The Isolation and General Properties of Escherichia coli Malonyl Coenzyme A-Acyl Carrier Protein Transacylase*

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SUMMARY

Malonyl coenzyme A-acyl carrier protein transacylase of Escherichia coli was purified 4800-fold by procedures which included chromatography on DEAE-cellulose, Sephadex G-100, Sephadex G-75, DEAE Sephadex, and preparative polyacrylamide gel electrophoresis. The purified enzyme was shown to be homogeneous by electrophoresis on polyacrylamide gels, by sedimentation equilibrium centrifugation, and by amino acid analysis. A molecular weight of 36,660, obtained with carboxymethylated enzyme by sedimentation equilibrium measurements, agreed with determinations on the native enzyme by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (36,500) and by Sephadex G-100 column chromatography (37,000). An $s_{20,w} = 2.31$ S was determined for the enzyme by sedimentation velocity measurements. Amino acid analysis and determination of the isoelectric point (pH 4.65) both showed the enzyme to be acidic. The purified enzyme was inhibited by $N$-ethylmaleimide and to a lesser extent by iodoacetamide, but inhibition by both reagents was significantly increased at pH values above 7.3. Preincubation of the enzyme with malonyl-CoA protected against inactivation by both sulfhydryl reagents. Reduction of aged enzyme preparations by incubation with dithiothreitol was shown to stimulate enzyme activity approximately 5-fold. These experiments suggest that a reduced sulfhydryl group(s) on the enzyme is required for maximal catalytic activity.

The de novo synthesis of fatty acids in Escherichia coli has been shown to require the initial transacylation of acetyl (Reaction 1)

\[ CH_3CO--S-CoA + HS-ACP = \]

\[ CH_3CO--S-ACP + CoA-SH \]

and malonyl groups (Reaction 3) from coenzyme A to acyl carrier protein (2-4). The transfer of acetate and malonate to ACP converts these acyl groups to thioester forms which are characteristic of acyl intermediates in E. coli fatty acid synthesis and which are strictly required for the condensation reactions catalyzed by &ketoacyl-ACP synthetase (Reactions 2 and 4).

\[ CH_3CO--S-CoA + HS-CoA = \]

\[ CH_3CO--S-ACP + CoA-SH \]

\[ HOOCCH_2CO--S-ACP + CH_3CO--S-ACP = \]

\[ CO_2 + HS-CoA + CH_3COCH_2CO--S-ACP \]

The formation of acetyl-ACP in this scheme is followed by transfer of the acetyl group to a sulfhydryl site on the condensing enzyme (E$\text{cond}$) with the formation of an acetyl-E$\text{cond}$ intermediate and the release of ACP (Reaction 2). Transacylation of malonate from CoA to ACP (Reaction 3) provides the substrate for the condensation of acetyl-E$\text{cond}$ and malonyl-ACP (Reaction 4).

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The abbreviation used is: ACP, acyl carrier protein.
The mechanism proposed by Joshi and Wakil (6) was analogous to that first suggested for malonyl transacylase observed in yeast fatty acid synthetase (7, 8) and similar also to the mechanism later extended to the same enzyme in the pigeon liver fatty acid synthetase (9, 10). The transfer of malonate to ACP by the E. coli enzyme was suggested to proceed through the intermediate esterification of malonate to a hydroxyamino acid residue, presumably serine, in the active site of the enzyme.

Since the reported insensitivity of purified E. coli malonyl transacylase to sulfhydryl reagents contradicted earlier observations with impure enzyme (9, 5) as well as a previous report from this laboratory using purified enzyme (11), a more detailed examination of the enzyme’s sensitivity to these agents was undertaken. A report is made here of the isolation of E. coli malonyl transacylase by a procedure yielding enzyme with a specific activity 2.8 times that previously reported for pure enzyme (6). The enzyme thus isolated appeared to be homogeneous by several criteria, and its physical properties including molecular weight, amino acid composition, sedimentation coefficient, and isoelectric point are described. Enzyme purified by this method was found to be stimulated by the reducing agent, dithiothreitol, and sensitive to inhibition by sulfhydryl poisons. In an accompanying paper a report is made on the isolation of a malonyl-enzyme intermediate, the characterization of its chemical and enzymatic properties, and the identification of a serine residue as the site of malonyl binding to the enzyme.

