Proc. Natl. Acad. Sci. USA
Vol. 76, No. 1, pp. 214–218, January 1979
Biochemistry

Bovine kidney 3-methylcrotonyl-CoA and propionyl-CoA carboxylases: Each enzyme contains nonidentical subunits*

(mitochondria/immunoprecipitate/pig heart propionyl-CoA carboxylase)

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Communicated by Lewis H. Sarett, October 25, 1978

ABSTRACT **3-Methylcrotonyl-CoA carboxylase (MCase; EC 6.4.1.4) and propionyl-CoA carboxylase (PCase; EC 6.4.1.3) have been obtained in highly purified form from bovine kidney mitochondria. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate revealed that each enzyme is composed of nonidentical subunits, including a smaller biotin-free subunit (M_r 62,000 and 58,000 for MCase and PCase, respectively), and a larger biotin-containing subunit (M_r 80,000 and 74,000 for MCase and PCase, respectively). The possibility that these subunits were derived from a single, larger precursor polypeptide via proteolysis was explored by purification and electrophoresis of each enzyme in the presence of protease inhibitors, but no evidence for proteolysis was obtained. Specific antisera directed towards each enzyme were prepared. The anti-PCase preparation was used to precipitate crossreacting PCase from a pig heart extract. Analysis of the immunoprecipitate obtained revealed a biotin-containing polypeptide (M_r 78,000) and a biotin-free polypeptide (M_r 55,000), suggesting that pig heart PCase also contains nonidentical subunits analogous to those seen in the kidney mitochondrial MCase and PCase. A bipartite subunit structure may be a common feature in mammalian MCase and PCase.**

Deficiencies in two biotin-containing enzymes, propionyl-CoA carboxylase (PCase; EC 6.4.1.3) and 3-methylcrotonyl-CoA carboxylase (MCase; EC 6.4.1.4), are responsible for the genetically inherited diseases, propionicacidemia and 3-methylcrotonylglycinuria, respectively, in humans (see refs. 1–4 for a review). In order to determine the structural defect(s) in the dysfunctioning enzymes, basic information on the structure of the corresponding normal enzymes is needed. We have been working to obtain purified PCase and MCase from a convenient animal source for such studies. Bovine kidney mitochondria represent a rich source of MCase (unpublished data) and of PCase, and we have recently obtained highly purified preparations of both enzymes. Based on published properties of crystalline pig heart PCase (5) and in analogy with other biotin enzymes from animal tissues, including acetyl-CoA carboxylase (6–8) and pyruvate carboxylase (9), we had assumed that PCase and MCase from animal sources would each contain one type of multifunctional subunit with all three catalytic subsites fused into a single polypeptide chain (see refs. 10 and 11 for a review). We report here the unexpected finding that mitochondrial MCase and PCase both contain two different types of subunits, a structural pattern of biotin enzymes heretofore ascribed only to bacterial biotin enzymes (10–12).‡ The significance of this finding in relation to the evolution of biotin enzymes and to the interpretation of recent genetic studies on human PCase deficiency will be discussed.

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EXPERIMENTAL PROCEDURE

Enzyme Purification. MCase and PCase were purified from bovine kidney mitochondria as outlined below. Mitochondria, isolated by differential centrifugation (15), were disrupted with a French pressure cell, and a supernatant containing membrane fragments plus soluble proteins was obtained by low-speed centrifugation. MCase, associated with the inner membrane, was precipitated with polyethylene glycol (Carbowax 6000, Fisher) (unpublished data), leaving PCase in the supernatant. MCase was isolated from the precipitate as follows: (*i*) "solubilization" of the enzyme by resuspension of the pellet in buffer; (*ii*) elution at 0.1 M KCl from a DEAE-cellulose column; (*iii*) gel filtration through a Sepharose 4B column; and (*iv*) ion exchange on a DEAE-agarose column. PCase was isolated from the supernatant fraction by procedures similar to those published for pig heart and bovine liver PCase (5, 16), including: (*i*) elution at 0.1 M KCl from a DEAE-cellulose column; (*ii*) elution at 0.26 M potassium phosphate from a hydroxyapatite column; (*iii*) ammonium sulfate fractionation at 35–55% saturation; (*iv*) gel filtration through a Sepharose 4B column; and (*v*) ion exchange on a DEAE-agarose column. A detailed description of these procedures will be published elsewhere. In some experiments, described in the text, protease inhibitors were included in the tissue homogenization and mitochondrial breakage steps.

