Properties and Subunit Composition of the Pig Heart 2-Oxoglutarate Dehydrogenase*

(Received for publication, November 21, 1973)

KICHIKO KOIKE,† MINORU HAMADA, NOBUTUKI TANAKA, KIN-ICHI OTSUKA, KYOKO OGASAHARA, AND MASAHITO KOIKE

From the Department of Pathological Biochemistry, Atomic Disease Institute, Nagasaki University School of Medicine, Sakamoto-cho, Nagasaki-shi, 852, Japan

SUMMARY

The 2-oxoglutarate dehydrogenase, one of the component enzymes of the 2-oxoglutarate dehydrogenase complex, has been highly purified. The enzyme has a sedimentation coefficient (s~20,w) of 10.3 S and diffusion coefficient (D~20,w) of 3.92 x 10^-7 cm^2 s^-1. The weight average molecular weight was estimated to be about 216,000 from the sedimentation equilibrium data. The content of right-handed α-helix in the enzyme molecule was estimated to be about 34% by both optical rotatory dispersion and circular dichroism. The enzyme was found to contain 1 molecule of protein-bound thiamine-PP per mole; some other kinetic and protein chemical properties are also reported.

In 6 M guanidine hydrochloride (pH 8.0), the enzyme was dissociated into its subunits and the molecular weight of subunit was estimated to be 97,000 from the sedimentation equilibrium data. On polyacrylamide gel electrophoresis in sodium dodecyl sulfate the enzyme was dissociated into its subunits with an estimated molecular weight of 113,000. The results indicate that 1 molecule of 2-oxoglutarate dehydrogenase consists of two similar subunits, which contains NH2-terminal alanine.

EXPERIMENTAL PROCEDURE

Materials

2-Oxoglutaric acid was obtained from Kyowa Hakko Kogyo, Tokyo. Takadiastase was a gift from Doctors G. Sunagawa and Y. Yusa, Sankyo Company, Tokyo. Permutit (50 to 80 mesh) for thiamine assay was obtained from the Japan Vitamin Society. B-Galactosidase, phosphorylase a, catalase, and albumin, aldolase, chymotrypsinogen, and cytochrome c were obtained from Boehringer Mannheim. Bovine serum albumin and diisopropylfluorophosphate were obtained from Sigma, St. Louis; and carbonic anhydrase was from Worthington Biochemical Corporation, Freehold, N. J. Lipoamide dehydrogenase was prepared from the 2-oxoglutarate dehydrogenase complex as described by Tanaka et al. (2). The sources of all other chemicals are as described previously (1-3).

Methods

Protein was determined by the phenol method (4) with bovine serum albumin as the standard. All other methods were carried out as described in previous papers (1-3).

Enzyme Assays—A ferricyanide-linked assay of 2-oxoglutarate dehydrogenase, based on Equation 4, was carried out at 25°C spectrophotometrically as described by Massey (5).

2-Oxoglutarate + 2 Fe(CN)₆³⁻ + H₂O → Succinate + CO₂ + 2 Fe(CN)₆⁴⁻ + 2 H⁺ (4)

The reaction mixture (2 ml) contained 1.34 μmoles of potassium ferrocyanide, 100 μmoles of potassium phosphate buffer (pH 6.5),...
0.1 mM of thiamine-PP, 1 mM of calcium chloride, 6.7 mM of potassium 2-oxoglutarate (pH 6.5), and 1.34 mM of bovine serum albumin and enzyme. Reaction was begun by the addition of 2-oxoglutarate (6.7 mM, 0.1 ml) and followed at 240 nm by a recording spectrophotometer. Ferricyanide-linked 2-oxoglutarate dehydrogenase activity was also determined by the modified method (6) of Hager (7) for pyruvate dehydrogenase, using potassium 2-oxoglutarate at a concentration of 17.5 mM.

Physicochemical Properties—The purity of the purified enzyme was determined by the method of Edman as modified by Eriksson and Bjöquist (32). Phenylthiohydantoin, separated by thin layer chromatography on Silica Gel F254 plates (from E. Merck) developed in chloroform, were scraped off and quantitated with 0.5% ethyl acetate in methanol. The ammonium sulfate step was determined from the absorbance of the extract at 289 nm. NH2-terminal groups were also identified by the dansyl-2-aminoethylaminobutyric acid method of Hartley (33) on a polyamide sheet.

