Purification of the Subunits of Transcarboxylase by Affinity Chromatography on Avidin-Sepharose*

Melvin Berger and Harland G. Wood

From the Department of Biochemistry, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106

SUMMARY

Transcarboxylase consists of a central 12 S subunit and three peripheral 5 S subunits each of which is linked to the central subunit by two 1.3 S biotin carboxyl carrier proteins. The subunits from dissociated transcarboxylase have been difficult to isolate because conditions which stabilize them also promote their reassociation to the intact enzyme. In this paper, we describe the use of avidin-Sepharose to adsorb the enzyme from crude extracts or partially purified transcarboxylase of propionibacteria. After removing impurities by washing the column with phosphate buffer at pH 6.5, in which the transcarboxylase is stable, the enzyme is dissociated first by elution at pH 8 yielding a fraction containing mostly 12 S subunit which can be rapidly stabilized against dissociation to 6 S without the problem of reconstitution because the 1.3 S and most of the 5 S subunits are not eluted. The second elution is at pH 9 which yields the 5 S subunit by dissociation from the 1.3 S biotin subunit and the 1.3 S subunit remains bound to the avidin. The 12 S and 5 S subunits are further purified by glycerol density gradient centrifugation or by chromatography on Bio-Gel. Very active enzyme can be reconstituted from these subunits upon the addition of the 1.3 S subunit.

EXPERIMENTAL PROCEDURE

Materials and Methods

Transcarboxylase was isolated from Propionibacterium shermanii and assayed as previously described (6). Purification was carried through chromatography on cellulose phosphate except as noted in the text. Specific activity is defined as micromoles of oxalacetate formed per min per mg of protein.

Recent studies in this laboratory indicate that a portion(s) of the 1.3 S subunit provides the link between the 5 S and 12 S subunit such that in the absence of this peptide reconstitution of the enzyme does not occur (5). This fact suggested that, if the 1.3 S could be removed from a mixture of the dissociated subunits, the 12 S subunit could be stabilized by lowering the pH and raising the ionic strength but it would not combine with 5 S and both could be isolated in good yield.

We describe here the adsorption of transcarboxylase onto avidin-Sepharose and the subsequent stepwise elution of the 12 S and 5 S subunits whereas the 1.3 S subunit remains bound to the avidin. The addition of concentrated phosphate buffer to the eluates allows the rapid stabilization of the 12 S subunit and its isolation.

* This investigation was assisted by Grant AM 12245 from the National Institutes of Health.

1 The sedimentation coefficient of the 5 S subunit was not determined until recently and is approximately 5.8 S as shown here and in the accompanying paper (2).
formed using the method of Martin and Ames (9), and an automatic fractionator (ISCO model 640) was used. The sedimentation coefficients were determined in a Spinco model E analytical ultracentrifuge equipped with schlieren optics, electronic speed control, and rotor temperature indicator control. Double sector cells were used.

Sepharose 4B was obtained from Pharmacia, cyanogen bromide from Biochem Chemical Co., 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-HCl from the Ott Chemical Co., biotin from Calbiochem, (2'-4Cl)biotin (20 mCi per mmol) from Amer sham-Shell, and avidin from Worthington Biochemical Corp. Different lots of avidin had specific activities varying from 11.6 to 12.6 units per mg and all of them were used without further purification. DNase was from P-L Biochemicals, Inc. and pronase from Calbiochem. All other biochemicals were of the highest purity commercially available.