A crude bovine-kidney cytosol fraction containing MCase was isolated by differential centrifugation (15). The enzyme in the 126,000 × *g* supernatant was concentrated by ammonium sulfate fractionation between 35 and 55% saturation and then resuspended in 10 mM potassium phosphate, pH 7.0/20% (vol/vol) glycerol/0.1 mM dithioerythritol/0.1 mM EDTA. A crude preparation of pig heart PCase was obtained by extracting pig heart as described (5), and then concentrating the enzyme as described above for kidney cytosolic MCase.

Immunological Techniques. Rabbit antisera were obtained after subcutaneous injections of purified bovine kidney PCase or MCase in incomplete Freund's adjuvant (17). The antisera were partially purified by ammonium sulfate precipitation, and then resuspension in and dialysis against 50 mM sodium phosphate/0.3 M glycine at pH 7.8 (17, 18). The immunoglobulin fractions in the PCase antiserum and control antiserum were

Abbreviations: MCase, 3-methylcrotonyl-CoA carboxylase; PCase, propionyl-CoA carboxylase; $NaDodSO_4$, sodium dodecyl sulfate.

* A preliminary account of this work was presented at the June 1978 meeting of the American Society of Biological Chemists.

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‡ While this manuscript was in preparation, two reports appeared relating to nonidentical subunits in eukaryotic biotin enzymes. Merton Utter communicated the information that his group has also detected biotinyl and non-biotinyl polypeptides, corresponding to the subunits of PCase, isolated by avidin affinity chromatography of mitochondrial extracts (13). In addition, Meyer and colleagues have shown that an acyl-CoA carboxylase from the nematode *Turbatrix aceti* also contains two types of subunits (14).

purified further by chromatography on DEAE-cellulose (18). Immunodiffusion of MCase or PCase preparations and their respective partially purified antisera was carried out in 1% (wt/vol) agarose (Sigma) containing 0.1 M potassium phosphate (pH 7.2), 0.9% (wt/vol) NaCl, and 0.2% sodium azide (17). After standing at 4°C for 72 hr, the plates were washed twice with saline and photographed. PCase was immunoprecipitated by mixing the appropriate purified immunoglobulin fraction with the pig heart PCase preparation in 0.1 M potassium phosphate, pH 7.2/0.1 mM phenylmethylsulfonyl fluoride at 30°C for 30 min; then an excess of goat anti-rabbit immunoglobulin (Miles) was added. After incubation for an additional 30 min at 30°C and then overnight at 4°C, immunoprecipitates were collected by centrifugation at 12,800 × *g* for 15 min. In some cases the resulting supernatants were assayed for PCase activity. After the immunoprecipitates were washed twice with 1.8% (wt/vol) NaCl/0.1 mM phenylmethylsulfonyl fluoride, they were dissolved in the sodium dodecyl sulfate ($NaDodSO_4$) sample solution described below, but supplemented with 0.1 mM phenylmethylsulfonyl fluoride, by heating in a boiling water bath for 5 min. They were then analyzed by $NaDodSO_4$ gel electrophoresis.

Polyacrylamide Gel Electrophoresis. Unless noted otherwise, electrophoresis in the presence of $NaDodSO_4$ was carried out at pH 7.2 as described (19) with the following modifications: (*i*) a slab gel apparatus was used; (*ii*) the electrode and separating gel buffer was 50 mM sodium phosphate, pH 7.2/0.1% $NaDodSO_4$; (*iii*) the separating gel was overlaid with a sample well gel containing 3% acrylamide, 0.08% *N,N'*-methylenebisacrylamide, 10 mM sodium borate (pH 7.2), and 0.1% $NaDodSO_4$ and polymerized with 0.1% *N,N,N',N'*-tetramethylethylenediamine/0.02% ammonium persulfate; (*iv*) samples were applied in 10–15 μl of 1% $NaDodSO_4$/20% glycerol/1% 2-mercaptoethanol/10 mM disodium phosphate/0.005% bromphenol blue, usually after boiling for 2 min; and (*v*) samples were overlaid with a cap gel (to fill each well) containing 1% agar (Noble agar, Difco), 0.1% $NaDodSO_4$, and 10 mM sodium borate (pH 7.2). Electrophoresis was initiated at 10 V until the tracking dye entered the separating gel, when the voltage was increased to 50 V to complete the run. Electrophoresis under nondenaturing conditions was also performed on slab gels with (*i*) a 4.5% acrylamide/0.12% *N,N'*-methylenebisacrylamide/0.12 M Tris·HCl, pH 7.2, separating gel; (*ii*) a sample well gel like that described above but with $NaDodSO_4$ omitted; (*iii*) samples applied in and 10 mM sodium borate, pH 7.2/20% glycerol/0.005% bromphenol blue; and (*iv*) samples overlaid with agar without $NaDodSO_4$ as in (*v*) above. Electrophoresis was performed at 4°C as above, with 50 mM sodium phosphate (pH 7.2) as the electrode buffer. Gels were stained as described (12).

