δ-Aminolevulinic Acid Synthetase

I. STUDIES IN LIVER HOMOGENATES

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SUMMARY

δ-Aminolevulinic acid synthesis has been demonstrated in both porphric and normal liver homogenates. A study of the factors which affect δ-aminolevulinic acid production indicates that the enzyme δ-aminolevulinic acid synthetase can be measured in liver homogenates, with use of added glycine and endogenously generated succinyl coenzyme A. δ-Aminolevulinic acid production in this system is greater than in the isolated mitochondrial system previously used.

The generation of succinyl coenzyme A from endogenous substrates is close to the optimal amount for δ-aminolevulinic acid production in liver homogenates.

The determinations of hepatic δ-aminolevulinic acid production in a number of normal species agree well with estimates of the δ-aminolevulinic acid required for heme enzyme synthesis.

The effects of factors influencing the synthesis of aminocetone and δ-aminolevulinic acid in liver homogenates were compared and found to be markedly different.

Two aminoketones of physiological importance have previously been described. The first, δ-aminolevulinic acid, is the aliphatic precursor of porphyrins and a source of 1-carbon fragments (1,2). The second, aminocetone, may also serve as a source of 1-carbon fragments (3). Both are produced by the enzymatic acylation of glycine, succinyl coenzyme A being used for δ-aminolevulinic acid synthesis (4-7) and acetyl coenzyme A for aminocetone (8-11). Through the action of threonine dehydrogenase, aminocetone can also be produced by the oxidation of threonine (10, 12-15).

Both δ-aminolevulinic acid synthetase (16, 17) and aminocetone synthetase (10, 11) are mitochondrial enzymes. Previous studies of δ-aminolevulinic acid synthetase performed in microorganisms (9, 14, 18-23), avian erythrocytes (7, 8, 24, 25), and mammalian liver (16, 17, 26) indicate this enzyme to be rate-controlling in the heme synthetic pathway. The enzyme is inducible (16, 27, 28), and its induction is inhibited by carbohydrate feeding (“glucose effect”) (17). Demonstration of δ-aminolevulinic acid synthesis in homogenates has not been achieved previously. Previous determinations of hepatic δ-aminolevulinic acid production have been performed only on the mitochondria isolated from the livers of animals treated with either 3,5-dicarbethoxy-1,4-dihydrocollidine (16) or allylisopropylacetamide (17). These assays have utilized both glycine and various tricarboxylic acid cycle intermediates as substrates. The present study demonstrates δ-aminolevulinic acid synthesis and the factors which affect it in homogenates under conditions where δ-aminolevulinic acid utilization is negligible. A simple method is described for the measurement of the enzyme in homogenates which gives much higher values of δ-aminolevulinic acid synthetase than methods previously used (16, 17). The level of δ-aminolevulinic acid synthetase in normal liver has been determined, and its relationship to the kinetics of heme enzyme synthesis is discussed.

MATERIALS AND METHODS

Allylisopropylacetamide was provided by Hoffmann-LaRoche. ALA-hydrochloride and CoA were purchased from Calbiochem. Succinyl coenzyme A was prepared by the method of Simon and Shemin (29) and determined by reaction with hydroxylamine (30). Phosphate buffers were prepared by adding sodium hydroxide to monobasic potassium phosphate.

ALA synthetase was induced in the livers of female Sprague-Dawley rats (120 to 150 g) by two subcutaneous injections of allylisopropylacetamide (400 mg per kg) at 24-hour intervals. Starting 24 hours before the first dose of allylisopropylacetamide, food was withheld until sacrifice (18 to 24 hours after the last dose of allylisopropylacetamide). Animals were sacrificed by decapitation, and livers were homogenized in 3 volumes of medium as described in the figures and tables. Most incubations were carried out in a total of 2 ml containing 0.5 ml of homogenate in 25-ml Erlenmeyer flasks at 37° with shaking in air. Six such flasks were used to obtain sufficient supernatant for chromatography. Except when indicated, the production of ALA by liver homogenates was determined with 0.1 ml of glycine as the only substrate addition. Other conditions in these experiments are indicated under “Results” and in the appropriate tables and figures. Incubations were terminated by addition of 0.5 ml of 25% trichloracetic acid to each flask. Usually, 10 ml of