Polyacrylamide gel electrophoresis in 8 M urea was performed using the 5.3% gel Tris-urea system of Jovin et al. (10) with the following modifications: the upper gel was omitted, and an Ortec pulsed constant power supply (model 4100) was used. The discharge capacitance was set at 100 μF and the current was set at 4 mA. The run time was about 2 hours and 2 l/min tap water was used for cooling. Solid urea was added to the sample (about 1 mg of protein per ml of 0.1 M phosphate buffer, pH 6.5) to bring the final concentration of urea to 8 M. Mercaptoethanol was added to each sample to a concentration of 50 mM, and the samples were immediately placed in a boiling water bath for 10 min. Then the samples were removed and allowed to cool to room temperature overnight. A small drop of 0.66% bromphenol blue was added to each sample and aliquots of 10 to 100 μl were applied to the gels. For gels run in the absence of urea, the 7.5% acrylamide-Tris system of the same authors was used (10). Again, the upper gel was omitted and glycerol was added to the sample solutions for density stabilization. The samples were not boiled and mercaptoethanol was not used in the absence of urea. Urea gels were run in the absence of urea at 10% glycerol which was used in addition to the dye is displaced from the avidin and the pink color disappears. Titration is this manner indicated that the packed resin had a biotin-binding capacity of 0.04 μmol of biotin per ml of avidin-Sepharose, in good agreement with the reported results (11).

Preparation of Avidin-Sepharose—Sepharose 4B was activated with cyanogen bromide and avidin was then coupled to it by the method of Botda and Bodansky (11). In a typical preparation, 20 ml of packed Sepharose were activated with 2 g of cyanogen bromide and then 20 mg of avidin was used for the coupling. About 70% of the protein became attached to the matrix as indicated by monitoring the A100, the absorbance of the effluent of the washed resin. The resin turns pink when mixed with 2(4'-hydroxyazobenzene)-benzoic acid (Sigma) which binds to the avidin and upon the addition of HCl the dye is displaced from the avidin and the pink color disappears. Titration is this manner indicated that the resin had a biotin-binding capacity of 0.04 μmol of biotin per ml of avidin-Sepharose, in good agreement with the reported results (11).

Preparation of Biotin-Sepharose—Sepharose 4B was activated with cyanogen bromide and ethylenediamine was added to prepare aminomethyl-Sepharose by the method of Cuatrecasas and Anfinsen (12). In a typical preparation, 30 ml of settled aminomethyl-Sepharose in H2O were stirred gently at room temperature. Biotin (300 mg) was dissolved in 30 ml of dimethylformamide, and 11.5 x 10^4 cm^2 of [2-14C]biotin (in 0.4 ml of H2O, 20 mCi per mmol) were added as a tracer. This solution was added to the Sepharose suspension with continuous stirring. The pH of the mixture was 4.7. Then, during continued stirring, 1 g of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-HCl in 3 ml of H2O was added to the biotin and Sepharose mixture. The pH rose to 5.5 within minutes and was adjusted to 4.5 with 1 N HCl. This pH adjustment was repeated at 45 min and 1 and 2 hours and the mixture was stirred for 20 hours at room temperature. The resin was then washed on a coarse scintillator glass funnel with 2.4 liters of 50% dimethylformamide, 0.05 M Tris-HCl, 2 liters of 0.05 M Tris-HCl, and 2 liters of H2O. Aliquots of the resin suspension (0.5 and 1.0 ml) were prepared for counting in a liquid scintillation spectrometer by suspending them in 10 ml of Aquasol (New England Nuclear Corp.) with sufficient H2O to form a stiff gel at room temperature. There were about 10,500 cpm per ml of Sepharose, corresponding to 1.1 μmol of biotin per ml.

Later preparations using a second addition of carbodiimide after 10 hours of mixing yielded approximately double the amount of biotin coupled to the Sepharose. The Sepharose derivative was stored as aqueous suspensions containing 0.04% sodium azide as a preservative and gave no evidence of decomposition after several months at 4°C.

**RESULTS**

Adsorption of Transcarboxylase onto Avidin-Sepharose Followed by Elution and Purification of Subunits—After a number of preliminary experiments such as dissociating the transcarboxylase before applying it to avidin-Sepharose or mixing the transcarboxylase with avidin in free solution followed by chromatography on biotin-Sepharose, the following procedure was adopted. The transcarboxylase containing [3H]biotin (35 mg with a specific activity ~20 which had decreased to 12.6 after storage at 0°C) was mixed with 29 ml of 0.25 M potassium phosphate buffer, pH 6.5. This solution and 10 ml of avidin-Sepharose (packed by low speed centrifugation) was placed in a 50 ml screw-capped tube. The tube was rotated end over end overnight at 5°C. These conditions were chosen to allow maximum contact of the enzyme with the avidin-Sepharose, whereas the relatively high ionic strength and low pH were chosen to prevent the enzyme from undergoing dissociation.