**EXPERIMENTAL PROCEDURE**

**Materials**

Malonyl-CoA and CoA were obtained from P-L Biochemicals Inc. N-Ethylmaleimide, iodoacetamide, iodoacetic acid, pep- pantethine, DPNH as well as β-hydroxyacyl-CoA dehydroge- nase (pig heart, 195 units per mg) were purchased from Sigma Chemical Co. The molecular weight standards, pepsin and ovalbumin (twice crystallized) were obtained from Worthington Biochemical Corp., myoglobin (horse heart tissue crystallized) and cytochrome c (horse heart) from Mann Biochemicals Inc., and bovine serum albumin (Fraction V, fatty acid poor) from Pentex Biochemicals. Urea (ultra pure) and guanidine HCl (ultra pure) were products of Schwarz-Mann Biochemicals Inc. Whatman DEAE-cellulose ion exchange resins were obtained through II. Reeve Angel and Co., Sephadex gels were purchased from Pharmacia Fine Chemicals Inc., and hydroxylapatite (Bio- Gel HTP) was from Bio-Rad Laboratories. Radioactive [1,3-14C]malonyl-CoA (18.5 and 19.8 mCi per mmole) and [1-14C]iodoacetic acid (13.9 mCi per mmole) were obtained from New England Nuclear Inc. ACP was prepared by the method of Majerus et al. (12), and acetyl-ACP by the method of Alberts et al. (13). The enzyme β-ketoacyl-ACP synthetase was purified according to the method of Prescott and Vagelos (14).

**Methods**

Malonyl Transacylase Assays—Two assay procedures were used for the measurement of malonyl transacylase activity. A coupled spectrophotometric assay was used during purification procedures and inhibitor studies. The formation of malonyl-ACP by malonyl transacylase was coupled to the condensation of malonyl-ACP and acetyl-ACP by β-ketoacyl-ACP synthetase and the reduction of acetocacyl-ACP by β-hydroxyacyl-CoA dehydrogenase in the presence of DPNH. The routine assay mixture contained 10 μmoles of potassium phosphate, pH 7.0, 2.5 μmoles of dithiothreitol, 0.1 μ mole of EDTA (pH 7.0), 30 μmoles of DPNH, 0.4 μg of β-hydroxyacyl-CoA dehydrogenase (106 μmoles per min per mg of protein at 37°), 2.0 μmoles of acetyl-ACP, 15 μ moles of malonyl-CoA, 5 μg of β-ketoacetyl-ACP synthetase (3 amoles per min per mg of protein at 25°), and 0.08 to 0.8 milliunits of malonyl transacylase in a total volume of 0.1 ml. Reactions were started by the addition of malonyl transacylase, and the oxidation of DPNH was measured spectrophotometrically at 340 nm in a Gilford recording spectrophotometer at 25°. Under the above conditions the change in absorbance was linear for a minimum period of 4 min. A unit of enzyme activity is defined as an amount of enzyme catalyzing the formation of 1.0 μmole of DPN per min under the assay conditions.

Alternatively, a precipitation assay was used in which malonyl transfer from [14C]malonyl-CoA was followed directly by measuring the formation of acid insoluble [14C]malonyl-ACP. The assay conditions described by Alberts et al. (15) were used with certain modifications. Incubation mixtures contained 2.5 μmoles of potassium phosphate, pH 6.8, 50 nmoles of EDTA (pH 7.0), 0.5 μmole of dithiothreitol, 5.0 nmoles of reduced ACP, 5.0 nmoles of [1,3-14C]malonyl-CoA (18.5 to 19.8 mCi per mmole), and 0.08 to 0.8 milliunits of enzyme in a total volume of 0.05 ml. The assays were begun by the addition of [14C]malonyl-CoA and terminated after 1.0 min of incubation at 25° by addition of 0.4 ml of 10% trichloroacetic acid. Bovine serum albumin (1.0 mg) was added to assure complete precipitation of the [14C]malonyl-ACP, the insoluble material was separated by centrifugation, and the pellet was washed twice with 10% trichloroacetic acid (0.4 ml) and once with diethyl ether (0.4 ml). [14C]Malonyl groups were released from protein by incubation of the washed pellet in 0.2 ml of 1 N NaOH at 37° for 15 min. The alkaline solutions were transferred to 20 ml of Bray’s solution (16) and radioactivity was measured in a Packard Tri-Carb model 3380 liquid scintillation spectrometer equipped with an absolute activity analyzer (AAA-model 544). The nonspecific precipitation of radioactivity was less than 1% of experimental values in this procedure. Protein was determined by the microbiuret method of Munkres and Richards (17) or by the method of Lowry et al. (18).