Tryptic Digestion and Peptide Mapping—Peptide mapping of the 2-oxoglutarate dehydrogenase was performed on Whatman No. 3MM filter paper using 24 hours, transferred into a small test tube, and lyophilized. The dried sample was dissolved in 0.1 ml of 0.1 M Tris-HCl buffer (pH 8.5) containing 6 mM guanidine HCl and 0.1 M 2-mercaptoethanol, followed by incubation at 100°C for 5 min in an An-D rotor with a 12-mm double sector cell equipped with one such a cell and F/20. For determination of the subunit molecular weight, the protein samples previously dialyzed against deionized and redistilled water at 4°C before subjection to gel electrophoresis. About 0.1 mg of protein sample was dialyzed against deionized and redistilled water at 4°C for 24 hours, transferred into a small test tube, and lyophilized. The dried sample was dissolved in 0.1 ml of 0.1 M Tris-HCl buffer (pH 8.5) containing 6 mM guanidine HCl and 0.1 M 2-mercaptoethanol, followed by addition of 1 ml of 0.1 M diisopropylfluorophosphate in 1-propanol to avoid proteolysis (15). After 24-hour treatment at room temperature under nitrogen, the protein was carboxymethylated essentially as described by Glibe and Perham (16), dialyzed against deionized and distilled water, and lyophilized. Carboxymethylated protein samples were either incubated at 37°C for 2 hours or were heated at 100°C for 5 min in 0.01 mM sodium phosphate buffer (pH 7.0) containing 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol. Similar results were obtained by both procedures. The gels were stained with 0.25% Coomassie brilliant blue for 40 min at room temperature and destained by diffusion in methanol-acetic acid (14).

Optical Rotatory Dispersion and Circular Dichroism—Optical rotatory dispersion and circular dichroism were recorded at 25°C on a JASCO model ORD/UV 5 spectropolarimeter equipped with a circular dichroism attachment. Optical rotatory dispersion and circular dichroism intensities were, respectively, calibrated with an aqueous solution of sucrose to give an [α]20 of 0.65 (17) and with an aqueous solution of d-10-camphorsulfonic acid at 290 nm to give an εL - εR of 2.20 (18). Optical rotatory dispersion results are expressed in terms of mean residue rotation, [m°], and circular dichroism results in terms of molecular ellipticity, [θ]. Both parameters have units of degrees-cm² per d mole. The mean residue weight in all calculations is taken to be 112, which was calculated from amino acid composition. The content of right-handed α helix was calculated from both [m°]222 as described by Blout et al. (10) and [θ]222 by Holzwarth and Dorf (20).

Thiamine-PP Determination—Thiamine-PP content was determined by the method of Kaziro (21) with yeast pyruvate dehydrogenase (2-oxo-acid carboxylase, EC 4.1.1.1), which was prepared by the modified procedure (22) of Green et al. (23). Thiamine-PP was also assayed spectrofluorometrically by the thiochrome method of Fujiiwara and Matsui (24) after conversion to free thiamine by takadiastase.

The content of thiol groups was determined spectrophotometrically with 5,5'-dithiobis(2-nitrobenzoic acid) in the presence of 6 mM guanidine HCl in 0.01 mM potassium phosphate buffer (pH 8.0) (23), and with p-chloromercuribenzoate in the absence (20) and presence (27) of 8 mM urea in 0.05 M potassium phosphate buffer (pH 7.0).

Amino Acid Analyses—Amino acid analyses were performed by the method of Spackman et al. (28). Iodoacetamide protein was hydrolyzed with 1 ml of 5 M constant boiling HCl in vacuo at 110°C for 24 to 72 hours (29), and the hydrolysate was analyzed on a Beckman model 116 amino acid analyzer. Serine and threonine contents were calculated by extrapolation to zero time. Half-cystine was determined as cysteic acid after performic acid oxidation (30). Tryptophan and tyrosine were estimated spectrophotometrically by the method of Goodwin and Morton (31).