A 1.4 cm (inside diameter) column is prepared as follows. A Saran screen bed support and a layer of glass wool are placed on the bottom and a layer of biotin-Sepharose is poured on it and allowed to settle to a height of 2 cm. The biotin-Sepharose retains avidin and/or its subunits either as a complex with subunits of transcarboxylase or as free avidin. A layer of glass wool is then placed above the biotin-Sepharose and then a 2-cm layer of avidin-Sepharose is added, and the column is washed with approximately 10 ml of 0.25 M phosphate buffer, pH 6.5. The enzyme-avidin-Sepharose mixture is then applied to the column at room temperature. The effluent from the column is collected in 5-ml fractions which are kept in an ice bath. Elution is with 0.25 M phosphate buffer, pH 6.5, until the protein concentration becomes lower than 0.05 mg per ml as evidenced by the absorption at 280 nm (Fig. 1). This elution removes most of the impurities but leaves the intact enzyme bound to the avidin-Sepharose. Elution at room temperature is then begun with 0.05 M Tris-PO4, pH 8.0, containing 20% glycerol (Fig. 1). This buffer promotes dissociation of the transcarboxylase to the 12 S and 6 S subunits and the glycerol stabilizes the 12 S subunit (4). The 6 S subunit remains bound to the avidin-Sepharose because it contains the biotin. To prevent dissociation of the eluted 12 S subunit to the 6 S subunits, the fractions are collected in ice-cooled tubes containing 0.5 ml (one-tenth of the total fraction volume) of 1.5 M phosphate buffer, pH 6.5. Some 5 S subunit does elute with the 12 S subunit but no reassociation occurs because the 1.3 S subunit remains bound to the avidin-Sepharose. A further elution with 0.05 M Tris-Cl, pH 9.0, causes dissociation of the 6 S to the 5 S subunit leaving the 1.3 S still bound to the avidin-Sepharose. Because some of the 12 S subunit does elute at this time, the fractions are again collected over 1.5 M phosphate buffer, pH 6.5, and kept on ice. The elution pattern of the protein is given in Fig. 1 and the results are summarized in Table 1. The fractions from each of the elution buffers are pooled and the proteins precipitated with 70% saturated (NH4)2SO4. Prior to precipitation, the eluate in Tris-PO4, 20% glycerol is dialyzed against 0.1 M phosphate buffer, pH 6.5, to remove the glycerol which interferes with the precipitation.

**Analysis by Ultracentrifugation and Polyacrylamide Gel Electrophoresis of Eluates from Avidin-Sepharose Chromatography**—
Fig. 1. Elution profile from chromatography of partially purified transcarboxylase on avidin-Sepharose. Arrows indicate buffer changes. See text for details.

**TABLE I**

Summary of chromatography of partially purified transcarboxylase on avidin-Sepharose

For details, see text and Fig. 1.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Enzymatic activity</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
<td>Total</td>
</tr>
<tr>
<td>mg</td>
<td>units</td>
<td>units/mg</td>
</tr>
<tr>
<td>Starting material</td>
<td>35</td>
<td>440</td>
</tr>
<tr>
<td>Eluate with potassium phosphate buffer (pH 6.5)</td>
<td>16.8</td>
<td>1.17</td>
</tr>
<tr>
<td>Eluate with Tris-SO₄, pH 8, 20% glycerol</td>
<td>9.8</td>
<td>2.25</td>
</tr>
<tr>
<td>Eluate with Tris-Cl, pH 9</td>
<td>7.7</td>
<td>0.60</td>
</tr>
<tr>
<td>Total recovery</td>
<td>34.3</td>
<td>4.02</td>
</tr>
</tbody>
</table>