**Analytical Polyacrylamide Gel Electrophoresis**—The method of Davin (10) was used in the preparation of polyacrylamide disc gels for analysis of the enzyme. Tubes containing 1.0 ml of 15% polyacrylamide separating gel (5 × 50 mm), 0.2 ml of stacking gel, and sample containing 10 to 100 μg of protein in a volume of 0.1 ml were subjected to electrophoresis at a current of 1 ma per tube at room temperature using a Tris-glycylglycine (pH 8.5) electrode buffer system. Samples were applied as a 5% glycerol solution layered beneath the upper electrode buffer which contained bromphenol blue tracking dye. At the end of electrophoresis the gels were cooled in an ice bath and then stained overnight in a solution of 0.01% Coomassie blue in 12.5% trichloroacetic acid. Analysis of gels for radioactivity or enzyme activity was performed on 1- to 2-mm slices of unstained gels cut with a razor blade. Elution of enzyme from the gels was performed by mincing the gel slice in a volume of 0.1 ml potassium phosphate, pH 6.9, containing 0.01 M dithiothreitol and 0.001 M EDTA and storing overnight at 4°.

**RESULTS**

Purification of Malonyl Transacylase

Previously published procedures (2, 5, 6, 15) for the purification of malonyl transacylase from extracts of E. coli were modi-
fied in these experiments to permit both an increased yield of pure enzyme of higher specific activity and the simultaneous isolation of β-ketoacyl-ACP synthetase. The sequence of purification steps used in this scheme is described below and the results are reported in Table I.

Ammonium Sulfate Fractionation—All of the following purification procedures, except where indicated, were conducted at 4°C. Five pounds of frozen E. coli B (full log, enriched medium, from Grain Processing Corp., Muscatine, Iowa), were thawed in 5 liters of buffer containing 0.02 M potassium phosphate, pH 7.0, 0.05 M 2-mercaptoethanol, and 0.001 M EDTA (Buffer A). The suspended cells were broken by passage through a Manton-Gaulin submicron disperser at 9,000 p.s.i., and the ruptured cells were brought to 25% saturation with ammonium sulfate. The precipitate was removed by centrifugation in a refrigerated Sorvall RCB-B at 15,000 x g for 30 min, and the pellet was resuspended in Buffer A to a protein concentration of 20 mg per ml. The resulting suspension, which contained 5% ammonium sulfate, was again ruptured by passage through the disperser and brought to 25% saturation with solid ammonium sulfate. The precipitate was removed by centrifugation, and the supernatant was combined with the original supernatant. After dilution to a protein concentration of 20 mg per ml the pooled supernatants were brought to 55% saturation with solid ammonium sulfate. The precipitate which was removed by centrifugation was dissolved in a minimal volume of Buffer A and dialyzed overnight against 40 volumes of the same buffer.

A streptomycin sulfate step for removal of nucleic acids was sometimes employed at this stage. When used, streptomycin was added as a concentrated solution in the proportion of 2 g per g of protein to a protein solution of conductivity 1.5 mmhos. The precipitate was allowed to form for 30 min and was removed by centrifugation.