1 The abbreviations used in this paper are: ALA, δ-aminolevulinic acid; pyrrole (ALA), 2 methyl-3 acetyl-4 propionic acid pyrrole.
ALA and aminocetone were determined by conversion to pyroles (pyrrole (ALA) and 2,4-dimethyl-3-acetylpyrrole) and separation of the pyroles by chromatographic methods previously described (31) followed by reaction with modified Ehrlich's reagent in 2 M perchloric acid and glacial acetic acid (32). When porphobilinogen was measured, the incubations were terminated by addition of 1 ml of dialyzed iron and 1 drop of a saturated solution of copper sulfate (33). Porphobilinogen was then measured by reaction with modified Ehrlich's reagent (32). Total porphyrin production was determined by adjusting the trichloroacetic acid supernatant to pH 4.5 with sodium acetate and extracting this with 5 volumes of ethyl acetate (34). To ensure complete conversion of porphyrinogens to porphyrins (35), the samples were exposed to a weak light for 30 min prior to extraction with ethyl acetate. The porphyrins were then extracted into 3 N HCl and were determined fluorometrically with the use of a coproporphyrin standard. The separation of the ALA and 2,4-dimethyl-3-acetylpyrroles on a Dowex 1-acetate resin (31) was repeatedly checked by paper chromatography of the pyroles in butanol-ammonium hydroxide and butanol-acetic acid systems (32). When normal liver was used, it was necessary to concentrate the pyrrole (ALA) under reduced pressure 50 to 100 times for identification by paper chromatography.

ALA synthesis in porphyric rat liver mitochondria was determined by a previously described modification (17) of the method of Granick and Urata (16). Mitochondria were isolated by a minor modification of the method of Schneider (36).

RESULTS

The ability to measure ALA synthetase activity in homogenates is based on the use of conditions which permit maximal ALA formation with little or no conversion of ALA to porphobilinogen by ALA dehydratase or utilization by the succinate-glycin cycle (1, 2). The optimum conditions for the measurement of ALA synthetase can be derived from the effects of the variables presented in Figs. 1 to 4 and Tables I and II.

EDTA enhances ALA production and reduces aminocetone synthesis in porphyric liver homogenates containing 75 mM Tris-HCl, pH 7.2, as buffer (Fig. 1). Not shown is that aminocetone production in liver homogenates is even more profoundly inhibited (by about 90%) in 10 mM EDTA and 75 mM sodium-potassium phosphate buffer (pH 7.0). A maximal 2- to 3-fold rise in ALA is provided by 0.5 to 10 mM EDTA (Fig. 1). This increase is the result of (a) inhibition of ALA dehydratase (Fig. 2) and (b) enhancement of ALA synthetase (Table I). The individual contributions of these two effects of EDTA to the total enhancement of ALA accumulation resulting from EDTA are determined by measuring the accumulation of ALA, porphobilinogen, and porphyrins in the presence and absence of EDTA (Table I). The decrease of ALA conversion to porphobilinogen and porphyrins resulting from a given concentration of EDTA represents the amount of increase of ALA accumulation accounted for by inhibition of ALA dehydratase by that concentration of EDTA. In the presence of 10 mM EDTA, about 20% of the increased ALA accumulation at 30 min and 27% at 1 hour is due to inhibition of ALA dehydratase (Table I). These values may be somewhat low since ALA might be converted to heme and bilirubin in the present system, and neither of these compounds was measured. Fig. 2 demonstrates that 10 mM EDTA almost completely blocks the conversion of ALA to porphobilinogen in the present system. The lower curve in Fig. 2 (0.1 mM ALA) represents a starting concentration of ALA which is in 2- to 4-fold excess of that produced by the incubation of porphyric liver for 1 hour. This inhibitory effect of EDTA on ALA dehydratase has previously been demonstrated on the purified enzyme from ox liver (33) and rabbit reticulocytes (37), where it has been shown to be of the noncompetitive type.

In addition, ALA dehydratase activity is diminished in the
Fig. 2. Conversion of ALA to porphobilinogen as a function of EDTA concentration. Normal rat liver was homogenized in 3 volumes of 0.9% sodium chloride solution containing 0.5 mM EDTA and 10 mM Tris-HCl, pH 7.4. Each flask contained 0.5 ml of homogenate, 150 μmoles of Tris-HCl, either 0.2 μmoles (●—●) or 10 μmoles (●—●) of ALA and EDTA as shown in a final volume of 2.0 ml and with a pH of 7.2. The incubations were in air at 37° for periods up to 1 hour. Porphobilinogen was determined as outlined under "Materials and Methods."

