Decarboxylation of Glycine by Serine Hydroxymethyltransferase in the Presence of Lipoic Acid*

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Serine hydroxymethyltransferase and the glycine cleavage system are both present in liver mitochondria and both bind glycine to form a pyridoxal 5'-phosphate carbanionic quinoid species. Lipoic acid has been shown to have the ability to intercept the carbanionic intermediate formed from the binary complex of serine hydroxymethyltransferase and glycine and form an intermediate adduct which is ultimately processed to yield CO2 and a methylamine adduct. Kinetic studies have shown that the lipoic acid-dependent decarboxylation of glycine catalyzed by serine hydroxymethyltransferase proceeds through a sequential mechanism.

This lipoic acid-dependent decarboxylation catalyzed by serine hydroxymethyltransferase is similar to the initial reaction of the glycine cleavage system to the lipoic acid-dependent decarboxylation of glycine by the P-protein alone suggesting that both enzymes could serve in lieu of each other.

It has been demonstrated that serine hydroxymethyltransferase can bind glycine and catalyze the exchange of the pro-S-α-proton similar to that found with the P-protein (Schirch and Jenkins, 1964). It has also been shown that lipoic acid can replace the H-protein as an acceptor of the carbanion (Hiraga and Kikuchi, 1980). Since serine hydroxymethyltransferase is capable of catalyzing amino acid transformations of various other B6 enzymes, we postulated that serine hydroxymethyltransferase, when in the presence of lipoic acid, could catalyze the decarboxylation of glycine to mimic the glycine cleavage system.

Results presented in this text suggest that serine hydroxymethyltransferase and P-protein both can account for the decarboxylation of glycine in mitochondria. Having this activity associated with serine hydroxymethyltransferase makes it conceivable that the complete degradation of serine to 2 mol of N5, N10-methylenetetrahydrofolic acid and 1 mol each of CO2 and NH3 could be initiated by a single mitochondrial pyridoxal 5'-phosphate enzyme.

MATERIALS AND METHODS2

Glycine, N5-methylenetetrahydrofolic acid, (Baron salt), B6 lipic acid (Schirch and Gross, 1968), L-allo-threonine, L-phenylalanine, L-seryl 2-mercaptoethanol, 2-mercaptoethanol (Schirch and Gross, 1968), glycine, N11-methyltetrahydrofolic acid, 5-aminolevulinic acid, pyridoxal 5'-phosphate (Novo, N.P.V.A., NADH, nicotinamide adenine dinucleotide (reduced form); NAD, nicotinamide adenine dinucleotide; DTNB, 5,5'-dithiobis(nitrobenzoic acid); HEPES, 4-(2-hydroxyethyl) piperazineethanesulfonic acid; DTT, dithothreitol.

2Materials and Methods and Tables I and II are presented in miniprint as prepared by the authors. The miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-2629, cite authors, and include a check or money order for $2.00 per set of photocopies.

2Photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

2The following abbreviations are used: PLP, pyridoxal 5'-phosphate; NADH, nicotinamide adenine dinucleotide (reduced form); DTNB, 5,5'-dithiobis(nitrobenzoic acid); HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DTT, dithothreitol.

3Centrifugations were carried out on a 5 cm, 30 cm ultracentrifuge column that was previously equilibrated with SMM buffer B. Washing the column with the same buffer removed unbound

The pyridoxal 5'-phosphate-dependent P-protein of the glycine cleavage system, present in the mitochondrion of hepatic tissue, reacts with glycine initiating its conversion to CO2, NH3, and N5,N10-methylenetetrahydrofolic acid through a methylene intermediate. The methylene is attached in a thioether linkage to the bound lipoic acid moiety of the H-protein of the glycine cleavage complex (glycine synthase, EC 2.1.2.10) (Hiraga and Kikuchi, 1980) as follows:1

\[
\text{CHO} + \text{glycine + acetaldehyde} \rightarrow \text{CH}_2\text{CHO} + \text{acetaldehyde} \quad \text{(a)}
\]

(Schirch and Gross, 1968)

\[
\text{L-allo-Threonine} \rightarrow \text{glycine + acetaldehyde} \quad \text{(b)}
\]

(Schirch and Gross, 1968)

\[
3\text{-Hydroxy-α-trimethyllysine} \rightarrow \text{glycine + butyrobetaine aldehyde} \quad \text{(c)}
\]

(Hulse et al., 1978)

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1 P and H in the circles represent the P-protein containing pyridoxal 5'-phosphate and the H-protein containing lipoic acid, respectively.