NH2-terminal Group Analyses—NH2-terminal groups were determined by the method of Edman as modified by Takahashi (36). Phenylthiohydantoin, separated by thin layer chromatography on Silica Gel F254 plates (from E. Merck) developed in chloroform, were scraped off and quantitated with 0.5% ethyl acetate in methanol. The ammonium sulfate step was estimated from the absorbance of the extract at 289 nm. NH2-terminal groups were also identified by the dansyl-2-aminoethylaminobutyric acid method of Hartley (33) on a polyamide sheet.

Tryptic Digestion and Peptide Mapping—Peptide mapping of the 2-oxoglutarate dehydrogenase was performed on Whatman No. 3MM filter paper as described by Katz et al. (34). Performic acid-oxidized protein was digested for 10 hours at 37°C with trypsin (substrate to enzyme ratio of 50:1) in 0.2 M ammonium bicarbonate (pH 8.3). The peptides (2 to 2.5 mg) were separated by a combination of paper chromatography and electrophoresis. Chromatography was performed with 1-butanol-pyridine-acetic acid-water (15:10:10:12) followed by electrophoresis in pyridine-acetic acid-water (1:1:1) at 3000 volts for 10 hours at 4°C. The tryptic digest was also identified by the dansyl-2-aminoethylaminobutyric acid method of Hartley (33) on a polyamide sheet.

Preparation of 2-Oxoglutarate Dehydrogenase—Pig heart 2-oxoglutarate dehydrogenase, which was isolated from the 2-oxoglutarate dehydrogenase complex as previously reported (2), was further purified by ammonium sulfate fractionation between 0.23 and 0.36 saturation. The purified enzyme had a specific activity of about 450 in the ferricyanide-linked 2-oxoglutarate dehydrogenase assay, and did not show any lipase soteinyltransferase or lipomino dehydrogenase activity. The ammonium sulfate step increased the specific activity by 10 to 15%. The purified enzyme was stored at -18°C in 0.05 M potassium phosphate buffer (pH 7.0) containing 0.5 mM EDTA for over 6 months without significant loss of activity. This preparation lost less than 10% of its activity by freezing at -18°C and thawing once.

RESULTS

Physicochemical Properties—The purity of the purified enzyme was examined by acrylamide-agarose composite gel electrophoresis. The enzyme protein migrated as a single band. The protein migrated as a single band. The protein was purified by ammonium sulfate fractionation between 0.23 and 0.36 saturation. The purified enzyme had a specific activity of about 450 in the ferricyanide-linked 2-oxoglutarate dehydrogenase assay, and did not show any lipase soteinyltransferase or lipomino dehydrogenase activity. The ammonium sulfate step increased the specific activity by 10 to 15%. The purified enzyme was stored at -18°C in 0.05 M potassium phosphate buffer (pH 7.0) containing 0.5 mM EDTA for over 6 months without significant loss of activity. This preparation lost less than 10% of its activity by freezing at -18°C and thawing once.

The abbreviations used are: [TPP], enzyme-bound thiamine-PP; [LipS], enzyme-bound lipoyl acid; [suecinyl-S-Lip-SH], enzyme-bound S-suecinyl dihydropicolinic acid; danyal, 5-dimethylaminophthalene-1-sulfonyl.
enzyme showed only one band (cf. Fig. 5B) on polyacrylamide gel electrophoresis in sodium dodecyl sulfate, indicating the homogeneity of the preparation.

The sedimentation velocity pattern of the enzyme showed a single major component and one minor component, as shown in Fig. 1. The reciprocal plot of $s_{20,w}$ against protein concentration was virtually linear, and $s_{20,w}$ was estimated as 10.3 S. The diffusion coefficient also showed a slight concentration dependence and the value extrapolated to infinite dilution ($D_{20,w}$) was $3.92 \times 10^{-7}$ cm$^2$ s$^{-1}$. From these values of $s$ and $D$ the molecular weight was calculated as 236,000, using for the partial specific volume a value of 0.732 ml per g, calculated from the amino acid composition.