Molecular weights of subunits were determined by constructing calibration curves (19) with the following polypeptides [M_r values from Weber and Osborn (19) except for transferrin (20)]: phosphorylase (94,000), transferrin (76,600), catalase (60,000), fumarase (49,000), aldolase (40,000), and yeast alcohol dehydrogenase (37,000).

Detection of Biotinyl Subunits. Prior to electrophoresis on $NaDodSO_4$ gels, MCase or PCase preparations were mixed with 5 μg of avidin (Sigma) or avidin saturated with *d*-biotin. After 10 min at room temperature, the samples were applied (without boiling) to the gels for the electrophoresis. A second procedure for detecting biotin involved slicing Coomassie-stained protein bands from $NaDodSO_4$/polyacrylamide gels, hydrolyzing in 1.5 M H_2SO_4, and assaying free biotin in the neutralized hydrolysate microbiologically (21) with *Saccharomyces cerevisiae* (ATCC 9896).

Other Techniques and Materials. MCase and PCase were assayed by $^{14}CO_2$ fixation as described (22), except that PCase was assayed in the presence of 100 mM KCl (23). Protein was determined by a microbiuret method (24). Radioactivity was determined by liquid scintillation counting in toluene/Triton X-100 (2:1, vol/vol) containing 6 g of 2a70 (Research Products Int., Elk Grove Village, IL) per liter. The sources of other materials used have been described (12, 22).

RESULTS

Bovine kidney mitochondria have proven to be a good source of both MCase (unpublished data) and PCase. Analysis of each purified enzyme by polyacrylamide gel electrophoresis is shown in Fig. 1. Under nondenaturing conditions, a single major band of protein was seen for purified MCase (Fig. 1, gel a). When an unstained companion gel was sliced and assayed for MCase activity, all the activity was coincident with the single protein band. A similar analysis of a purified PCase preparation (Fig. 1, gel b) revealed one major and one minor protein band. Slicing and assay of companion gels revealed PCase activity associated with both protein bands, and both bands yielded identical subunit patterns when analyzed separately on $NaDodSO_4$/polyacrylamide gels (see below). The faster migrating minor band appears to be an altered form of PCase which increases upon aging the enzyme, even when stored at −80°C.

Analysis of each purified enzyme on $NaDodSO_4$/polyacrylamide gels revealed two subunits in MCase (Fig. 1, gel c) and in PCase (Fig. 1, gel d). These subunits are designated the A and B subunits,¶ based on results described below. Determination of subunit molecular weights by a calibration curve as described (19), gave values of 62,000 and 80,000 for the A_m and B_m subunits of MCase, respectively, and 58,000 and 74,000 for the A_p and B_p subunits, respectively.

¶ By the nomenclature of Schiele *et al.* (25), A subunit is the biotin-free subunit and B subunit is the biotin-containing subunit; the subscript denotes whether the subunit is from MCase (e.g., A_m and B_m) or PCase (e.g., A_p and B_p).