Fig. 3. ALA (●—●) and aminoacetone (AA) (○—○) production in porphyric rat liver homogenate as a function of glycine concentrations. Porphyric rat liver was homogenized in 3 volumes of 0.9% sodium chloride solution containing 0.5 mM EDTA and 10 mM Tris-HCl, pH 7.4. Each flask contained 0.5 ml of homogenate, 150 μmoles of Tris-HCl, 20 μmoles of EDTA, and glycine as shown in a final volume of 2.0 ml, pH 7.2. Incubations were in air at 37° for periods up to 1 hour. Incubations were terminated by addition of ¼ volume of 25% trichloracetic acid.

This suggests that, under the conditions of the present method, there is essentially no utilization of ALA by the succinate-glycine cycle. The principal effect of EDTA is that of enhanced ALA synthesis. A stimulatory effect of EDTA on ALA formation from glycine and succinyl-CoA was first demonstrated by Gibson.
Laver, and Neuberger (8) in particles from the red cells of anemic chickens and later from glycine and α-ketoglutarate in porphyrin guinea pig liver mitochondria by Granick and Urata (16), who suggested that the effect is due to mitochondrial stabilization. EDTA blocks active water uptake by mitochondria (38).

As shown in Table II, there is a constant rate of production of ALA and aminoacetone by porphyric liver homogenates for at least 60 min in Tris-HCl buffer, but only for about one-half as long in potassium-sodium phosphate buffer. This difference may result from orthophosphate-induced swelling of mitochondria (39) with resulting loss of ability to carry out oxidative phosphorylation (40). The pH optimum is 7.0 to 7.2 at 20° in either Tris-HCl or potassium-sodium phosphate buffer. Aminoacetone formation in both potassium-sodium phosphate and Tris-HCl buffer increased with decreasing pH over the range.

**TABLE II**

Comparison of effects of phosphate and Tris-HCl buffers on generation of ALA and aminoacetone with time

Porphyric rat liver was homogenized in 3 volumes of 0.9% sodium chloride solution containing 0.5 mM EDTA at a pH of 7.4. Each incubation mixture, in a final volume of 2.0 ml, consisted of 0.5 ml of homogenate; 200 μmoles of glycine; and either 150 μmoles of potassium-sodium phosphate buffer, pH 7.0, or 20 μmoles of Tris-HCl buffer, pH 7.2. The incubations were in air at 37° for periods up to 1 hour. Incubations were terminated by the addition of a 1/4 volume of 25% trichloroacetic acid.

**TABLE I**

Effect of EDTA on ALA, porphobilinogen, and porphyrin synthesis in porphyric rat liver homogenate

Porphyric rat liver was homogenized in 3 volumes of 0.9% sodium chloride solution containing 10 mM Tris, pH 7.4. Each incubation mixture consisted of 0.5 ml of homogenate, 150 μmoles of Tris-HCl, 20 μmoles of EDTA, and liver homogenate as shown in a final volume of 2.0 ml, pH 7.2. Incubations were in air at 37° for periods up to 1 hour. Incubations were terminated by the addition of 1/4 volume of 25% trichloroacetic acid.

- The total increase of ALA due to a given concentration of EDTA was obtained by subtracting the values in Column 2 (ALA, 30 min and 60 min) in the absence of EDTA from those obtained in the presence of the particular concentrations of EDTA used. The increase of ALA due to inhibition of ALA dehydratase was calculated by subtracting the ALA accounted for by the sum of porphobilinogen and porphyrins (Columns 3 and 4) when EDTA was used from this sum in the absence of EDTA. The difference (not given in the table) of the total increase of ALA due to EDTA minus the increase of ALA due to inhibition of ALA dehydratase for a given concentration of EDTA represents the increase of ALA synthesis due to EDTA.
studied (from 7.8 to 6.2 in phosphate and from 7.8 to 7.2 in Tris). In three experiments, aminoacetone production was 3 to 4 times greater in Tris than in phosphate buffer at pH 7.2 and 10 mM EDTA.

ALA synthesis in porphyric liver homogenates is maximal with 100 to 300 mM glycine (Fig. 3). The $K_m$ values for ALA synthetase and aminocetic synthetase in this system are about 5 mM and greater than 150 mM, respectively. This $K_m$ of ALA synthetase for glycine is similar to that found by Gibson et al. (8) and Brown (24) for the enzyme from amnionic chicken erythrocyte particles. The $K_m$ for glycine of partially purified ALA synthetase from *Rhodopsseudomonas spheroides* is 280 $\mu$M (23). Neither of the curves passes through the origin because of the presence of a small amount of endogenous glycine and, in the case of aminocetic, threonine (10, 12-15).