In the meniscus depletion sedimentation runs, the plots of the logarithm of the vertical displacement ($c$) of a single fringe versus the square of the radial distance ($r^2$) were virtually linear, indicating homogeneity of the protein, as shown in Fig. 2. Values of the weight average molecular weight of the purified preparation of 2-oxoglutarate dehydrogenase at different initial protein concentration are summarized in Table I. At the initial concentration of 0.04 g/100 ml, values for several different preparations of 2-oxoglutarate dehydrogenase at different initial concentrations of 0.04 g/100 ml, values for several different preparations of 2-oxoglutarate dehydrogenase were obtained from the interference photograph of the equilibrium distribution in a 12-mm double sector cell with a 4 mm solution column at 12,590 rpm and 20° in an An-D rotor for 45 hours. Units of $r$ are radial distance in centimeters, and units of $c$ are fringe displacement in millimeters.

Thiamine-PP Content—The isolated 2-oxoglutarate dehydrogenase contained all the protein-bound thiamine-PP in the 2-oxoglutarate dehydrogenase complex, as reported previously (2). The 2-oxoglutarate dehydrogenase activity of the purified enzyme was not stimulated by exogenous thiamine-PP even in the presence of Ca$^{2+}$. This observation suggests that thiamine-PP was not dissociated from the enzyme during isolation. The thiamine-PP content of the enzyme was estimated to be about 1 mole per mole of the enzyme (mol wt 216,000) by both the enzymatic assay of thiamine-PP and the chemical analysis of total thiamine.

Thiol Content—The content of thiol groups and half-cystine residues is summarized in Table II. Approximately 32 moles of each were detected per mole of the enzyme (mol wt 216,000), indicating the absence of disulfide bridges.

Kinetic Properties—As shown in Table III, the ferricyanidedlinked oxidation of 2-oxoglutarate catalyzed by 2-oxoglutarate...
TABLE II

Content of total thiol groups and half-cystine in 2-oxoglutarate dehydrogenase

<table>
<thead>
<tr>
<th>Methods</th>
<th>Thiol group or half-cystine</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTNB(^b) in 6 M guanidine HCl (pH 8.2)</td>
<td>34</td>
</tr>
<tr>
<td>CMB(^c) in 0.05 M potassium phosphate buffer (pH 7.0)</td>
<td>32</td>
</tr>
<tr>
<td>CMB(^c) in 8 M urea in 0.05 M potassium phosphate buffer (pH 7.0)</td>
<td>31</td>
</tr>
<tr>
<td>Total half-cystine(^d) as cysteic acid</td>
<td>32</td>
</tr>
</tbody>
</table>

\(^a\) Average molecular weight of 216,000.
\(^b\) 5,5'-Dithiobis(2-nitrobenzoic acid) (25).
\(^c\) p-Chloromercuribenzoate (26, 27).
\(^d\) Determined by amino acid analysis after performic acid oxidation (30).

TABLE III

Effect of phosphate concentration on 2-oxoglutarate dehydrogenase activity

Each reaction mixture contained the standard 2-oxoglutarate dehydrogenase assay components, 100 \(\mu\)moles of histidine buffer (pH 6.5) in place of potassium phosphate buffer, the indicated concentration of potassium phosphate buffer (pH 6.5), and 20 \(\mu\)g of 2-oxoglutarate dehydrogenase in a final volume of 2 ml. Reaction was begun by the addition of 2-oxoglutarate and followed at 420 nm.

<table>
<thead>
<tr>
<th>Amount of potassium phosphate added ((\mu)moles)</th>
<th>Specific activity ((\mu)moles/hr/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>176</td>
</tr>
<tr>
<td>10</td>
<td>242</td>
</tr>
<tr>
<td>20</td>
<td>282</td>
</tr>
<tr>
<td>40</td>
<td>339</td>
</tr>
<tr>
<td>60</td>
<td>392</td>
</tr>
<tr>
<td>80</td>
<td>418</td>
</tr>
<tr>
<td>100</td>
<td>440</td>
</tr>
<tr>
<td>200</td>
<td>497</td>
</tr>
<tr>
<td>400</td>
<td>515</td>
</tr>
<tr>
<td>800</td>
<td>497</td>
</tr>
</tbody>
</table>

dehydrogenase required the presence of dibasic phosphate ion (potassium salt). Therefore, the standard assay system contained 100 \(\mu\)moles of potassium phosphate buffer (pH 6.5) in a final volume of 2 ml to give nearly full activity.