Sedimentation velocity profiles of the starting material and of the proteins eluted from the avidin-Sepharose column are shown in Fig. 2. Transcarboxylase with a typical double peak of \( s_{20,W} = 16 \) S, 18 S plus a substantial amount of slower moving material, was present in the starting material (Fig. 2A). This slow moving material was the major component of the eluate with phosphate buffer (Fig. 2B), from which all of the transcarboxylase apparently was removed by adsorption onto the avidin-Sepharose. The eluate with Tris-SO₄-glycerol at pH 8 had a major peak at \( \sim 12 \) S and a smaller amount of \( 6 \) S material (Fig. 2C). There also was a very small amount of protein which sedimented faster than the \( 12 \) S subunit; this may represent a slight amount of intact transcarboxylase and/or complexes of the subunits with avidin which were not trapped by the biotin-Sepharose because of saturation of the biotin-binding site of the avidin or inaccessibility of the biotin of the enzyme to the avidin. The eluate with Tris-Cl, pH 9, contained mostly the \( \sim 5 \) S material with a small amount of 12 S material (Fig. 2D).

Polyacrylamide gel electrophoresis in 8 M urea of the starting material (Fig. 3A) gave the two major bands characteristic of transcarboxylase, the faster moving band being the 2.5 S₅ peptide arising from the 5 S₅ subunit and the slower moving band, the 2.5 S₇ peptide from the 12 S₇ subunit (13). The eluate with phosphate, which contains the impurities, gave bands with the same mobility as the transcarboxylase peptides (Fig. 3B). Immunological studies showed that antibodies prepared against
the subunits of transcarboxylase cross-reacted with this material, suggesting that at least part of the protein in the eluate with phosphate buffer consisted of peptides from transcarboxylase. The eluate with Tris-SO₄ (pH 8)-20% glycerol (Fig. 3C) gave only two bands, one of which corresponds to the slower moving 2.5 Sn peptide and was denser than the faster moving band which corresponds to the 2.5 SE peptide (13). The eluate with Tris-Cl, more of the 5 SE subunit.

Further Purification of 12 Sn Subunit and 5 Ss Subunit by Glycerol Gradient Centrifugation—The eluate with Tris-Cl (pH 9) contained about 0.8 mg of protein was layered on top of each gradient tube. Fractions of 0.1 ml were collected from the top of the gradients using an ISCO model 640 density gradient fractionator. The fractions from all six of the gradients run at one time were collected in the same tubes, such that the final volume of each fraction was 0.6 ml. Fractions between the arrows were pooled as indicated in the text.

FIG. 4. Separation of 12 Sn and 5 Ss subunits by glycerol density gradient centrifugation. A, eluate with Tris-Cl (pH 9). The gradients were 10 to 30% glycerol in 0.1 m phosphate buffer at pH 6.5. Centrifugation was at 30,000 rpm for 16 hours at 40,000 rnm in a Beckman SW 56 rotor. The proteins applied to the gradients had been precipitated with ammonium sulfate and then resuspended in and dialyzed against 0.1 m phosphate buffer, pH 6.5. Approximately 0.2 ml of solution containing about 0.8 mg of protein was layered on top of each gradient tube. Fractions of 0.1 ml were collected from the top of the gradients using an ISCO model 640 density gradient fractionator. The fractions from all six of the gradients run at one time were collected in the same tubes, such that the final volume of each fraction was 0.6 ml. Fractions between the arrows were pooled as indicated in the text.

FIG. 5. Sedimentation profiles of combined pools from glycerol gradients as described in Fig. 4. A, 12 Sn subunit (\(s_{20,w} = 12.2\)); B, 5 Ss subunit from eluate with Tris-Cl (pH 9) (\(s_{20,w} = 5.97\)); and C, 5 Ss subunit from eluate with Tris-Cl (pH 9) (\(s_{20,w} = 5.49\)). The peaks from the gradients were dialyzed to remove glycerol and precipitated with 70% saturated ammonium sulfate. They were resuspended in 0.1 m phosphate buffer and dialyzed 16 hours at 4°C against the same buffer. Centrifugation was at 48,000 rpm at 4°C. The profiles shown were taken after 60 min. A, 1.75 mg per ml; B, 1.69 mg per ml; and C, 1.75 mg per ml.

