese source</th>
<th>Temperature of sedimentation run</th>
<th>Protein concentration*</th>
<th>( s_{20,w} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef kidney</td>
<td>6.3( ^\circ )</td>
<td>0.05</td>
<td>3.26</td>
</tr>
<tr>
<td>Beef kidney</td>
<td>5.0( ^\circ )</td>
<td>0.06</td>
<td>3.12</td>
</tr>
<tr>
<td>Beef liver</td>
<td>5.8( ^\circ )</td>
<td>0.10</td>
<td>3.23</td>
</tr>
<tr>
<td>Beef liver</td>
<td>6.0( ^\circ )</td>
<td>0.20</td>
<td>3.24</td>
</tr>
<tr>
<td>Beef liver</td>
<td>15.6( ^\circ )</td>
<td>0.60</td>
<td>3.13</td>
</tr>
</tbody>
</table>

*In addition to the enzyme, solutions contained ammonium sulfate at 0.18 M, glycine at 0.16 M, and Tris and Na\( \text{SO}_3 \) both at 10\( ^{-3} \) M, pH 7.6.

\[
\hat{s}_{20,w} = \left[ \frac{2.303}{60} \left( \frac{d \log x}{dt} \right) \right] \left[ \frac{\eta - \eta_0}{\eta_0} \right] \left[ \frac{1 - \frac{P_{\text{prot}}}{P_{\text{r}}}}{1 - \frac{V_{\text{prot}}}{V_{\text{r}}}} \right]
\]

where \( d \log x/dt \) was calculated by the method of least squares as the slope of the best fitting straight line connecting the points [log distance of schlieren maximum from axis of rotation (cm.)]

\( \eta/\eta_0 \) was determined by capillary viscometry, \( \rho_e \) pycnometrically. Sörbo's (4) value for \( V \) was used.

**DISCUSSION**

The principal modification which has made possible the crystallization of beef kidney rhodanese is the introduction of a fractionation step involving DEAE-cellulose. The use of this ion exchanger in a batchwise procedure rather than as a column has been necessitated by the instability of rhodanese in contact with the exchanger under the conditions required for selective elution.

The beef kidney and liver rhodaneses do not differ in either crystal form or sedimentation behavior.3 In these properties and in specific activity, as in the other biochemical properties reported previously (5), the two enzymes appear to be identical. Some biochemical genetic implications of such findings have previously been discussed (5).

**SUMMARY**

A method for preparing crystalline beef kidney rhodanese has been developed. The same procedure has been found useful as a rapid, small scale method for preparing crystalline beef liver rhodanese. The procedure includes a batchwise fractionation with \( N, N \)-diethylaminomethyl cellulose as well as ammonium sulfate and acetone fractionation steps based on Sörbo's procedure for the crystalline liver enzyme. The crystalline beef kidney and beef liver rhodaneses are indistinguishable in specific activity, crystal form, and sedimentation rate.

**REFERENCES**


2 The difference between the \( s_{20,w} \) value 3.2 obtained for crystalline liver rhodanese in this work and the value 3.0 previously reported by Sörbo (4) may be related to the differences in the buffers used for the sedimentation runs. A buffer of high ionic strength containing glycine and thiosulfate was considered preferable here, since under these conditions all of the enzymatic activity could be recovered from the analytical cell after sedimentation at low temperatures.
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