When the ferricyanide-linked 2-oxoglutarate dehydrogenase assay was carried out in potassium phosphate buffer the optimal pH was found to be approximately 6.5, as shown in Fig. 4. The optimal temperature at this pH was determined by the modified method (6) of Hager (7) and found to be near 36°. The activation energy of the 2-oxoglutarate dehydrogenase reaction was calculated to be \(1.75 \times 10^4\) cal per mole of the enzyme. The heat stability of the enzyme was measured by estimating the activity remaining when samples of the enzyme in 0.05 M potassium phosphate buffer (pH 7.0) containing 0.5 mM EDTA were heated for 5 min at different temperatures. Loss of activity was negligible up to 25°, but rapid beyond 30°.

Substrate specificities are shown in Table IV. The results indicate that 2-oxoglutarate dehydrogenase is very specific for the oxidation of 2-oxoglutarate, but does show some activity with \(\alpha\)-ketoadipate and \(\alpha\)-ketopimelate. Similar results were obtained with the 2-oxoglutarate dehydrogenase complex (1), which contains 2-oxoglutarate dehydrogenase bound noncovalently to lipoate succinyltransferase (2). The \(K_m\) (apparent) value for 2-oxoglutarate under the assay condition is 0.038 mM.

2-Oxoglutarate dehydrogenase contained about 32 moles of thiol group per mole of the enzyme and its dehydrogenase activity was completely inhibited only by high concentration of \(p\)-chloromercuribenzoate (enzyme to \(p\)-chloromercuribenzoate molar ratio of 1:200). However, the role of thiol group is being investigated further.

Amino Acid Composition—The results of amino acid analyses of the enzyme are summarized in Table V. The final recovery of all amino acids was calculated to be 98%. The partial specific volume (\(\phi\)) of the enzyme calculated from the amino acid composition (8) was 0.732 ml per g.

NH\(_2\)-terminal Residue—The Edman method revealed alanine as the NH\(_2\)-terminal residue, and the amount present was estimated as 2 moles per mole of the enzyme (mol wt 216,000).

The result indicates that 2-oxoglutarate dehydrogenase probably consists of 2 polypeptide chains. Dansyl-alanine was likewise the only derivative revealed by the dansyl method.

Peptide Mapping—The peptide map of the tryptic digest of performic acid-oxidized enzyme showed a total of 86 ninhydrin-positive spots, 32 of which contained arginine. The amino acid analysis showed the presence of 46 lysine residues and 45 arginine residues per subunit (mol wt 97,000). These data suggest...
that the two subunits of 2-oxoglutarate dehydrogenase are probably similar, if not identical.

**Polyacrylamide Gel Electrophoresis in Sodium Dodecyl Sulfate—**
The 2-oxoglutarate dehydrogenase, which was treated with 1% sodium dodecyl sulfate containing 1% 2-mercaptoethanol after reduction and alkylation in 6 M guanidine HCl (pH 8.5) and 0.1 M 2-mercaptoethanol, showed a single band (Fig. 5B) with estimated molecular weight 113,000 (Fig. 6), on electrophoresis in 5% polyacrylamide gel in 0.1% sodium dodecyl sulfate. The

<table>
<thead>
<tr>
<th>Amino acid composition of 2-oxoglutarate dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amino acid</strong></td>
</tr>
<tr>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Threonine</td>
</tr>
<tr>
<td>Serine</td>
</tr>
<tr>
<td>Glutamic acid</td>
</tr>
<tr>
<td>Proline</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Half-cystine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Tyrosine</td>
</tr>
<tr>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
<tr>
<td>Tryptophan</td>
</tr>
</tbody>
</table>

*Average molecular weight of 216,000.

b Determined as cysteic acid after performic acid oxidation (30).

c Estimated spectrally according to the procedure of Goodwin and Morton (31).