DEAE-cellulose-23 Batching—The dialyzed or streptomycin-treated extract was applied to DEAE-cellulose-23 equilibrated with Buffer A (15 mg of protein per ml of gravity settled resin) by direct mixing in a fritted bottom funnel (Bel-Art Products). The resin containing the adsorbed enzyme was washed twice with 1.5 volumes of Buffer A containing 0.125 M LiCl (conductivity, 5.3 mmhos). Malonyl transacylase was eluted with two washes of 1.5 volumes of Buffer A containing 0.20 M LiCl (conductivity, 8.5 mmhos). The eluate containing enzyme was reduced to a volume of 200 ml by pressure filtration under nitrogen (UM-10, Diaflo Ultrafiltration Membrane, Amicon Corp.) and equilibrated by dialysis against 40 volumes of Buffer A to remove excess LiCl.

DEAE-cellulose-52 Gradient—The dialyzed eluate from the preceding step was adjusted to a LiCl concentration of 0.075 M (conductivity, 3.5 mmhos) and applied to a column of DEAE-cellulose-52 (10 mg of protein per ml of resin) equilibrated with Buffer A containing 0.075 M LiCl. After washing with 1 volume of the equilibrating buffer, the column was eluted with a 10-volume linear gradient of 0.075 to 0.20 M LiCl in Buffer A (conductivity, 3.5 to 8.5 mmhos). Peak malonyl transacylase activity was found at a conductivity of 5.8 mmhos. The pooled fractions containing the enzyme were dialyzed overnight against 20 volumes of Buffer A.

### Table I

**Purification of malonyl-CoA-ACP transacylase from Escherichia coli**

| TREATMENT                      | TOTAL ACTIVITY (μmoles/min) | TOTAL PROTEIN (g) | SPECIFIC ACTIVITY (μmoles/min/mg) | PURIFICATION FOLD | % RECOVERY |
|--------------------------------|-----------------------------|-------------------|-----------------------------------|-------------------|------------|
| Step                           |                             |                   |                                   |                   |            |
| 1 Crude Extract                | 85,500                      | 223.5             | 0.38                              | -                 | 100        |
| 2 55-95% Ammonium Sulfate      | 76,025                      | 76.2              | 0.99                              | 2.6               | 89.0       |
| 3 DEAE-23 Batching             | 64,108                      | 18.6              | 3.44                              | 9.0               | 75.0       |
| 4 DEAE-52 Gradient             | 43,200                      | 3.1               | 13.78                             | 36.0              | 50.5       |
| 5 Hydroxylapatite              | 38,367                      | 1.4               | 26.4                              | 68.9              | 44.8       |
| 6 Sephadex G-100               | 52,820                      | 0.310             | 170.3                             | 444.1             | 61.8       |
| 7 Sephadex G-75                | 48,300                      | 0.109             | 443.1                             | 1156.9            | 56.5       |
| 8 DEAE-Sephadex                | 36,600                      | 0.027             | 1312.6                            | 3427.1            | 42.8       |
| 9 Preparative Polyacrylamide Gel Electrophoresis | 32,940 | 0.017 | 1850.5 | 4831.8 | 38.5 |

*a* Protein was measured as described in the text.

*b* All measurements were performed using the spectrophotometric assay described in "Experimental Procedure."
Hydroxylapatite Balancing—The dialyzed eluate from the previous step (concentration, 1.5 mmhos) was mixed with hydroxylapatite (50 g dry weight adsorbent per g of protein) which had been washed with several volumes of Buffer A. This step was performed in a fritted glass funnel under vacuum. Under these conditions the enzyme was not retained by the hydroxylapatite and was recovered quantitatively in the filtrate. The filtrate was concentrated by pressure filtration to a volume of 40 ml.

Sephacryl G-100 Chromatography—Molecular sizing of the purified eluate was first performed on a 1.35-liter column (4.5 x 85.0 cm) of Sephadex G-100 (40 to 120 mesh) equilibrated and eluted with Buffer A containing 0.01 M dithiothreitol in place of 2-mercaptoethanol (Buffer A-dithiothreitol). 2-Mercaptoethanol was replaced by dithiothreitol as the reducing agent in all subsequent purification steps. The 40-ml sample was applied by layering as a 5% glycerol solution under a solution of Buffer A-dithiothreitol. The column was eluted at a rate of 0.3 ml per min. Fractions were collected every 8 min and assayed for protein by measuring absorbance at 280 nm and for malonyl transacylase activity. Tubes containing enzyme were pooled and concentrated by pressure dialysis to a volume of 9.0 ml.