In the present method, ALA production is a linear function of enzyme concentration from 0.05 to 0.70 ml of homogenate (1:3) per 2 ml of incubation mixture (Fig. 4).

Of the citric acid cycle intermediates studied (citrate, $\alpha$-ketoglutarate, succinate from 0.1 to 10 mM, and succinyl-CoA from 0.1 to 3 mM), only $\alpha$-ketoglutarate at 1 mM and succinyl-CoA with or without additional ATP significantly increased ALA production in rat liver homogenates. However, these increases are small (10% to 121% of control activity with succinyl-CoA concentrations of 0.1 to 2.0 mM and 131% of control activity with 2.0 mM succinyl-CoA and 10.0 mM ATP). $\alpha$-Ketoglutarate at 1 mM increased ALA production only to 115% of controls. About 30 to 50% of the added succinyl-CoA is hydrolyzed in 10 min in normal or porphyric rat liver homogenates. Maintenance of 0.5 mM and 1.0 to 2.0 mM succinyl-CoA in the incubation mixture by addition of that compound at 10-min intervals resulted in ALA synthesis of 118 and 124% that of controls, respectively. Since it has been demonstrated that succinyl-CoA is utilized by porphyric rat liver mitochondria for the synthesis of ALA (17), the above data would suggest that there is nearly optimal synthesis of succinyl-CoA in the liver homogenate system. Concentrations of 10 mM $\alpha$-ketoglutarate, citrate, and succinate produce varying degrees of inhibition of ALA synthetase (to levels of 70, 82, and 81% of control activity, respectively). Similar effects of these compounds have been observed in a number of other systems (16, 18, 24, 25). The mechanism of inhibitory effects of certain tricarboxylic acid cycle intermediates on ALA formation in chick erythrocytes has been examined in detail by Brown (24). He concluded that inhibition of ALA formation by certain concentrations of succinate is caused by its conversion to oxalacetate, which in turn inhibits the decarboxylation of $\alpha$-ketoglutarate to succinyl-CoA. Malonate diminished the inhibition by succinate, presumably by decreasing oxalacetate formation from the latter. The inhibitory effect on ALA formation of high concentrations of $\alpha$-ketoglutarate is thought to result from the direct inhibition of the condensation of glycine and succinyl-CoA (24).

Addition of 1, 10, and 50 mM pyruvate reduced the production of ALA from glycine and the endogenous generations of succinyl-CoA to 98, 90, and 78%, respectively, of control levels. This effect of high concentrations of pyruvate on ALA production has been observed in other systems (3, 7). As expected, pyruvate augmented aminoacetone synthesis under the conditions of the present system, and 1, 10, and 50 mM pyruvate increased aminoacetone production 45, 78, and 190%, respectively, above that of controls. ATP, 10 mM, slightly stimulated (115% of control values) and 1 and 10 mM ADP inhibited ALA formation (to 86 and 50% of control values). These effects have been observed also in porphyric guinea pig mitochondria (16). ADP would favor conversion of succinyl-CoA to succinate by succinic thio kinase, whereas ATP would enhance succinyl-CoA accumulation. Glucose (1 and 10 mM), GTP (10 mM), glutamate (1 and 10 mM), $\beta$-methyltetrahydroethanol (1 and 5 mM), and NAD (0.5 and 5 mM) did not significantly affect ALA production from glycine and endogenous succinyl-CoA. MgCl$_2$ (20 mM) did not increase ALA formation in this system despite the high concentrations of EDTA routinely utilized and the previous demonstrations in other systems that Mg$^{++}$ enhanced succinyl-CoA formation, thereby increasing ALA synthesis (16, 18, 25).

Neither the addition of 0.2 or 1 mM pyridoxyl phosphate nor the addition of 0.1 to 5.0 mM deoxyxypiridoxine significantly affected ALA production by porphyric rat liver homogenates. However, L-penicillamine, but not D-penicillamine, was markedly inhibitory (31 and 12% of control values with concentrations of 1 and 5 mM, respectively). This inhibition was reversed by addition of pyridoxyl phosphate. Laver, Neuberger, and Udenfriend (22) observed similar effects of D- and L-penicillamine on ALA synthesis in chicken red cell particles. Endogenous pyridoxyl phosphate in rat liver is probably already sufficient for maximum ALA formation. Burnham and Lascelles (23) have estimated the $K_m$ for pyridoxyl phosphate of a partially purified ALA synthetase from *R. spheroides* to be about 4 $\mu$M. The inhibition by deoxyxypiridoxine of pyridoxyl phosphate-requiring enzymes is variable. Dietrich and Borrie (41) have shown that apocystine desulfhydrase is a pyridoxal phosphate enzyme which is not affected by deoxyxypiridoxine because it is unable to combine with that compound (41). Although both enantiomorphs of penicillamine can react with pyridoxyl phosphate to form thiazolidines, only the L form is a significant antagonist of pyridoxyl phosphate in biological systems (42, 43).