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Table 1

| Purification step | Protein (mg) | Activity (Sp Act) |
|-------------------|-------------|------------------|
| Host treatment    |             | 52000            |
| pH 3.9 buffer B   |             | 1260             |
| pH 3.9 buffer C   |             | 469              |
| pH 7.0 buffer B   |             | 0.01             |
| pH 7.0 buffer C   |             | 0.22             |
| pH 8.0 buffer B   |             | 220              |
| pH 8.0 buffer C   |             | 220              |
| pH 9.0 buffer B   |             | 2                  |
| pH 9.0 buffer C   |             | 2                 |
| pH 10.0 buffer B  |             | 10.1              |
| pH 10.0 buffer C  |             | 10.1              |
| pH 11.0 buffer B  |             | 15.7              |
| pH 11.0 buffer C  |             | 15.7              |

Purification of Bovine Serum Hydroxymethyltransferase

Activity was determined routinely using the pyruvyl-
serine assay (Ulevitch and Kalten, 1977). One unit of
enzyme activity is defined as the amount that catalyzed
the formation of 1 µmol of glycine. The enzyme was
loaded onto a column of Macropore™ Gel Red A equilibrated
with 0.02M phosphate buffer, pH 7.0. The column was washed
with 0.02M phosphate buffer, pH 7.0, and 0.02M buffer to remove
most excess proteins. The enzyme was eluted from the 0.02M buffer
with the active fractions being pooled and dialyzed against 0.02M
buffer B.

The dialyzed enzyme was loaded onto a 1.5 x 10 cm column of
Macropore™ Gel Red A equilibrated with 0.02M phosphate
buffer B. The column was washed with 0.02M phosphate buffer,
and 0.02M buffer to remove excess proteins. The enzyme was eluted
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and 0.02M buffer to remove excess proteins. The enzyme was eluted
from the 0.02M buffer with the active fractions being pooled
and dialyzed against 0.02M buffer B.

Table 2

| Purification step | Protein (mg) | Activity (Sp Act) |
|-------------------|-------------|------------------|
| Host treatment    |             | 52000            |
| pH 3.9 buffer B   |             | 1260             |
| pH 3.9 buffer C   |             | 469              |
| pH 7.0 buffer B   |             | 0.01             |
| pH 7.0 buffer C   |             | 0.22             |
| pH 8.0 buffer B   |             | 220              |
| pH 8.0 buffer C   |             | 220              |
| pH 9.0 buffer B   |             | 2                  |
| pH 9.0 buffer C   |             | 2                 |
| pH 10.0 buffer B  |             | 15.7              |
| pH 10.0 buffer C  |             | 15.7              |

Table 2

Purification of Mitochondrial Bovine Serum Hydroxymethyltransferase

Activity was determined routinely using the pyruvyl-
sereine assay (Ulevitch and Kalten, 1977). One unit of
enzyme activity is defined as the amount that catalyzed
the formation of 1 µmol of glycine.

Table 3

| Purification step | Protein (mg) | Activity (Sp Act) |
|-------------------|-------------|------------------|
| Host treatment    |             | 52000            |
| pH 3.9 buffer B   |             | 1260             |
| pH 3.9 buffer C   |             | 469              |
| pH 7.0 buffer B   |             | 0.01             |
| pH 7.0 buffer C   |             | 0.22             |
| pH 8.0 buffer B   |             | 220              |
| pH 8.0 buffer C   |             | 220              |
| pH 9.0 buffer B   |             | 2                  |
| pH 9.0 buffer C   |             | 2                 |
| pH 10.0 buffer B  |             | 15.7              |
| pH 10.0 buffer C  |             | 15.7              |

Table 3

Activity was determined routinely using the pyruvyl-
sereine assay (Ulevitch and Kalten, 1977). One unit of
enzyme activity is defined as the amount that catalyzed
the formation of 1 µmol of glycine.