Sephacryl G-75 Chromatography—A more restrictive sizing of the eluate was next accomplished by chromatography on Sephadex G-75. The sample, which had been concentrated and made 5% in glycerol, was applied to a 320 ml (2.5 x 90.0 cm) Sephadex G-75 (10 to 40 mesh) column equilibrated with Buffer A-dithiothreitol. The column was eluted with the same buffer at a flow rate of 0.15 ml per min, and fractions were collected every 10 min. Fractions containing enzyme were pooled. The pH of the solution was adjusted to 8.5 by addition of NaOH, and the dithiothreitol concentration was increased to 0.05 M. Reduction of thiols groups of the enzyme was accomplished by incubation at 25°C for 15 min. The pH of the solution was then reduced to 7.0 with HCl, and the conductivity was brought to 4.5 mmhos by addition of 10 mM LiCl.

DEAE-Sephadex Gradient—A portion of the pooled eluate (40 mg of protein) from the previous step was applied to a DEAE-Sephadex A-50 column (2.5 x 25.0 cm, 44 ml) equilibrated with Buffer A-dithiothreitol containing 0.12 M LiCl (conductivity, 5.5 mmhos) under gravity flow conditions, and the column was washed with 1 volume of equilibrating buffer. The column was eluted with a 10-volume linear gradient of 0.12 to 0.21 M LiCl in Buffer A-dithiothreitol (conductivity, 5.5 to 9.0 mmhos) by gravity flow at a rate of 0.33 ml per min. Fractions were collected every 6 min and analyzed for protein and malonyl transacylase activity. The fractions containing enzyme were subjected to analysis by standard 15% polyacrylamide gel electrophoresis. The gel was increased to a final concentration of 0.4% N,N'-methylenebisacrylamide. Fifteen milliliters of separating gel (15% acrylamide-0.4% bisacrylamide), polymerized with ammonium persulfate, and 5 ml of stacking gel, polymerized with riboflavin and ultraviolet light, were used for the electrophoresis of 10 mg of protein. Polymerization, electrophoresis, and elution of the gels were carried out at 4°C. The sample, 2 to 3 ml containing 10 mg of protein, 5% glycerol, and bromphenol blue tracking dye, was applied to the top of the stacking gel, and Tris-glycylglycine buffer, pH 8.5, was layered above. Electrophoresis was begun at 8 ma and continued until the tracking dye entered the separating gel; the amperage was then increased to 12. Eluting buffer (0.2 M imidazole-HCl, pH 7.0, 0.01 M dithiothreitol, 0.001 M EDTA) was initially pumped at a flow rate of 0.4 ml per min until the tracking dye was eluted; the flow rate was increased to 0.8 ml per min during protein collection. A profile of enzyme activity eluted from the gel by this procedure is shown in Fig. 2. Fractions were examined by standard analytical polyacrylamide disc gel electrophoresis. Those fractions which showed a single band upon staining with Coomassie blue (Fractions 885 to 885) were pooled for further characterization. The yield of pure enzyme by this procedure represented 25 to 30% of the material applied to the gel. The impure enzyme of the tail fractions was subjected to repeat electrophoresis to improve the recovery. The total yield of enzyme activity from the initial electrophoresis ranged from 80 to 90%, but this yield decreased when the procedure was repeated.

Comments on Purification Procedure—The purification of malonyl transacylase by this procedure provided enzyme in good yield which appeared homogeneous by several criteria. Electrophoresis of 60 μg of pure enzyme on standard polyacrylamide disc gels at pH 8.5 revealed a single band that stained with Coomassie blue (Fig. 3). A second gel, that was not stained, was sliced and the slices were eluted with Buffer A-dithiothreitol. Enzyme assays of the eluted material indicated that malonyl transacylase was present at the same Rf as the single protein band. The use of 15% polyacrylamide gels in this analysis was found necessary for the discrimination of contaminating proteins which were observed to have Rf characteristics similar to that of the enzyme. Electrophoresis on 10% polyacrylamide gels containing 0.1% sodium dodecyl sulfate at pH 7.0 also revealed a single protein band which was stained with a solution of Coomas-
FIG. 2. Preparative polyacrylamide gel electrophoresis of malonyl transacylase. Electrophoresis of DEAE-Sephadex purified malonyl transacylase (10 mg) on 15% polyacrylamide gel, pH 8.5, was conducted as described in the text. Fractions (0.8 ml) were analyzed for malonyl transacylase activity (○—○). Fractions between the vertical bars were pooled, concentrated by pressure dialysis, and analyzed by standard polyacrylamide disc gel electrophoresis. Fractions included by the large arrow were found to contain enzyme which was homogeneous by disc gel electrophoresis.