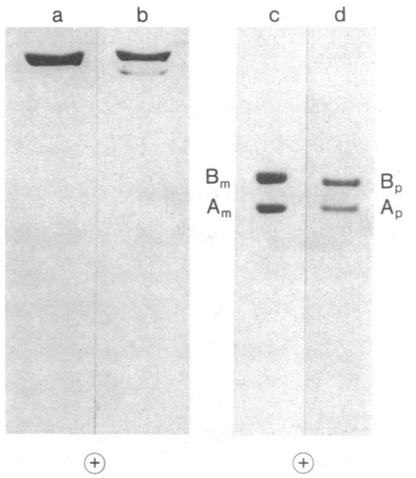

FIG. 1. Polyacrylamide gel electrophoresis of purified bovine mitochondrial MCase and PCase. Gels a and b, 4.5% acrylamide gels containing 5 μg of MCase (gel a) or 5 μg of PCase (gel b). Gels c and d, 7.5% acrylamide gels containing 0.1% $NaDodSO_4$ with either 5 μg of MCase (gel c) or 5 μg of PCase (gel d) applied. The designation of the subunits is described in a footnote. Although the migration of the A_m and A_p subunits appears identical in gels c and d, when run on the same slab gel side by side or mixed together, the A_p subunit migrated slightly faster than the A_m subunit, consistent with its lower molecular weight (see the text).

The localization of the biotin prosthetic group was determined by an avidin-binding procedure prior to $NaDodSO_4$ electrophoresis as shown for PCase in Fig. 2. Avidin binds tightly to the biotinyl prosthetic groups of this enzyme, and remains bound even in the presence of $NaDodSO_4$ (26) as used in the electrophoresis procedure. Under these conditions, the mobility of the biotinyl subunit was greatly reduced due to avidin binding, as can be readily seen for PCase in Fig. 2 (gel c) compared to untreated PCase (Fig. 2, gel a). Control experiments using biotin-saturated avidin (Fig. 2, gel b) showed no effect on the B_p subunit, consistent with a specific binding of avidin to the biotinyl moiety of this polypeptide. Similar results (data not shown) were obtained with MCase, indicating that avidin binds only to the large subunit. These results were verified by microbiological bioassays for biotin with *S. cerevisiae* (21), during which stained gels were sliced and protein-bound biotin released by acid hydrolysis was detected by a growth response of the yeast. (This type of biotin analysis is illustrated in Fig. 4.) In each case, biotin was associated exclusively with the large subunits and not the small subunits, consistent with their designation as the B or A subunits, respectively.

We have considered the possibility that detection of discrete A and B subunits in bovine kidney MCase and PCase could be due to partial proteolysis of larger precursor polypeptides during $NaDodSO_4$ treatment or during the enzyme isolation procedures. Such a phenomenon has recently been documented with the related acyl-CoA carboxylase, acetyl-CoA carboxylase, isolated from chicken or rat liver (6, 7). When purified preparations of MCase and PCase were treated with EDTA, *o*-phenanthroline, or phenylmethylsulfonyl fluoride, each at 1 mM, prior to addition of $NaDodSO_4$ sample buffer for the electrophoresis, the subsequent electrophoretic pattern was identical to that seen in Fig. 1 (slots c and d) and no larger potential precursor polypeptides were seen.

In a separate experiment, both MCase and PCase were purified from a kidney preparation in which the tissue was homogenized in buffered sucrose supplemented with the protease inhibitors EDTA (1 mM), *o*-phenanthroline (1 mM), and diisopropylfluorophosphate (0.1 mM). Then mitochondria were isolated and broken in a buffer supplemented with these same inhibitors; small aliquots of each enzyme were purified to near homogeneity. $NaDodSO_4$ electrophoretic analysis of each also revealed the same pattern of A and B subunits, and again no larger precursor polypeptides were noted. Similar experiments with a wider range of protease inhibitors will be needed to firmly establish this point.

We initiated immunochemical studies of the purified enzymes with the goal of preparing specific antisera that might be used to purify crossreacting MCase or PCase directly from crude tissue extracts via immunoabsorbant techniques (27). Antisera directed against either MCase or PCase were elicited by injection of the respective purified bovine mitochondrial enzymes into rabbits. As shown in Fig. 3, immunodiffusion tests revealed single precipitin bands between each purified enzyme and its respective antiserum, and no precipitin bands with a control antiserum. Both enzymes, purified from tissue and mitochondria treated with protease inhibitors as described above, showed identity reactions (17) with the corresponding enzyme purified in the absence of protease inhibitors.

In an experiment to test for crossreactivity between each enzyme and the opposing antiserum, it was shown that MCase did not crossreact effectively with anti-PCase and PCase did not crossreact with anti-MCase at the levels of enzymes and antisera that yielded the precipitin reactions described above.