Table 4

| Assay Procedures | Activity (Sp Act) |
|------------------|------------------|
| Serine hydroxymethyltransferase activities were determined by both the cleavage of D- and L-serine hydroxymethyltransferase, respectively (Ulevitch and Kalten, 1977) and the formation of glycine. | |
Glycine Deacylating

FIG. 1. Effect of lipoic acid on the glycine carbamion generated by serine hydroxymethyltransferase in the presence of N5-methyltetrahydrofolate. Each sample contained in a total volume of 1.0 ml: 50 mM potassium phosphate buffer, pH 7.5, 0.5 mg/ml of enzyme, 75 mM glycine, and 40.0 nM N5-methyltetrahydrofolate acid except C, in which N5-methyltetrahydrofolate was omitted. The solutions contained lipoic acid at the concentrations indicated and their corresponding spectra were recorded as subsequently defined. ○, 0.0 mM; ■, 2.0 mM; □, 4.0 mM; and ▲, 6.0 mM.

FIG. 2. Lipoic acid quenching of the anionic quinoid species. This double reciprocal plot allowed for the calculation of the dissociation constants for the ternary complex titrated as described in Fig. 1. The sources titrated were: ○, mitochondrial form from porcine liver, and the cytosolic forms from ■, beef; ●, sheep; and ▲, pork livers.

the assay procedure and thus the catalytic nature of the reaction between glycine and lipoic acid, we demonstrated that the rate of reaction obeyed saturation kinetics with respect to concentration of substrates as well as concentration of enzymatically active bovine serine hydroxymethyltransferase.

The serine hydroxymethyltransferase from beef liver was also used to investigate the kinetic mechanism of the decarboxylation of glycine in the presence of lipoic acid. From the data presented in Fig. 4, a sequential mechanism for the glycine-lipoic acid oxidoreductase and decarboxylation reaction can be suggested. From the linear replots of the lines in Fig. 4 and computer analysis, the $K_m$ values were determined to be 27.0 ± 0.8 mM for glycine and 12.7 ± 0.3 mM for lipoic acid.

In order to determine if bovine serine hydroxymethyltransferase was able to catalyze decarboxylation of the glycine-lipoate adduct, the reaction was carried out using [1-14C]glycine. In the reaction between [1-14C]glycine and lipoic acid in the presence of serine hydroxymethyltransferase a significant amount of [14C]CO$_2$ was released and trapped in KOH and no decarboxylation occurred in the absence of either lipoic acid or enzyme. In the presence of lipoic acid, the decarboxylation reaction proceeded at a rate of 1 nmol/min/(mg of enzyme) of [14C]CO$_2$ produced. This rate of decarboxylation of glycine agrees with the relative rate of formation of the methylamine-lipoate adduct indicating a stoichiometric relationship among the products.

Inhibition of Reactions Involving a Glycine Anion by Lipoic Acid—After demonstrating that lipoic acid can add to the glycine anion and cause decarboxylation, it was also desirable to determine if lipoic acid could be involved in other reactions catalyzed by bovine serine hydroxymethyltransferase in which a glycine anion is an intermediate. To investigate this possibility, the reaction involving the cleavage of β-phenylserine to benzaldehyde and glycine was studied in the presence of lipoic acid. Lipoic acid was observed to have a competitive inhibitory effect on the cleavage reaction as illustrated in Fig. 5. The inhibition constant, $K_i$, for lipoic acid was determined to be 0.92 ± 0.02 mM from computer analysis.

Reactions of the Glycine Carbamion with Coenzyme A Derivatives—We have now established that serine hydroxymethyltransferase, in addition to its role in reactions a–c stated previously, can also catalyze the decarboxylation of glycine illustrated in Reaction d.

Glycine + Lipoic acid → CO$_2$ + methylamine-lipoate (d)

We found it of interest to investigate the possibility that serine hydroxymethyltransferase could catalyze condensation reactions with acyl coenzyme A derivatives to mimic reactions catalyzed by the following pyridoxal 5'-phosphate-dependent enzymes, δ-aminolevulinate synthase and aminoaacetoacetyl synthase. These reactions involve the condensation of a acyl coenzyme A or succinyl coenzyme A and glycine in a manner similar to lipoic acid and glycine. To carry out this study, we generated the carbanionic quinoid complex of glycine, serine hydroxymethyltransferase, and N5-methyltetrahydrofolic acid and titrated it with incremental increases in concentration of the acyl coenzyme A derivatives of interest, and the spectral change of the band was monitored at 505 nm. Through such experiments, it was determined that even at concentrations as high as 10 mM of each of the acyl coenzyme A derivatives, no spectral change occurred.