FIG. 3. Polyacrylamide disc gel electrophoresis of malonyl transacylase at various stages of purification. Aliquots of enzyme from the indicated purification steps were subjected to electrophoresis on 15% polyacrylamide gels in a Tris-glycylglycine, pH 8.5, buffer system according to the method of Davis (19). Tube A (75 μg) from DEAE-52 gradient; Tube B (85 μg) from hydroxylapatite; Tube C (80 μg) from Sephadex G-100, Tube D (82 μg) from Sephadex G-75; Tube E (50 μg) from DEAE-Sephadex; Tube F (60 μg) from preparative electrophoresis.

FIG. 4. Molecular weight determination of malonyl transacylase by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Carboxamidomethylated (A) and native (B) malonyl transacylase (30 μg) were subjected to electrophoresis with the indicated standards (30 μg) on 1.4 ml gels containing 10% polyacrylamide and 0.1% sodium dodecyl sulfate according to the method of Shapiro et al. (20). Sample preparations and gel staining were conducted as described in the text.

Characterization of Malonyl Transacylase

Sodium Dodecyl Sulfate-polyacrylamide Gel Electrophoresis—An estimate of the molecular weight of malonyl transacylase purified through the DEAE-Sephadex step was made by electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate according to the method of Shapiro et al. (20). Enzyme was reduced by incubation in 0.1 M Tris-HCl, pH 8.6, containing 0.05 M dithiothreitol for 15 min at 25°C. An aliquot of malonyl transacylase was alkylated by incubation with iodoacetamide at a final concentration of 50 mM for 30 min at 25°C. The alkylation was stopped by the addition of a 5-fold excess of 2-mercaptoethanol. Both the reduced native enzyme and the alkylated enzyme were dialyzed overnight against buffer containing 0.1 M sodium phosphate, pH 7.2, 1% 2-mercaptoethanol, and 1% sodium dodecyl sulfate and subjected to electrophoresis on 10% polyacrylamide gels containing 0.1% sodium dodecyl sulfate. The proteins were located in the gels by staining for 16 hours with a solution of 0.1% Coomassie blue in 12% trichloroacetic acid and 5% methanol. Electrophoretically purified enzyme was also found to be homogeneous from its sedimentation properties and from amino acid analyses described below.

The maximum specific activity of pure enzyme (1850 units per mg) obtained by this procedure is 2.8 times greater than that previously reported (6). Although the coupled spectrophotometric assay used in obtaining this value differs from the assay procedures previously employed for this enzyme, a comparison with the acid precipitation assay used by others showed that the two methods gave similar results.
Sedimentation Equilibrium Centrifugation—An estimate of the molecular weight of reduced and carboxymethylated malonyl transacylase by equilibrium centrifugation was made using the high speed meniscus depletion method of Yphantis (22). Malonyl transacylase which had been purified by preparative electrophoresis was reduced and alkylated as described above. Centrifugation of the samples was conducted under the identical conditions and the protein concentration across each cell was recorded photographically at 19 and 23 hours with the use of Rayleigh interference optics (Fig. 6). The molecular weight of carboxymethylated enzyme determined by this method is 36,660. A similar value was obtained for all three protein concentrations tested.