The feasibility of using these antisera for analysis of crude enzyme preparations was demonstrated in the following experiment. The anti-PCase preparation was found, by immunodiffusion analysis, to crossreact with extracts containing PCase from the mitochondria of bovine liver, rat liver, rat kidney, and pig heart. Because the pig heart enzyme has been extensively studied (5, 28), except with respect to its subunit composition, we attempted to analyze its subunits by direct $NaDodSO_4$ gel electrophoresis of the immunoprecipitate obtained. Such experiments were hampered initially because the anti-PCase–PCase precipitates obtained were unstable upon washing, even when bovine kidney mitochondrial PCase was

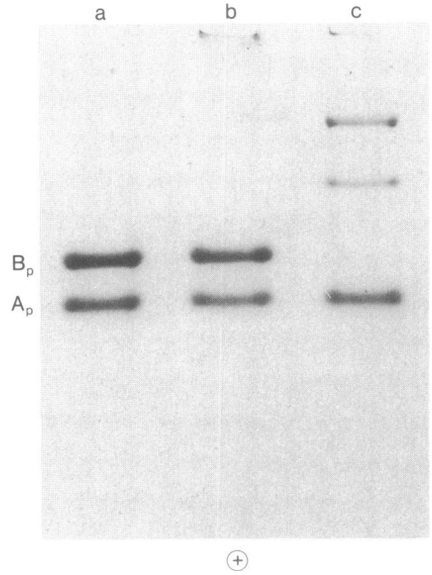

FIG. 2. Detection of the biotinyl subunit of PCase by avidin binding and subsequent $NaDodSO_4$/polyacrylamide gel electrophoresis. Five micrograms of PCase (5 μg) was used for electrophoresis. Gel a, PCase; gel b, PCase plus 5 μg of biotin-saturated avidin; gel c, PCase plus 5 μg of avidin. Samples were not boiled before application to the gel. Under these conditions, free avidin (a basic protein; ref. 26) did not penetrate into the gel and a distinct protein band corresponding to the avidin subunit was not seen.

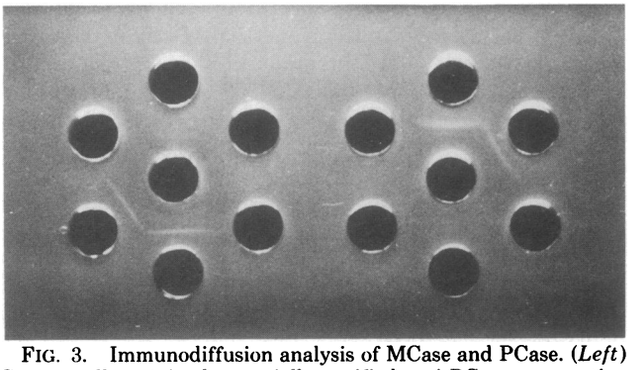

FIG. 3. Immunodiffusion analysis of MCase and PCase. (*Left*) Center well contained a partially purified anti-PCase preparation (0.44 mg of protein); (*Right*) center well contained a partially purified anti-MCase preparation (0.38 mg of protein). Samples in the outer wells were set up identically. Starting with the top well and going clockwise, the outer wells contained: 5.9 milliunits of MCase purified in the absence of protease inhibitors; 5.1 milliunits of MCase purified in the presence of protease inhibitors; 0.51 mg of control partially purified antiserum; 5.4 milliunits of PCase purified in the absence of protease inhibitors; 2.3 milliunits of PCase purified in the presence of protease inhibitors; and 0.51 mg of control partially purified antiserum.

used as antigen. However, efficient yields of washed immunoprecipitates could be obtained by using goat anti-rabbit immunoglobulin to enhance precipitation of an anti-PCase–PCase complex, in which the anti-PCase was the immunoglobulin fraction purified from the anti-PCase antiserum. An immunotitration of pig heart PCase is illustrated in Fig. 4 *left*. The upper curve shows that when a control rabbit immunoglobulin fraction was used, there was little nonspecific precipitation, whereas specific precipitation occurred when the anti-PCase immunoglubulin fraction was used (lower curve).