Use of Avidin-Sepharose to Purify Subunits of Transcarboxylase from a Crude Extract of Propionibacterium shermanii—Thus far, transcarboxylase is the only biotin-containing enzyme detected in propionibacteria. It has been proposed that fatty acids may be formed by these bacteria by the use of transcarboxylase rather than acetyl-CoA carboxylase (14) because transcarboxylase is capable of forming malonyl-CoA from acetyl-CoA (1). It thus seemed possible that avidin-Sepharose affinity chromatography might be used to purify the subunits of transcarboxylase directly from crude extracts. The crude extract was prepared from 30 g of P. shermanii as previously described (6) except that a Sorvall Omni-Mixer was used instead of the Eppenbach colloid 

The analysis was done using polyacrylamide gel electrophoresis although the constituent peptides could be resolved by gel electrophoresis in the presence of 8 M urea (13). During the course of these investigations it was found that a procedure using a 7.5% acrylamide gel system buffered with Tris-Cl at pH 8.9 (10) does separate the subunits as illustrated in Fig. 7. When intact transcarboxylase in 0.1 M phosphate buffer, pH 6.5, is applied to the gel, the enzyme dissociates and three major bands are observed (Fig. 7A). When the transcarboxylase is dissociated at pH 9.0 to the 6 Sn, 5 Sn, and 1.3 Sn subunits, only two major bands are observed corresponding to the 6 Sn and 5 Sn subunits (Fig. 7B). The 1.3 Sn subunit is present in such low concentration and probably diffuses so much that it is not seen as a distinct band. The two major bands migrate at the same rate as the slowest and fastest components in the gel run on untreated transcarboxylase. The results obtained with the purified 5 Sn subunit are shown in Fig. 7C. Only one major band is observed, corresponding in position to the fastest moving band of the transcarboxylase dissociated at pH 9. Thus, the slowest moving band in the gels shown in Fig. 7, A and B represent the 6 Sn subunit. The results with the purified 12 Sn subunit are presented in Fig. 7D. Two major bands are observed, the slower being from the 6 Sn subunit which is formed by dissociation of the 12 Sn subunit during the electrophoresis at alkaline pH and the faster moving band is from the undissociated 12 Sn subunit. Other experiments have shown that when isolated 12 Sn is preincubated at pH 9, only one band is observed on the gels and the band corresponds to the 6 Sn band as seen here. At present, we have no explanation of why the 12 Sn subunit of molecular weight 360,000 migrates faster than the 6 Sn subunit of molecular weight 120,000.

The results of gel electrophoresis of the crude extract and eluates from the avidin-Sepharose column are shown in Fig. 8. Only a small amount of material is retained by the avidin-Sepharose and the gels of the original crude extract (Fig. 8A) and of the eluate with phosphate (Fig. 8B) are very similar. Comparison of the gel from the eluate with Tris-Cl, pH 8 (Fig. 8C) with those of Fig. 7 shows that it contained the 12 Sn subunit together with a large amount of the 5 Sn subunit. The eluate with Tris-Cl, pH 9 (Fig. 8D) contained predominantly the 5 Sn subunit as well as some 12 Sn and 6 Sn subunits. Sedimentation profiles of the protein in these eluates are shown in Fig. 9 and are in accord with the results from the gels.

**Separation of 5 Sn and 12 Sn Subunits by Chromatography on Bio-Gel**—Previous attempts to separate the subunits of transcarboxylase by gel filtration have been only partially successful.

---

**Table II**

Summary of chromatography of crude extract from *P. shermanii* on avidin-Sepharose

For details, see text. Note that in this case, the protein concentrations were determined using the microbiuret procedure of Zamenhof (8) with bovine serum albumin (Pentex) as a standard.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Transcarboxylase activity</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>mg</td>
<td>units</td>
</tr>
<tr>
<td>Eluate with phosphate buffer (pH 6.5)</td>
<td>888</td>
<td>0.00</td>
</tr>
<tr>
<td>Eluate with Tris-SO4, pH 8, + 20% glycerol</td>
<td>10.8</td>
<td>4.98</td>
</tr>
<tr>
<td>Eluate with Tris-Cl, pH 9</td>
<td>14.8</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>914</td>
<td>4.98</td>
</tr>
</tbody>
</table>