Association of Lipoamide Dehydrogenase Activity with Serine Hydroxymethyltransferase—The evidence presented in this text shows that the reaction between glycine and lipoic acid catalyzed by serine hydroxymethyltransferase mimics those reactions that occur between the P-protein and the H-protein of the glycine cleavage system. Another feature of the P-protein is a close association of this enzyme with the L-protein (lipoamide dehydrogenase activity) of the glycine cleavage system. To determine if this association was also observed with the various serine hydroxymethyltransferases, lipoamide dehydrogenase activity was monitored throughout the purifications of the various serine hydroxymethyltransferases. The results indicate that even nearly homogeneous serine hydroxymethyltransferase, in all cases, had some resid-

L. R. Zieske and L. Davis, unpublished data.
Glycine Decarboxylation

\[
\text{Glycine Decarboxylation} \\
\text{NH}_2\text{CH}_2\text{COOH} + \text{E-PLP-CHO} \rightleftharpoons \text{E-PLP-CH=N-CH}_2\text{COOH} \]

(a)

(b)

FIG. 3. Effect of lipoic acid on the carbanion generated from glycine.
This figure illustrates, schematically, what is occurring during the lipoic acid titration of the glycine carbanion, which is generated upon loss of the pro-S proton from the Schiff base.

\[
\text{HOOC(C}_2\text{H}_4\text{COOH} \rightleftharpoons \text{E-PLP-CH=N-CH}_2\text{COOH} \rightleftharpoons \text{E-PLP-CH=N-CH}_2\text{COOH} \]

(c)

(d)

FIG. 4. Double reciprocal plot of the initial velocity study of the methylamine-lipoate formation with glycine as the varied substrate at different fixed concentrations of lipoic acid. Detection of the product was accomplished using the di-thiobis(nitrobenzoic acid) assay described under "Materials and Methods." The concentrations of lipoic acid employed were: ○, 5 mM; ■, 10 mM; ◇, 15 mM; ▲, 20 mM.

uional lipoamide dehydrogenase activity remaining. During each step of the purification, however, the ratio of serine hydroxymethylase activity/lipoamide dehydrogenase activity did not remain constant and dramatically increased, i.e. from 36.5 prior to affinity chromatography to over 300 upon elution for the cytosolic bovine isoenzyme. This lipoamide dehydrogenase activity, when present in a ratio below 100, competed with serine hydroxymethyltransferase for lipoic acid, thus diminishing the rate of decarboxylation of glycine catalyzed by serine hydroxymethyltransferase. Therefore, homogeneous serine hydroxymethyltransferases were used throughout these studies.

DISCUSSION

Putative carbanion intermediates formed during the reaction of pyridoxal 5'-phosphate-dependent enzymes with their substrates have been very difficult to trap. Evidence in this manuscript demonstrates the ability of lipoic acid to intercept glycine carbanion intermediates formed from binary complexes of serine hydroxymethyltransferase and glycine. The only previous probes for such carbanion intermediates have been oxidation-reduction indicators such as hexacyanoferrate (III) (Healy and Christen, 1973; Shylapnikov and Karpeisky, 1969). In addition to trapping the glycine carbanion, lipoic acid forms an adduct which is processed further by the hy-
hydroxymethyltransferase to yield CO₂ and a methylamine lipoic acid adduct. The overall reaction is comparable to the initial reaction of the glycine cleavage system catalyzed by the P-protein component in which glycine is transferred to the lipoate moiety of the H-protein with subsequent loss of CO₂. In addition, the decarboxylation reaction catalyzed by serine hydroxymethyltransferase is identical with the lipoic acid-dependent decarboxylation of glycine by the P-protein alone. Therefore, both serine hydroxymethyltransferase and the P-protein component of the glycine cleavage complex will catalyze lipoic acid-dependent decarboxylation of glycine.