The immunoprecipitates obtained in this fashion were stable to washing. A $NaDodSO_4$ gel electropherogram of a pig heart PCase–anti-PCase precipitate (Fig. 4 *left*, arrow) is shown in Fig. 4 *right*, gel a. Five major protein bands are seen, corresponding to approximate molecular weights of 78,000, 62,000, 55,000, 50,000, and 22,000. The latter two represent the heavy and light chains, respectively, derived from the goat and rabbit immunoglobulin fractions, as shown by electrophoresis of a control rabbit immunoglobulin–goat anti-rabbit immunoglobulin precipitate (Fig. 4 *right*, gel b). The 62,000 M_r polypeptide seen in gel a (Fig. 4 *right*) is also present in this control immunoprecipitate, and is unidentified. The protein bands corresponding to 78,000 and 55,000 M_r probably represent the A and B subunits of PCase. To further explore this possibility, we sliced the stained gel, hydrolyzed it to release biotin, and then detected the biotin by bioassay (Fig. 4 *right*). The majority of the biotin on the gel was associated with the 78,000 M_r polypeptide, consistent with its identity as the B subunit of pig heart PCase. A smaller amount of biotin was found associated with the region of the gel containing the immunoglobulin H chain (and other polypeptides). The source of this biotin was traced to the goat anti-rabbit immunoglobulin antiserum used in this experiment, which precipitated some unidentified biotinyl polypeptide ($M_r \approx 50{,}000$) when mixed with the purified rabbit anti-PCase immunoglobulin even in the absence of the pig heart PCase preparation.

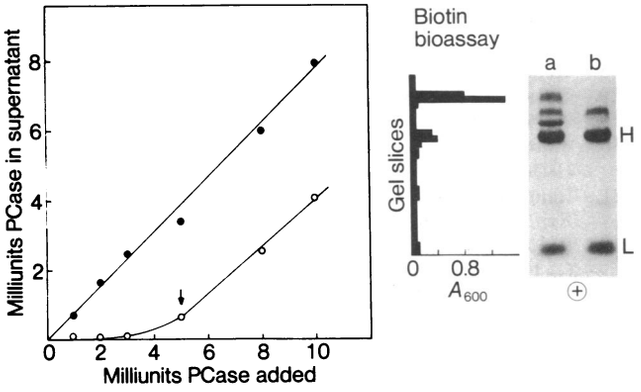

FIG. 4. Immunoprecipitation of pig heart PCase and $NaDodSO_4$/polyacrylamide gel electrophoresis of an immunoprecipitate. (*Left*) For immunoprecipitation of a pig heart PCase, control purified rabbit immunoglobulin (●) or a purified rabbit anti-PCase immunoglobulin fraction (○) was used. Arrow indicates the sample tube whose precipitate was analyzed on a 7.5% acrylamide gel containing 0.1% $NaDodSO_4$ (*Right*, gel a). (*Right*) Gel b, electrophoretic pattern obtained from a control immunoprecipitate. The H (heavy) and L (light) chains derived from the rabbit and goat immunoglobulins are designated. Only the center two-thirds of each gel (the only region with detectable protein bands) is shown. After photography of gel a, it was sliced into 2-mm portions near the H-chain region and 5-mm portions from the H-chain region to the L-chain region, and slices were analyzed for biotin. A_{600} is the absorbance of the yeast cultures after 72 hr of incubation.

DISCUSSION

As a group, each of the biotin-dependent carboxylases catalyzes a similar reaction sequence that involves the stepwise interaction of three distinct catalytic subsites, including a biotin carboxylation site, a transcarboxylation site, and a region containing the biotin prosthetic group that acts as a "CO_2" carrier between the other two sites. Depending on the source of the carboxylase, these three subsites are distributed on separate subunits or fused into one or two polypeptide chains, as recently reviewed by Obermayer and Lynen (10). The latter phenomenon is presumably a result of gene fusion during evolution of these enzymes (10, 11).

Of the biotin enzymes from animal sources, only two, pyruvate and acetyl-CoA carboxylases, have been subjected to intensive subunit analysis. Studies on pyruvate carboxylases isolated from numerous animal sources have shown that these enzymes are tetrameric assemblies of identical protomeric subunits (9). These subunits, containing all three subsites mentioned above, vary in molecular weight from 110,000 to 130,000. Acetyl-CoA carboxylases from rat liver (6, 29), chicken liver (7), and rat mammary gland (8) are now known to contain a single type of polypeptide chain, with a molecular weight of 220,000–250,000. These studies suggest that animal pyruvate and acetyl-CoA carboxylases have evolved the structural pattern of one type of multifunctional subunit.