---

**Fig. 6.** Polyacrylamide gel electrophoresis in 8 M urea of subunits purified by glycerol gradient centrifugation. A, 30 µg of 12 Sn subunit; B, 30 µg of 5 Sn subunit from eluate with Tris-SO4 (pH 8)-20% glycerol; and C, 30 µg of 5 Sn subunit from eluate with Tris-Cl (pH 9).
FIG. 9. Sedimentation profiles of Tris-\(SO_4\)-glycerol (pH 8) (A) and Tris-Cl (pH 9) (B) eluates from crude extract avidin-Sepharose experiment. Both eluates were precipitated with 70\% saturated ammonium sulfate then resuspended in 0.1 M potassium phosphate buffer, pH 6.5, and dialyzed at 4° against the same buffer. Both samples were run at 2.0 mg per ml. Photographs were taken 68 min after the centrifuge reached 48,000 rpm. \(S_{20,w} = 12.17\), 5.49 in (A), \(\sim 12\), 5.73 in (B).

FIG. 7. Polyacrylamide gel electrophoresis at pH 8.9 (in the absence of urea) of intact transcarboxylase, of transcarboxylase dissociated at pH 9, and of the isolated 12 \(S_h\) and 5 \(S_h\) subunits. A, 45 \(\mu\)g of intact transcarboxylase dialyzed 4 hours versus 0.1 M phosphate buffer, pH 6.5; B, 30 \(\mu\)g of transcarboxylase dialyzed 24 hours versus 0.05 M Tris-Cl, pH 9.0; C, 30 \(\mu\)g of 5 \(S_h\) subunit purified by glycerol gradients; and D, 30 \(\mu\)g of 12 \(S_h\) subunit purified by glycerol gradients. Gels were run as described under "Materials and Methods." Note that Gels C and D correspond to Gels B and A of Fig. 6, respectively.

FIG. 10. Separation of the 5 \(S_h\) and 12 \(S_h\) subunits by chromatography on a Bio-Gel A-1.5m column. A mixture of 5 \(S_h\) and 12 \(S_h\) subunits obtained from crude extracts by avidin-Sepharose chromatography was dialyzed to remove the glycerol, precipitated with \((\text{NH}_4)_2\text{SO}_4\) at 80\% saturation, suspended in 0.1 M phosphate buffer, pH 6.5, and then dialyzed against the buffer. Seventy milligrams of protein in 6.9 ml were applied to a column (101 X 2.3 cm) of Bio-Gel A-1.5m which had been equilibrated with this buffer and was eluted with the same buffer using a flow rate of 15 ml per hr. Fractions (2.5 ml) were collected and pooled as indicated. Fractions 87 to 97 contained 21.4 mg of protein with an \(S_{20,w} = 12.2\) S and Fractions 109 to 118, 20.5 mg with an \(S_{20,w} = 5.4\) S because the subunits interact and reassociate to form active enzyme (2). Because the 1.3 \(S_h\) subunit is required for this interaction (5) it seemed likely that a mixture of 12 \(S_h\) and 5 \(S_h\) subunits, which had been freed of the 1.3 \(S_h\) subunit by avidin-Sepharose chromatography, could be separated by gel filtration. The results are shown in Fig. 10 and demonstrate that in marked contrast to previous attempts to separate the subunits of transcarboxylase by gel filtration, the 12 \(S_h\) and 5 \(S_h\) subunits are clearly resolved if the 1.3 \(S_h\) subunit is first removed from the mixture.

**DISCUSSION**

In this paper, we describe the use of avidin-Sepharose to adsorb transcarboxylase from crude or partially purified extracts of *P. shermanii*. Impurities are then removed by washing the column with phosphate buffer in which the transcarboxylase as the complex with avidin is stable. Following this, the transcarboxylase is dissociated stepwise using buffers at pH 8 and pH 9
to obtain the 12 $S_H$ and 5 $S_E$ subunits whereas the 1.3 $S_E$ biotin carboxyl carrier protein remains bound to the avidin-Sepharose.

