

Interaction between the Component Enzymes of the Glycine Decarboxylase Multienzyme Complex¹

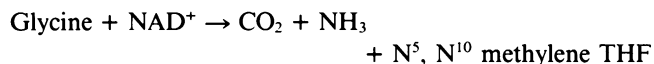
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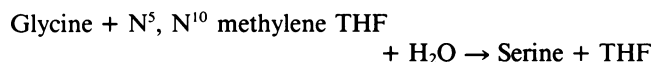
ABSTRACT

The glycine decarboxylase multienzyme complex comprises about one-third of the soluble protein of the matrix of pea (*Pisum sativum*) leaf mitochondria where it exists at a concentration of approximately 130 milligrams protein/milliliter. Under these conditions the complex is stable with an approximate subunit ratio of 2 P-protein dimers:27 H-protein monomers:9 T-protein monomers:1 L-protein dimer. When the complex is diluted it tends to dissociate into its component enzymes. This prevents the purification of the intact complex by gel filtration or ultracentrifugation. In the dissociated state the H-protein acts as a mobile cosubstrate that commutes between the other three enzymes and shows typical substrate kinetics. When the complex is reformed, the H-protein no longer acts as a substrate but as an integrated part of the enzyme complex.

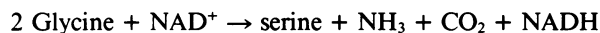
The glycine decarboxylase multienzyme complex (EC 2.1.2.10) is a key enzyme of the photorespiratory C-2 cycle of C₃ plants. The complex is located within the mitochondrial matrix where it catalyzes the conversion of glycine, NAD⁺, and THF into CO₂, NH₃, NADH, and N⁵, N¹⁰ methylene THF.



Mitochondria also contain serine hydroxymethyltransferase (EC 2.1.2.1), a matrix enzyme that catalyzes the conversion of glycine and methylene THF to serine.



The overall mitochondrial reaction is:



While glycine decarboxylase activity (often referred to as the

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² Abbreviations: C₃ photosynthesis, photosynthetic pathway where the first carboxylation reaction is catalyzed by ribulose 1,5-bisphosphate carboxylase/oxygenase; P-, H-, T-, and L-proteins, component proteins (subunits) of the glycine decarboxylase multienzyme complex; PLP, pyridoxal 5-phosphate; THF, tetrahydrofolate.

glycine cleavage system or glycine synthase) has been reported from the mitochondria of a broad range of plant and animal tissues (8) as well as the cytosol of a number of bacteria (10, 11), it is found at its highest level in the mitochondria of C₃ plant leaves. Indeed, glycine formed by the photorespiratory C-2 pathway is the predominant substrate oxidized by these mitochondria in illuminated leaf tissue (17).

The concentration of the complex in plant tissue is controlled by light. In etiolated leaves or nongreen tissues the amount of enzyme activity is low (1, 3, 6, 22) and following illumination of etiolated leaves the amount of enzyme activity increases about 10-fold (16, 22). The increase in activity results from a *de novo* synthesis of new proteins and the increase in protein synthesis is largely regulated at the transcriptional level (9). The time course for the increase in mRNA concentrations for the specific component proteins of the enzyme complex closely parallels the increase in enzyme activity in greening pea tissues (9, 12, 22).

The glycine decarboxylase complex consists of four different component proteins (2, 21). The 100 kD P-protein binds the PLP that forms the initial Schiff base with the α -amino group of glycine. The α -carboxyl of glycine is lost as CO₂ and the remaining methylamine moiety is passed to the lipoamide cofactor of the 13.9 kD H-protein. The lipoamide-bound methylamine group is shuttled to the 45 kD T-protein where the methylene carbon is transferred to THF to produce methylene THF and the amino nitrogen is released as NH₃. The last step of the reaction involves the oxidation of the resulting dihydrolipoamide of the H-protein by the 59 kD L-protein with the sequential reduction of FAD and NAD⁺.

Although substantial progress has been made in understanding the enzymology and molecular biology of the glycine decarboxylase complex from leaf tissue, little is known about the physical structure of the complex. We describe here a series of experiments designed to study this multienzyme complex and some of the unusual enzymological consequences of the unique interactions between the subunits.