For the other biotin enzymes known to be present in animal tissues, including MCase and PCase [and possibly isocitrate synthase (30)], only structural information on the PCase from pig heart has been published (5). Based on sedimentation data, the molecular weight of crystalline pig heart PCase was estimated to be 700,000. As stated by Ochoa and Kaziro (28), it "may be a tetramer of 4 primary units of molecular weight 175,000, each containing one molecule of biotin." It seems unlikely that the "primary unit" could have been a single polypeptide of M_r 175,000, since the enzyme dissociated into a single species sedimenting with a low sedimentation coefficient (2.55 S) when treated in 7 M urea (28). In light of the results described here on PCase from bovine kidney and pig heart, it seems very likely that PCase from each of these sources is composed of nonidentical subunits, including biotin-containing and biotin-free polypeptides. A similar conclusion has been drawn recently by Utter and his coworkers, who have examined PCase in rat and chicken liver mitochondria (ref. 3; M. F. Utter, personal communication). Further studies will be required to establish the subunit stoichiometry of native PCase from these sources.

Our analysis of purified bovine mitochondrial MCase shows that it also contains nonidentical subunits like those present in kidney and heart PCases. This type of subunit arrangement is similar to that previously described for bacterial MCases (12, 25), bacterial PCase (31), and bacterial pyruvate carboxylases (32). A recent report shows that PCase from the nematode *Turbatrix aceti* also contains two subunits (14). It might be concluded at this stage that a bipartite arrangement of subunits is the most common arrangement of subsites yet uncovered among the biotin enzymes.

We have not completely ruled out the possibility that the subunits seen in MCase and PCase are due in each case to limited proteolysis of a larger precursor polypeptide. Such a situation has been shown to occur with acetyl-CoA carboxylase from rat or chicken liver (6, 7, 29), where the enzyme is subject to highly specific proteolysis leading to the appearance of discrete smaller "subunits" unless protected by protease inhibitors. However, we have been unable to detect larger precursor polypeptides in MCase or PCase, even when the enzymes are purified in the presence of protease inhibitors like *o*-phenan-

throline, EDTA, phenylmethylsulfonyl fluoride, or diisopropylfluorophosphate, or when they are immunoprecipitated from freshly prepared tissue extracts in the presence or absence of such protease inhbitors.

It is clear that if kidney MCase and PCase subunits are not proteolytic artifacts, then the two enzymes do not share common biotinyl subunits. Even though these two enzymes are very similar structurally, the common catalytic elements, including the biotin carboxylation subsite and biotin attachment region present in each enzyme, probably evolved separately (10, 11), as we have also suggested to have occurred in the case of structurally homologous 3-methylcrotonyl-CoA and geranyl-CoA carboxylases of *Pseudomonas citronellolis* (12). Structural differences in the enzymes are also implied by the finding that there is no crossreaction between MCase and anti-PCase or between PCase and anti-MCase (Fig. 3).

The finding of two types of subunits in mammalian PCase is consistent with recent genetic studies on PCase deficiency in humans. Gravel *et al.* (3) have analyzed numerous pairwise crosses of PCase-deficient fibroblast cell lines, fused by inactivated Sendai virus to form heterokaryons, and have described two major complementation groups. Mutant cell lines from each group exhibit characteristic low PCase activity; when complementary cell lines are fused, PCase activity in the heterokaryons increases in a time-dependent and protein synthesis-dependent process (B. Wolf, personal communication). Although other interpretations are possible, these results are consistent with the possibility that mutations in the two classes represent mutations in different gene products, corresponding to either the A or B subunits described here.

It remains to be established whether the human enzyme contains two types of subunits. Recently we have found that human placental PCase (and MCase) crossreact with our antibody preparations, raising our expectations that small quantities of the human enzymes will be available (via immunoadsorption techniques) for analysis. Similar techniques presumably will be useful for isolation and characterization of defective forms of PCase and MCase from fibroblasts of patients with genetically inherited diseases affecting these enzymes.

We thank Drs. Merton Utter and Barry Wolf for communicating results of their research prior to publication. This research was supported by grants from the National Institutes of Health (HL 16628) and the National Science Foundation (PCM75-16251).