There are two main advantages of this procedure over the direct isolation of the subunits from dissociated transcarboxylase. The first is that purified enzyme is not required because quite pure subunit preparations are obtained even from crude extracts. The second advantage is that higher yields of the 12 $S_H$ subunit are obtained because the separation is relatively rapid, following which the 12 $S_H$ subunit can be stabilized against dissociation without loss because there is no reconstitution to the intact enzyme in the absence of the 1.3 $S_E$ subunit. The ability to stabilize the 12 $S_H$ also facilitates the purification of the 5 $S_E$ subunit because formation of the 6 $S_H$ subunit is minimal in this procedure. The results obtained when active transcarboxylase was reconstituted from these subunits will be presented by Wood et al. (2).

We have used the avidin-Sepharose procedure many times using from 1 to 20 ml of packed avidin-Sepharose and usually, about 50 units of transcarboxylase per ml of resin. All of these experiments gave similar results, with the amount of protein in the phosphate eluate depending on the nature of the starting material. We have found in mixing the avidin-Sepharose and transcarboxylase solution that the tube containing the mixture must be very full, or the protein will aggregate, apparently due to surface denaturation at the air-solution interface during the rotation of the tube.

In early experiments, the Tris-SO$_4$ and Tris-Cl eluates sometimes contained, in addition to a small amount of transcarboxylase activity, some material which inhibited added transcarboxylase. Thinking that this represented a complex of avidin or avidin subunits together with transcarboxylase which may have lost one or two peripheral subunits, we adopted the technique reported here, including the prolonged mixing and the layer of biotin-Sepharose at the bottom of the column to bind any avidin complex. These modifications have been successful in eliminating the problem of inhibitory material in the eluates, but a small amount of active transcarboxylase is still eluted. Although Green et al. (3) have shown that the biotinyl groups on transcarboxylase are in a relatively exposed position and may easily form a complex with avidin, it is possible that the coupling of avidin to the Sepharose matrix makes it difficult for all of the biotins in some of the transcarboxylase molecules to form complexes. Thus, some transcarboxylase molecules with only part of the peripheral subunits attached to the avidin could, on dissociation, be eluted with the uncomplexed peripheral subunit still attached to the 12 $S_H$ subunit. This has not been a major difficulty but may account for the small amount of transcarboxylase activity recovered in the eluates.

The results obtained when this method was used with the crude extract demonstrate that a two-step purification for the subunits is practical, i.e. affinity chromatography followed by separation of the 5 $S_E$ and 12 $S_H$ subunits by density gradient centrifugation or gel filtration. These experiments also indicate the possibility that there may be an excess of peripheral subunits in the intact cell, because the yield of 5 $S_E$ appears to be greater than the yield of 12 $S_H$ from the crude extract (Fig. 8, C and D). The cells may synthesize peripheral subunits which are not incorporated into active enzyme molecules, or the 18 $S$ form of the enzyme which is isolated may not be the native form of the enzyme. A 24 $S$ form of the enzyme has been described which contains an extra set of peripheral subunits (3, 4, 17). It is possible that one set of peripheral subunits is removed during the isolation procedures currently employed. Further investigations concerning this question are presently in progress.

The strong binding of biotin to avidin has suggested the use of these two compounds in several affinity chromatography systems (11, 18–20). The usefulness of these systems in the preparation of biotin enzymes has been limited, however, by the inability to dissociate the avidin-biotin complex without using strongly denaturing conditions such as 6 M guanidine-HCl and pH 1.5 (21). Because transcarboxylase dissociates at a mildly alkaline pH yielding non-biotin subunits, we have been able to use avidin-Sepharose to purify these subunits. Attempts to elute the 1.3 $S_E$ biotin carboxyl carrier protein using 2% sodium dodecyl sulfate have been only partially successful. The method described here may be applicable to the study of other biotin enzymes which dissociate to non-biotin subunits.

REFERENCES

Purification of the subunits of transcarboxylase by affinity chromatography on avidin-sepharose.
M Berger and H G Wood


Access the most updated version of this article at http://www.jbc.org/content/250/3/927

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/250/3/927.full.html#ref-list-1