**Sedimentation Equilibrium Analysis in 6 M Guanidine Hydrochloride—**
The molecular weight of the dissociated subunit of 2-oxoglutarate dehydrogenase was measured by the meniscus depletion sedimentation equilibrium method (10). The samples, which were previously dialyzed against water and lyophilized, were dissolved in 6 M guanidine HCl (pH 8.0) containing 0.1 M 2-mercaptoethanol and allowed to stand under nitrogen at 4° for 24 to 48 hours. The plots of the logarithm of the vertical displacement of a single fringe versus the square of the radial distance were virtually linear, as shown in Fig. 7. The weight average molecular weight of the dissociated subunit of the enzyme for two values of \( r \), the value for the native enzyme (0.732 ml per g) and a reduced value in guanidine (0.722 ml per g) (11), are given in Table VI. Using the value of 0.732 ml per g, the molecular weights obtained for several different preparations of the enzyme lay in the range of 96,000 to 99,500 at an initial concentration of 0.04 g/100 ml. The value of the weight average molecular weight of the subunit of the enzyme was taken to be 97,000 ± 4,000. This indicates two subunits per mole of 2-oxoglutarate dehydrogenase with a molecular weight of 216,000. On the other hand, the enzyme was dissociated into a single subunit with a molecular weight of 113,000 by gel

**Fig. 5 (left).** Polyacrylamide gel electrophoresis in sodium dodecyl sulfate of the 2-oxoglutarate dehydrogenase complex and its component enzymes. Each protein was treated with 1% sodium dodecyl sulfate containing 1% 2-mercaptoethanol for 2 hours at 37° and subjected to electrophoresis as described under "Methods." **A**, 2-oxoglutarate dehydrogenase complex; **B**, 2-oxoglutarate dehydrogenase; **C**, lipoamide dehydrogenase; **D**, lipoate succinyltransferase. The bands denoted by arrows indicate the subunits corresponding to: 1, 2-oxoglutarate dehydrogenase; 2, lipoamide dehydrogenase; 3, lipoate succinyltransferase.

**Fig. 6 (right).** Determination of the apparent molecular weights of the dissociated subunits of 2-oxoglutarate dehydrogenase by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Standard protein samples and 2-oxoglutarate dehydrogenase itself were subjected to electrophoresis exactly as described under "Methods." Mobility relative to the tracking dye was plotted against the known subunit molecular weight of each protein as described by Weber and Osborn (14). 1, bovine serum albumin (dimer); 2, B-galactosidase; 3, 2-oxoglutarate dehydrogenase (arrow); 4, phosphorylase a; 5, bovine serum albumin (monomer); 6, catalase; 7, lipoamide dehydrogenase; 8, ovalbumin; 9, aldolase; 10, carbonic anhydrase; 11, chymotrypsinogen.
Similar subunits with an estimated molecular weight of 113,000 dehydrogenase, which is free of thiamine-PP (6). Attempts to dissociate thiamine-PP resulted in complete loss of activity. A single molecule of thiamine-PP tightly bound, in contrast to pyruvate dehydrogenase bound to the core enzyme, lipoate succinyltransferase, catalyzes the first step of the oxidative glutarate dehydrogenase complex has been isolated from pig heart muscle (2), further separated into three component enzymes, and reconstituted from the isolated component enzymes (2). One of the component enzymes of this multi-enzyme complex, 2-oxoglutarate dehydrogenase bound to the core enzyme, lipoate succinyltransferase, catalyzes the first step of the oxidative decarboxylation of 2-oxoglutarate. Various properties of the enzyme have now been described in detail in this paper.

As reported in a recent paper of this series, the mammalian 2-oxoglutarate dehydrogenase complex has been isolated from the Kelin-Hartree particle preparation from pig heart muscle (1), further separated into three component enzymes, and reconstituted from the isolated component enzymes (2). One of the component enzymes of this multi-enzyme complex, 2-oxoglutarate dehydrogenase bound to the core enzyme, lipoate succinyltransferase, catalyzes the first step of the oxidative decarboxylation of 2-oxoglutarate. Various properties of the enzyme have now been described in detail in this paper.