MATERIALS AND METHODS

Pea (*Pisum sativum*) leaf mitochondria were isolated from young plants by differential centrifugation and purified on Percoll-polyvinylpyrrolidone gradients (5). The soluble enzymes were released from the mitochondria (800 mg protein) by three freeze-thaw cycles in 5 mM Mops, 5 mM Tris, 1 mM glycine, 1 mM EDTA, 1 mM octylglucoside, 2 mM β -mercaptoethanol, 20 μ M PLP (pH 7.2). After removal of membrane

fragments by ultracentrifugation, the resulting crude matrix extract was concentrated on an Amicon XM 300 membrane (14). The P-, H-, T-, and L-proteins of the glycine decarboxylase complex were isolated from the crude matrix extract by a combination of gel filtration and ion exchange chromatography as described earlier (2).

Glycine decarboxylase activity was assayed spectrophotometrically by measuring glycine-dependent NADH formation (2, 14) in a reaction medium containing 5 mM Mops, 5 mM Tris, 1 mM EDTA, 1 mM $MgCl_2$, 1 mM β -mercaptoethanol, 20 μ M PLP, 2 mM NAD^+ , and 0.8 mM THF (pH 7.2). The reactions were maintained under a stream of argon to assure anaerobic conditions in order to prevent the oxidation of THF. The reactions were initiated by the addition of glycine.

H-protein activity was assayed by the method of Motokawa and Kikuchi (13, 20). The reaction medium contained 0.1 M KPi (pH 7.4), 20 mM EDTA, 10 μ g yeast lipoamide dehydrogenase (EC 1.8.1.4), 2 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and 1.5 mM NADH in a final volume of 0.6 mL. The reaction was initiated with the addition of the equivalent of up to 15 μ g of purified H-protein. Thirty seconds was allowed for any reactions with the β -mercaptoethanol in the crude or purified H-protein preparation before monitoring the reaction spectrophotometrically at 412 nm. Multiple samples were often analyzed in 96 well microtiter plates and quantified on an ELISA plate reader. The reaction was linear for at least 5 min and the reaction rate was directly proportional to the amount of added H-protein.

P-protein activity was measured as the rate of glycine- $H^{14}CO_3$ exchange reaction in the presence of excess added H-protein (21). L-protein was measured as the lipoamide-dependent NADH oxidation (2). T-protein activity was estimated by the rate of glycine-dependent NAD^+ reduction in the presence of excess P-, L-, and H-proteins (2, 21, 22).

The amount of P-, H-, and T-protein in crude mitochondrial extracts was measured by an ELISA assay (22). Purified proteins were used as a standard and monospecific polyclonal antibodies against the P-, T-, and H-proteins were prepared in rabbits.

RESULTS AND DISCUSSION

Subunit Interactions within the Glycine Decarboxylase Complex

The physical structure of the glycine decarboxylase multienzyme complex has never been completely resolved. While the individual component enzymes of the complex can readily be separated, attempts to isolate an intact complex have met with only limited success (18, 19). Figure 1 shows the fractionation of the purified component enzymes and the concentrated matrix enzymes on identical sucrose density gradients. The individual subunits moved through the gradients in relation to their molecular weights. P-protein moved the furthest (Fig. 1A), H-protein moved the least (Fig. 1C), and L-protein migrated to an intermediate position (Fig. 1B). Because of the difficulty in assaying T-protein, this component enzyme was not analyzed. The concentrated matrix fraction was then separated on an identical gradient and each of the resulting fractions was analyzed for P-, H-, and L-protein activity as

