1. Introduction

Previously, a purification procedure for citrate lyase (EC 4.1.3.6) of *Rhodopseudomonas gelatinosa* has been described [1] which because of the instability of the enzyme in some purification steps and of the low yields is not suited for the preparation of large amounts of citrate lyase. In connection with studies on the enzymes which modify citrate lyase by acetylation or deacetylation [2,3] it was necessary to prepare large amounts of enzyme. The procedure used for this purpose is reported here. It involves 2 very effective crystallization steps and yields preparations with a specific activity much higher than measured with purified citrate lyases from other sources. Sodium dodecylsulfate gel electrophoresis revealed the presence of 3 types of subunits in the enzyme as has been shown for citrate lyase from other microorganisms [4–7].

2. Materials and methods

2.1. Growth of bacteria

*Rhodopseudomonas gelatinosa* DSM 149 was grown in 20 l carboys anaerobically in the light for 36 h. The medium contained 30 mM sodium citrate as carbon source, otherwise it was composed as in [2]. The cells were harvested with a Cepa continuous centrifuge, and the cell paste was stored at −20°C.

2.2. Electrophoresis

Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed in tubes and vertical slabs using 7.5% gels as in [8].

The electrophoresis was carried out in 50 mM sodium phosphate buffer (pH 7.0) + 0.1% SDS at a current of 6 mA/tube for 3.5 h and a power of 10 W for 6 h for the slab, respectively. Sample preparation: Citrate lyase crystals were washed twice with 1 ml 0.3 M ice-cold sodium phosphate buffer (pH 7.0). The sediment was dissolved in 10 mM sodium phosphate buffer (pH 7.0) and adjusted to 1 mg protein/ml. The solution was then incubated with 2% SDS and 1% 2-mercaptoethanol for 3 min at 100°C. After staining with Coomassie blue the gel was scanned for protein $A_{669}$ with a Vitatron TLD 100. Protein determinations were performed according to [9].

2.3. Enzyme assay

Cells of *R. gelatinosa* used for enzyme purification contained citrate lyase in the inactive HS-form. For determination of enzyme activity it was necessary to acetylate the enzyme chemically with acetic anhydride [10]. The test system was described [1].

Citrate lyase from *Streptococcus diacetilactis* was purified by the method in [11].

2.4. Materials

DEAE-Cellulose (DE 52) was purchased from Whatman Biochem., Maidstone, Kent and Alumina Cy gel from Serva, Heidelberg. The chemicals and enzymes were from E. Merck, Darmstadt or from Boehringer, Mannheim.

3. Results

3.1. Purification and crystallization of citrate lyase

Unless otherwise specified all steps were carried
out at 0–4°C. Frozen cells, 120 g, were suspended in 360 ml 75 mM potassium phosphate buffer (pH 7.2) containing 3 mM MgCl₂ and 1 mM dithioerythritol (DTE). The cells were disrupted by passing the suspension through a French press at 9500–13 500 N/cm². Cell debris was removed by centrifugation at 20 000 X g for 45 min, and the supernatant (cell extract) was used for purification of citrate lyase.

A 1% (w/v) solution of cetyltrimethylammonium bromide (CTAB) 0.4 vol., was added dropwise to the cell extract with stirring. The precipitation was removed by centrifugation at 20 000 X g for 45 min and the supernatant was subjected to precipitation with Alumina C γ gel. Alumina C γ gel (20 mg dry wt/ml) 0.5 vol., was carefully mixed with the supernatant, and the precipitate was removed by centrifugation as above. DTE was added to the supernatant to give 1 mM final conc., then the supernatant was concentrated at room temperature with the hollow fiber system DC 2 HF 10 (Amicon, N.V., Oosterhout) to 100 ml.

The protein solution from the above step was applied to a column (5 cm diam X 25 cm) of DEAE-cellulose which had been equilibrated against the same buffer as used for cell extract preparation. The column was washed with 1200 ml of this buffer and citrate lyase was eluted with 0.1 M potassium phosphate buffer (pH 7.2) containing 0.1 M KCl, 3 mM MgCl₂ and 1 mM DTE at 80 ml/h flow rate. Fractions, 6 ml, were collected and citrate lyase eluted with fractions 55–75.

The specific activity of citrate lyase prepared by the above procedure was 3-times higher than that obtained with the previous procedure. Since the presence of 2 types of subunits with mol. wt 61 000 and 30 000 was reported for the previous preparation [1] the crystalline enzyme preparation was also subjected to SDS–PAGE. It is apparent from fig.2 that citrate lyase of R. gelatinosa contains 3 types of subunits as reported for this enzyme from Enterobacter aerogenes [4–6] and from S. diacetilactis [7,12]. The 3 protein bands corresponded to mol. wt 53 700, 35 500 and 12 000, respectively (fig.3). In order to establish the reliability of our procedure the electrophoresis was run with citrate lyase from S. diacetilactis as a marker protein. Values of 53 700, 35 500 and 12 000 were obtained for the subunits,
### Table 1

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume (ml)</th>
<th>Total protein (mg)</th>
<th>Total activity (U)</th>
<th>Spec. act. (U/mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell extract</td>
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<td>19 320</td>
<td>1.5</td>
<td>100</td>
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<tr>
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<td>7920</td>
<td>17 380</td>
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<td>90</td>
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<tr>
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<td>15 180</td>
<td>5.7</td>
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<tr>
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<td>336</td>
<td>12 800</td>
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<tr>
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<td>26</td>
<td>9300</td>
<td>358</td>
<td>48</td>
</tr>
</tbody>
</table>

### 4. Discussion

The property of citrate lyase from *R. gelatinosa* to crystallize from a partially purified, concentrated protein solution allowed the preparation of homogeneous citrate lyase in large amounts. The enzyme obtained had spec. act. 360 U/mg protein which was much higher than obtained with the previous purification procedure or reported for the citrate lyases from *S. diacetilactis* and *Ent. aerogenes*. A possible reason for this could be that this purification procedure did not involve gel filtration steps. During purification of citrate lyase from *R. gelatinosa* these steps were always associated with a considerable decrease of the yield [1], and the final enzyme preparations presumably contained considerable amounts of denatured enzyme.

SDS–PAGE showed that citrate lyase of *R. gelatinosa* is also composed of 3 types of subunits. This subunit composition conforms to the model of the hexameric structure of citrate lyase as proposed [1] on the basis of electron microscopic studies.

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References