In two of four experiments, 10 mM malonate increased ALA synthesis during the first 30 min of the incubation by 10 to 30% above that of the controls. In all cases, there was inhibition at the end of 60 min (50 to 80% of control activity). This suggests that malonate at first increases succinyl-CoA concentration and later decreases it through inhibition of succinic dehydrogenase. Arsenite, 1 mM; glyoxalate, 10 mM; and hydroxylamine, 10 mM, reduced ALA production in liver homogenates to 18, 63, and 28% of control levels, respectively. Inhibition by these compounds was constant throughout the 60-min incubations. The inhibition by glyoxalate results from its condensation with endogenous oxalacetate to form oxalomalate, an inhibitor of aconitase (14).

Production of ALA by porphyric liver homogenates was generally linear with time for 60 to 120 min (Fig. 5). However, when ALA synthesis exceeded 800 mmoles per g of liver, the rate of ALA production significantly decreased before 60 min even in the presence of exogenous succinyl-CoA. This indicates that product inhibition of ALA synthetase in rat liver is consistent with a similar observation of Lascelles in *R. spheroides* (22).

Incubation of porphyric liver homogenates in air rather than nitrogen or oxygen resulted in the greatest production of ALA for periods of 1 to 2 hours (Table III). This is in contrast to the observation of Granick and Urata (16) that porphyric guinea pig liver mitochondria synthesize 3 times as much ALA from...
citrate and glycine in nitrogen as in air. In the present system, nitrogen may be inhibitory during relatively long incubations as a result of its depression of the citric acid cycle. The ratio of surface area to incubation volume significantly affects ALA production in the present system. An optimum ratio was obtained when liver homogenates in 2-ml volumes were incubated in 25-ml Erlenmeyer flasks.

On the basis of the above observations, the following method was generally employed for the assay of ALA synthetase in liver homogenates. Liver was homogenized in 3 volumes of 0.9% sodium chloride solution containing 0.5 mM EDTA and 10 mM Tris, pH 7.4. The incubation mixture contained 0.5 ml of homogenate, 200 µmoles of glycine, 200 µmoles of EDTA, 150 µmoles of Tris-HCl buffer at a final pH of 7.2 and in a total volume of 2.0 ml. The incubations were in air at 37° and were terminated by the addition of 0.5 ml of 25% trichloracetic acid. When a species other than the rat was studied, 0.4 µmole of pyridoxal phosphate was routinely added to the incubation mixture.

A comparison of ALA formation by porphyric rat liver homogenate with that of mitochondria from the same liver is seen in Fig. 5. No citric acid cycle intermediates were added to the homogenate, whereas the mitochondrial preparation contained 2 mM succinyl-CoA (17). The homogenate produced larger amounts of ALA with linear relationship to time for a longer period than the mitochondrial preparation.

![Fig. 5. ALA production in porphyric liver. Comparison of ALA synthesis in homogenate (○—○) and mitochondrial systems (△-△) with the same porphyric liver. Conditions for the measurement of ALA synthesis in porphyric rat liver homogenates were identical with those for Table III (in air). ALA production in isolated mitochondria was determined by incubating mitochondria equivalent to 0.2 to 0.4 g of liver with 600 µmoles of glycine, 4 µmoles of succinyl-CoA, 0.4 µmole of pyridoxal phosphate, 10 µmoles of EDTA, and 100 µmoles of phosphate buffer, pH 6.0, in a final volume of 2.0 ml. The incubations were carried out in air at 37° and terminated by addition of 0.5 ml of 25% trichloracetic acid.](image-url)