1. Alberts, A. W. & Vagelos, P. R. (1972) *Enzymes* (Academic, New York), 3rd Ed., Vol. 6, pp. 37–82.
2. Weyler, W., Sweetman, L., Maggio, D. C. & Nyhan, W. L. (1977) *Clin. Chim. Acta* **76,** 321–328.
3. Gravel, R. A., Kam-Fong, L., Skully, K. J. & Hsia, Y. E. (1977) *Am. J. Hum. Genet.* **29,** 378–388.
4. Wolf, B., Hsia, Y. E. & Rosenberg, L. E. (1978) *Am. J. Hum. Gen.*, in press.
5. Kaziro, Y., Ochoa, S., Warner, R. C. & Chen, J. Y. (1961) *J. Biol. Chem.* **236,** 1917–1923.
6. Tanabe, T., Wada, K., Okazaki, T. & Numa, S. (1975) *Eur. J. Biochem.* **57,** 15–24.
7. Mackall, J. C. & Lane, M. D. (1977) *Biochem. J.* **162,** 635–642.
8. Ahmad, F., Ahmad, P. M., Pieretti, L. & Waters, G. T. (1978) *J. Biol. Chem.* **253,** 1733–1737.
9. Utter, M. F., Barden, R. E. & Taylor, B. L. (1975) *Adv. Enzymol.* **42,** 1–72.
10. Obermayer, M. & Lynen, F. (1976) *Trends Biochem. Sci.* **1,** 169–171.
11. Wood, H. G. & Barden, R. E. (1977) *Annu. Rev. Biochem.* **46,** 385–413.
12. Fall, R. R. & Hector, M. L. (1977) *Biochemistry* **16,** 4000–4005.
13. Swack, J. A., Zander, G. L. & Utter, M. F. (1978) *Anal. Biochem.* **87,** 114–126.
14. Meyer, H., Nevaldine, B. & Meyer, F. (1978) *Biochemistry* **17,** 1822–1827.
15. Clinkenbeard, K. D., Reed, W. D., Mooney, R. A. & Lane, M. D. (1975) *J. Biol. Chem.* **250,** 3108–3116.
16. Halenz, D. R., Feng, J. Y., Hegre, C. S. & Lane, M. D. (1962) *J. Biol. Chem.* **237,** 2140–2147.
17. Crowle, A. J. (1973) *Immunodiffusion* (Academic, New York), 2nd Ed.
18. Deutsch, H. F. (1967) in *Methods in Immunology and Immunochemistry*, eds. Williams, C. A. & Chase, M. W. (Academic, New York), Vol. 1, pp. 315–321.
19. Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244,** 4406–4412.
20. Bryce, C. F. A. & Crichton, R. R. (1971) *J. Biol. Chem.* **246,** 4198–4205.
21. Lichstein, H. C. (1955) *J. Biol. Chem.* **212,** 217–222.
22. Hector, M. L. & Fall, R. R. (1976) *Biochemistry* **15,** 3465–3472.
23. Giorgio, A. J. & Plaut, G. W. E. (1967) *Biochim. Biophys. Acta* **139,** 487–501.
24. Koch, A. L. & Putnam, S. L. (1971) *Anal. Biochem.* **44,** 239–245.
25. Schiele, U., Niedermeier, R., Stürzer, M. & Lynen, F. (1975) *Eur. J. Biochem.* **60,** 259–266.
26. Green, N. M. (1975) *Adv. Protein Chem.* **29,** 85–135.
27. Ruoslahti, E., ed. (1976) "Immunoadsorbents in Protein Purification." *Scand. J. Immunol.*, Suppl. 3, (Universitetsforlaget, Oslo).
28. Ochoa, S. & Kaziro, Y. (1965) *Compr. Biochem.* **16,** 210–249.
29. Inoue, H. & Lowenstein, J. M. (1972) *J. Biol. Chem.* **247,** 4825–4832.
30. Mattoo, A. K., Carabott, M. J. J., Keech, D. R. & Wallace, J. C. (1976) *Biochem. Soc. Trans.* **4,** 1058–1060.
31. Henrikson, K. P. & Allen, S. H. G. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37,** 1525.
32. Barden, R. E., Taylor, B. L., Isohashi, F., Frey, W. H., II, Zander, G., Lee, J. C. & Utter, M. F. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4308–4312.