Sedimentation Velocity—Sedimentation rate measurements of malonyl transacylase, which had been reduced by prior treatment with dithiothreitol as described above, were conducted using a synthetic boundary cell and a Spinco model E analytical ultracentrifuge equipped with schlieren optics. Fig. 7 illustrates the boundary peak observed and its rate of migration. The presence of a single absorbance peak, whose symmetry was maintained throughout the centrifugation, confirms the relative homogeneity of the protein sample. An $s_{20,w}$ of 2.31 S was calculated from this experiment. The partial specific volume, $\bar{V}$, used in this calculation (0.7389 cm$^3$ per g) was estimated from the observed amino acid composition by the method of Cohn and Edsall (23).

Amino Acid Composition—The results of amino acid analyses of electrophoretically pure malonyl transacylase after 24 and 72 hours of acid hydrolysis in 6 N HCl at 110° are shown in Table II. The presence of 6 half-cystine residues was indicated by the performic acid oxidation method of Moore (25) and confirmed by carboxymethylation using iodo[14C]acetic acid as described by Hirs (26). When the enzyme was reduced and then alkylated in 0.1 M Tris-HCl, pH 8.6, containing 6 M guanidine-HCl and labeled iodoacetic acid in 100-fold excess over the enzyme concentration for 10 min at 25°, there was good agreement between the number of carboxymethylcysteine residues detected by amino acid analysis and the amount of label in an aliquot of protein precipitated by addition of trichloroacetic acid. No attempt was made to quantitate the number of glutamine or asparagine residues present; however, an isoelectric point of pH 4.65 for the native enzyme, as determined by gel isoelectric focusing (Fig. 8), suggests the presence of a large number of free acidic residues.

pH Optimum—The effect of pH on the catalytic activity of malonyl transacylase was determined by direct measurement of the rate of malonyl-ACP formation. Fig. 9 indicates a broad

![Fig. 5. Molecular weight determination of malonyl transacylase by Sephadex G-100 column chromatography. Malonyl transacylase (1.0 mg, 1200 units) was applied to a column (2.5 x 100 cm, 420 ml) of Sephadex G-100 (120 mesh) and eluted as described in the text. Proteins were reduced prior to chromatography by incubation in 0.1 M Tris-HCl, pH 8.6, containing 0.005 M dithiothreitol for 20 min at 25°. The elution volumes of malonyl transacylase (MTA) and the indicated standards were determined by measuring fraction volumes and assaying for enzyme activity, absorbance at 410 nm (myoglobin) or turbidity in 5% trichloroacetic acid (450 nm).](http://www.jbc.org/)

![Fig. 6. Equilibrium sedimentation of malonyl transacylase.](http://www.jbc.org/) Carboxymethylated malonyl transacylase (0.3, 0.47, and 0.6 mg per ml), prepared as described in the text, was sedimented in a six chambered Yphantis cell at 32,000 rpm for 23 hours (6.8") using a Spinco model E ultracentrifuge. Protein concentration across each cell was recorded with Rayleigh interference optics. Fringe displacements, $\Delta Y$, in this figure represent recordings from a protein concentration of 0.47 mg per ml.

![Fig. 7. Schlieren peaks from sedimentation velocity centrifugation of malonyl transacylase. Malonyl transacylase (8.4 mg per ml; 1850 units per mg) was centrifuged in a synthetic boundary cell at 50,780 rpm and 4.3" using a Spinco model E ultracentrifuge. The position of the boundary was recorded photographically at 26, 38, 77, and 94 min (left to right) after boundary formation.](http://www.jbc.org/)
Amino acid composition of Escherichia coli malonyl-CoA-ACP transacylase

Values represent the results of a single analysis performed on samples hydrolyzed for the indicated number of hours. Carboxymethylated and performic acid oxidized samples were analyzed after 24 hours of hydrolysis. All analyses were performed by the method of Spackman et al. (24) using a Beckman model 120 C amino acid analyzer equipped with a long column containing Beckman Dowex 50 (AA-27) and a short column containing Beckman Dowex 50-X8 (AA-15) resins.