The pig heart 2-oxoglutarate dehydrogenase contains 1 mole of thiamine-PP. Recently, Pettit et al. (30) reported that E. coli 2-oxoglutarate dehydrogenase with a molecular weight of 190,000 was a dimer of identical polypeptide chains. The subunit molecular weight was estimated to be about 94,000 by sedimentation equilibrium ultracentrifugation in guanidine HCl and by gel electrophoresis in sodium dodecyl sulfate.

Studies of the binding sites for the core enzyme (lipoate succinyltransferase), the mode of association of this enzyme with the core enzyme, and the binding of thiamine-PP to 2-oxoglutarate dehydrogenase are still in progress.


discussion

As reported in a recent paper of this series, the mammalian 2-oxoglutarate dehydrogenase complex has been isolated from the Kelin-Hartree particle preparation from pig heart muscle (1), further separated into three component enzymes, and reconstituted from the isolated component enzymes (2). One of the component enzymes of this multi-enzyme complex, 2-oxoglutarate dehydrogenase bound to the core enzyme, lipoate succinyltransferase, catalyzes the first step of the oxidative decarboxylation of 2-oxoglutarate. Various properties of the enzyme have now been described in detail in this paper.

The pig heart 2-oxoglutarate dehydrogenase contains 1 molecule of thiamine-PP tightly bound, in contrast to pyruvate dehydrogenase, which is free of thiamine-PP (6). Attempts to dissociate thiamine-PP resulted in complete loss of activity. The 2-oxoglutarate dehydrogenase apparently consists of two similar subunits with an estimated molecular weight of 113,000 by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. By the sedimentation equilibrium ultracentrifugation in 6 M guanidine HCl, the molecular weight of the subunit was estimated to be 97,000 + 4,000 from the data calculated with two values of ρ, namely the value for the native enzyme and the value in guanidine HCl (11). The amino acid analysis showed the presence of 46 lysine residues and 45 arginine residues per subunit with a molecular weight of 97,000. A total of 66 ninhydrin-positive spots and 32 of arginine-containing spots were found on the peptide map of the native enzyme. The discrepancy between the ratio expected for lysine to arginine per subunit predicted from the amino acid composition for an α2 structure and those found in peptide map remains to be studied. These results, however, indicate that 2-oxoglutarate dehydrogenase consists of two similar, if not identical, subunits, and that either one of the two subunits or two subunits together contains 1 molecule of thiamine-PP. Given the content of thiamine-PP (1 mole per mole of the enzyme) it appears that a pair of subunits with N-terminal alanine bind one thiamine-PP as coenzyme.

Acknowledgments—We wish to thank Dr. K. Kawahara and Miss K. Ota, Department of Physical Chemistry, School of Pharmaceutical Sciences, Nagasaki University, for their advice in ultracentrifugal analyses. We are indebted to Miss M. Yoshida for her technical assistance. We also thank Dr. Jean O. Thomas, Department of Biochemistry, University of Cambridge, for her criticisms and kind help in the preparation of the manuscript.

REFERENCES


**TABLE VI**

**Weight average molecular weight of dissociated subunit of 2-oxoglutarate dehydrogenase**

<table>
<thead>
<tr>
<th>Initial concentration</th>
<th>Molecular weight</th>
<th>ρ = 0.732&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ρ = 0.722&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/100 ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>99,700</td>
<td>93,000</td>
<td></td>
</tr>
<tr>
<td>0.04</td>
<td>97,900</td>
<td>91,400</td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>95,900</td>
<td>89,800</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Value of ρ for the native enzyme (8).
<sup>b</sup> Assumed value of ρ in guanidine HCl (11).

Downloaded from http://www.jbc.org/ by guest on July 5, 2017
Properties and Subunit Composition of the Pig Heart 2-Oxoglutarate Dehydrogenase
Kichiko Koike, Minoru Hamada, Nobuyuki Tanaka, Kin-Ichi Otsuka, Kyoko Ogasahara and Masahiko Koike


Access the most updated version of this article at http://www.jbc.org/content/249/12/3836

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/249/12/3836.full.html#ref-list-1