The decarboxylation of glycine catalyzed by the hydroxymethyltransferase proceeds by a pathway which requires prior dissociation of the pro-S-α-proton from glycine. The resulting carbanion then adds reductively to lipoic acid with stoichiometric release of CO₂ and a methylamine adduct of lipoic acid. In addition to establishing a 1:1 stoichiometry for CO₂ release and methylamine-lipoate production, we were also interested in demonstrating that the rate of the reaction obeyed saturation kinetics with respect to the concentration of substrates and was proportional to the concentration of enzymatically active hydroxymethyltransferase. Both of these criteria were met and the reaction was observed to proceed through a sequential mechanism, with all substrates binding prior to the release of any products.

A sequential mechanism has also been suggested for the decarboxylation of glycine by the P- and H-proteins of the glycine cleavage complex (Hiraga and Kikuchi, 1980; Fujisawa et al., 1979) and other pyridoxal 5'-phosphate-dependent decarboxylases which required carbanion addition prior to loss of CO₂ from the amino acid cosubstrates (Zaman et al., 1973; McGilvray and Morris, 1971; Krisnangkura and Sweeley, 1976). Since the addition of the glycine carbanion to lipoic acid in this system is mechanistically similar to the addition of glycine carbanions to acyl-CoA derivatives in other enzyme systems, we tested acetyl-CoA and succinyl-CoA as acceptors for the glycine carbanion in the presence of serine hydroxymethyltransferase. These acyl-CoA derivatives were not acceptors for the glycine carbanion in the presence of serine hydroxymethyltransferase. However, the inability to trap the carbanion with these derivatives may be due to the inability of the acyl-CoA to bind to the binary complex or a result of some stereoelectronic factors. In reactions where an amino acid carbanion is observed to condense with acyl-CoA derivatives, the enzymes selectively remove the pro-R proton or its equivalent (Zaman et al., 1973; Krisnangkura and Sweeley, 1976) whereas serine hydroxymethyltransferase selectively removes the pro-S proton from glycine (Schirch and Jenkins, 1964). Further evidence for carbanion stereoselectivity is provided by experiments which showed lipoic acid to be unable to react with the L-phenylalanine carbanion generated with serine hydroxymethyltransferase. Since the proton abstraction from L-phenylalanine by the hydroxymethyltransferase is equivalent to loss of the pro-R proton from glycine, this observation might suggest that the pro-R carbanion is not accessible for lipoic acid condensation.

Structural specificity for lipoic acid was tested for by substituting oxidized dithiothreitol in its place; however, only lipoic acid was active. The lack of activity may result from dithiothreitol not binding, differences in redox potential, or ring strain. Significantly, the uniqueness of lipoic acid as a substrate in the decarboxylation of glycine would imply a specific binding site for it on the hydroxymethyltransferase. Other evidence for a lipoic acid binding site was obtained from studies which revealed the ability of lipoic acid to intercept glycine carbanion intermediates formed during the cleavage of β-threo-phenylserine by the hydroxymethyltransferase. The fact that this reaction is inhibited by lipoic acid would suggest that it is able to complete with protons for the glycine carbanion intermediate. Secondly, the fact that the inhibition is competitive is consistent with lipoic acid and phenylserine competing for the same enzyme form. Both observations previously discussed would suggest that lipoic acid binds at some unique site on serine hydroxymethyltransferase.

Previous interpretations of serine and glycine catabolism have implied that separate mitochondrial pyridoxal 5'-phosphate-dependent enzymes are required to carry out the complete degradative pathway of serine to CO₂, NH₃, and methylenetetrahydrofolic acid within the mitochondrion. One enzyme, serine hydroxymethyltransferase, generates glycine and methylenetetrahydrofolic acid from serine; then, it releases the glycine to the P-protein, the second enzyme, which initiates the further breakdown to CO₂, NH₃, and a second mole of methylenetetrahydrofolic acid. This manuscript suggests that this complete mitochondrial catabolic process could be initiated by the serine hydroxymethyltransferase without the transfer of glycine to a different enzyme, thus, eliminating the requirement for two separate pyridoxal 5'-phosphate-containing enzymes to process serine in mitochondria. However, final assessment of the ability of serine hydroxymethyltransferase to function alone or in lieu of the P-protein awaits experiments coupling the hydroxymethyltransferase with purified H-protein of the glycine cleavage system. This is required since the H-protein containing lipoic acid is 1000-fold more effective in enhancing the glycine decarboxylase activity of the P-protein than lipoic acid alone (Hiraga and Kikuchi, 1980) and is the natural aminomethyl group carrier.

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