| AMINO ACID | COMPOSITION | AVERAGE OR EXTRAPOLATED INTEGER |
|------------|-------------|--------------------------------|
|            | 24 HOURS   | 72 HOURS | |
| Lysine     | 15.2        | 16.0     | 16.0 |
| Histidine  | 4.7         | 4.4      | 5.0  |
| Arginine   | 8.8         | 9.0      | 9.0  |
| Aspartic Acid | 20.9      | 20.9     | 21.0 |
| Threonine  | 15.6        | 15.4     | 16.0 |
| Serine     | 14.3        | 12.1     | 16.0 |
| Glutamic Acid | 39.4      | 39.4     | 39.0 |
| Proline    | 18.9        | 19.2     | 19.0 |
| Glycine    | 25.3        | 25.6     | 25.0 |
| Alanine    | 58.3        | 56.3     | 57.0 |
| Valine     | 29.3        | 32.8     | 33.0 |
| Methionine | 11.8        | 12.1     | 12.0 |
| Isoleucine | 7.1         | 9.1      | 9.0  |
| Leucine    | 28.7        | 29.8     | 29.0 |
| Tyrosine   | 8.9         | 7.6      | 9.0  |
| Phenylalanine | 11.3       | 8.8      | 10.0 |
| Cysteine   | 5.8^d       | 6.0      | 6.0  |
| Total      |             |          | 331.0 |

^a Extrapolated value  
^b 72 hour value  
^c Determined as cysteic acid after performic acid oxidation (43)  
^d Determined as carboxymethylcysteine after alkylation with iodoacetic acid (43)

pH optimum from pH 6.5 to 8.5 and a decline in enzyme activity at pH values approaching the isoelectric point of the enzyme. The pH optimum indicated here is higher than that previously reported for impure enzyme, pH 6.0 to 7.0 (5).

Inhibition by Alkylating Agents—Malonyl transacylase purified by the procedure described in this report was inhibited by N-ethylmaleimide, but the degree of inhibition was dependent on the pH of the reaction (Table III). Whereas the enzyme was inhibited by only 37.5% when incubated with 11.5 mM N-ethylmaleimide for 15 min at pH 7.3, total inhibition occurred under the same conditions at pH 8.6. A similar pH effect was observed when iodoacetamide and iodoacetic acid were tested as alkylating agents; however, maximal inhibition by 25 mM concentrations of these reagents at pH 8.6 was only 74% and 52%, respectively. Malonyl-CoA at a concentration of 25 mM completely protected the enzyme against 25 mM concentrations (pH 8.6) of the alkylating agents, as measured both by effects on enzyme activity (not shown) and the binding of malonate to the enzyme (27).

Stimulation by Reduction with Dithiothreitol—The stimulation of malonyl transacylase activity by dithiothreitol, which was initially observed in the Sephadex G-100 step of enzyme purification when dithiothreitol replaced 2-mercaptoethanol as the reducing agent (Table I), was readily demonstrated with aged enzyme preparations. Enzyme which had been stored at 4°C for several weeks showed a 5-fold stimulation of catalytic activity when exposed to 0.05 mM dithiothreitol at pH 8.6 (Table IV). Although the thiol reducing properties of dithiothreitol have been reported to be quite rapid at pH 7.0 (28), it appeared that an enhancement of this stimulation occurred when the enzyme was reduced at pH 8.6. The more rapid reduction of the enzyme at pH 8.6 is consistent with the greater inactivation of the enzyme by the alkylating agents, N-ethylmaleimide and iodoacetamide, observed at higher pH values.

Discussion

Purification of malonyl transacylase by the method described in this report resulted in a maximum specific activity of the homogeneous enzyme (1850 units per mg) which exceeded by 2.8-fold that previously reported (653 units per mg) for the presumably pure enzyme (6). Although this discrepancy might be attributable in part to impurities, a more likely explanation would appear to reside in the relative reduction states of the enzyme in the different preparations. As seen in Table I, the use of dithiothreitol in place of 2-mercaptoethanol in the Sephadex G-100 and subsequent purification steps resulted in an increase in the total enzyme activity of the preparation. Evidence that this stimulation resulted from a reduction of the enzyme was derived from experiments in which dithiothreitol was added...
to a preparation of enzyme which was stored at 4°C for several weeks (Table IV). The effect of diethiothreitol in restoring activity to the enzyme would suggest that a sulphydryl group(s) sensitive to oxidation and reduction has an important structural or functional role in the catalytic process.

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