TABLE III

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<tr>
<th>Time (min)</th>
<th>ALA (µmoles/g liver)</th>
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<tr>
<td>30</td>
<td>115</td>
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<tr>
<td>60</td>
<td>184</td>
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<td>90</td>
<td>230</td>
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With the above described method for the assay of ALA synthetase, it has been possible to demonstrate consistently ALA production in the livers of normal rats and other species. The ranges of values of ALA synthetase activity, as determined by the present method, in 89 fasted and 24 fed Sprague-Dawley female rats, were 8 to 24 and 4 to 15 µmoles of ALA per g of liver per hour, respectively. Values for hepatic ALA production (millimicromoles of ALA per g per hour) by other species were: rabbit, 5, 12; dog, 15; monkey, 10; and turkey, 6. These are similar to the activities obtained from nine nonporphyric humans (range, 15 to 30) (26). That the ALA measured was not contaminated with aminocetone was confirmed by paper chromatography with both the butanol-acetic and butanol-ammonia systems (32). In the determination of hepatic ALA synthetase of some normal rats and in assays of all the other species, the pyrrole (ALA) derived from the incubations was concentrated on Dowex 1-acetate (31) and the resulting net optical density at 556 µm (optical density after incubation minus the initial optical density) upon reaction with modified Ehrlich's reagent (32) was between 0.050 and 0.100. A similar range of values for normal rat liver ALA synthetase was observed when the pyrrole (ALA) was not concentrated and the net optical density was in the range of 0.020. ATP, 10 mM, and succinyl-CoA, 2 mM, failed to enhance hepatic ALA synthesis in the normal species studied. Citrate, α-ketoglutarate, and succinate in 0.1 to 1 mM concentrations did not increase ALA production in the livers of normal rats, rabbits, or nonporphyric humans. Greater concentrations of these compounds were inhibitory.

**DISCUSSION**

Granick and Urata (16) first demonstrated that markedly elevated levels of ALA synthetase are present in the liver in experimental porphyria. Granick (27, 45) obtained evidence that a number of compounds, some of which are used therapeutically, may induce the enzyme in cultured chick embryo liver. It has also been shown that a pronounced elevation of hepatic ALA synthetase explains the increased porphyrin precursor excretion in the genetic disease, acute intermittent porphyria (26). This is of particular interest because the induction in this disease is of genetic origin, raising the question of an operator-constitutive mutation in man (26, 45, 46).

Since the enzyme which converts ALA to porphobilinogen (ALA dehydratase) is present in high levels in liver (33), it
had been assumed that measurement of ALA synthetase could not be achieved in the presence of ALA dehydratase. Because ALA dehydratase is present in the soluble portion of the cell (23), previous measurements of the enzyme in liver have been made in isolated mitochondria (16, 17). The present studies indicate that, in the presence of high concentrations of EDTA and in the absence of sulfhydryl activators, ALA conversion to porphobilinogen is almost completely blocked without inhibition of ALA production.

That the generation of succinyl-CoA from endogenous substrates is close to the optimal amount for ALA production in the present system is supported by several lines of evidence. (a) Of the tricarboxylic acid cycle intermediates studied, only small increases in ALA production could be demonstrated with succinyl-CoA (0.5 to 2 mM) and α-ketoglutarate (1 mM). (b) Estimates of tricarboxylic acid cycle capacities by several techniques (48, 49) indicate that only a small fraction (<1%) of the succinyl-CoA generated is required for maximal ALA production (17). (c) Under the conditions outlined here, ALA production from glycine and endogenous succinyl-CoA in liver homogenates is significantly greater than that generated from glycine and exogenous citric acid cycle intermediates in mitochondria (Fig. 5). ATP (10 mM) also increases ALA production in porphyrin liver homogenate, possibly as a result of enhanced formation of succinyl-CoA. However, the increase is small.

The levels of the heme enzymes are in part dependent on the rate of production of ALA since that step is rate-limiting in heme biosynthesis (16, 27). Based upon their previously determined turnover rates and content in rat liver, the range of requirements of catalase (50) and cytochrome c (51, 52) for hepatic ALA production are 3.8 and 0.5 nmol of ALA per g of liver per hour. Less certainty exists regarding the amount of ALA required for the synthesis of the other hepatic heme enzymes, cytochromes a, a₃, b, c, b₅, P-450, and tryptophan pyrrolase. However, calculations based on half-lives of 1 to 10 days for the first five of the enzymes and on 1 to 4 hemes per mole for tryptophan pyrrolase indicate that the ALA utilized for the synthesis of tricarboxylic acid cycle intermediates by several techniques (47). (c) Estimates of tricarboxylic acid cycle capacities by several techniques (48, 49) indicate that only a small fraction (<1%) of the succinyl-CoA generated is required for maximal ALA production (17).

REFERENCES

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