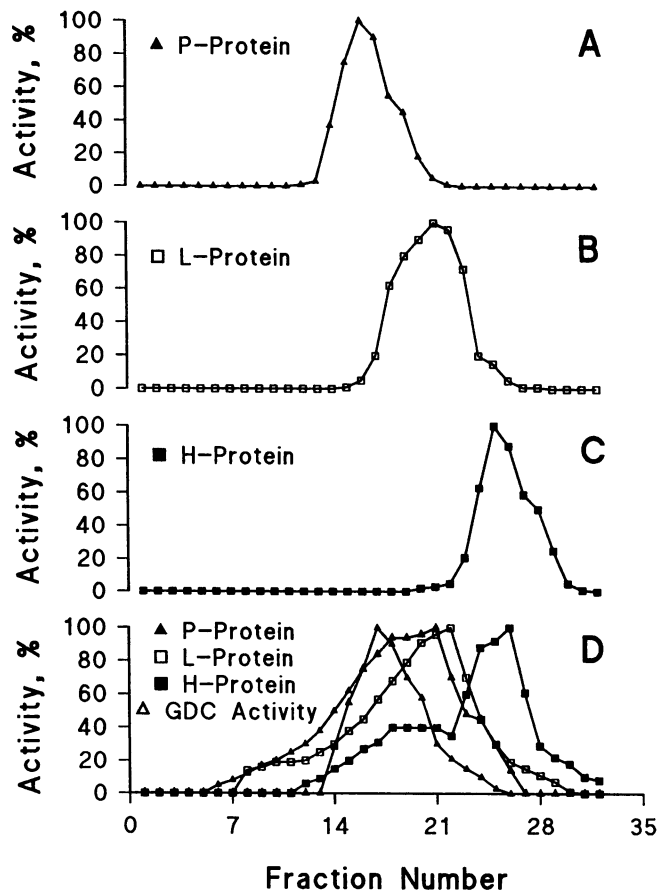


Figure 1. Separation of the matrix extract and the glycine decarboxylase component enzymes of a sucrose density gradient. Matrix extract (15 mg protein in a volume of 0.5 mL [D]) or the individual component enzymes (0.25 to 1.0 mg in 1.0 mL [A, B, C]) were loaded on a 10 to 30% sucrose density gradient that was centrifuged at 35,000 rpm for 16 h. The medium for the gradient was the same as used for the glycine decarboxylase assay. The samples were then separated into 1 mL fractions (from the bottom) and the amount of each enzyme and glycine-dependent NAD^+ reduction determined.

well as glycine decarboxylase activity which required the presence of all four subunits (Fig. 1D). The individual component enzymes of the glycine decarboxylase complex in the matrix extract moved through the gradient in a manner identical to the isolated proteins. The only exception was the H-protein where about 20% of the activity was found at higher sucrose density in the gradient than was observed with the purified H-protein. These results indicated that in the crude matrix extract the component enzymes of the glycine decarboxylase complex predominately behaved under these conditions as independent enzymes that moved through the gradient without any apparent interaction. A fraction of the large subunits was apparently bound to one or more of the large subunits and, as a result, migrated further into the gradient.

The only place where glycine decarboxylase activity was detected was in the center region of the gradient where the different component proteins fortuitously overlapped (Fig. 1D). Similar results have been noted when crude glycine decarboxylase preparations were applied to gel filtration col-

umns (2, 19). Both of these fractionation techniques separated the complex into its component proteins and only those fractions where the components overlapped showed glycine decarboxylase activity.

The ultracentrifugation and gel filtration analyses have been run under a broad range of conditions including the presence or absence of the substrates and cofactors (glycine, serine, PLP, NAD⁺, and THF), different pH values, varying salt concentrations, and with the inclusion of the divalent cations, Ca²⁺ and Mg²⁺. None of these alterations has increased the apparent stability of the complex.

While the results presented above suggest that there was limited interaction between the subunits of the glycine decarboxylase complex, other data suggested that the subunits do exist in a somewhat stable supermolecular complex (14). Each of the individual subunits of the glycine decarboxylase complex readily passed through an Amicon XM 300 (300 kD exclusion limit) ultrafiltration membrane and the smaller H- and T-proteins passed through a YM 100 membrane (100 kD cutoff) (Table I). This was consistent with the molecular masses for these component proteins estimated by gel filtration where the P-protein and L-protein behaved as dimers of 200 kD and 120 kD, respectively. However, when the components were presented together as a concentrated matrix extract, all of the glycine decarboxylase activity was retained along with all of the component enzymes (Table II, experiment 1). This indicated that all of the proteins involved in glycine decarboxylase activity were loosely associated into a complex within the matrix extract and that this complex had an apparent size higher than the 300 kD exclusion limit of these membranes. Although glycine decarboxylase activity was inhibited at high ionic strength (14), the complex was not dissociated with 0.2 M NaCl. The complex was also stable from pH 6.5 to 7.8 and at temperatures from 4°C to 20°C (Table II, experiment 2).

The complex appeared to be specific for the ratio of subunits present in the matrix extract. This was tested by adding extra H-protein and L-protein to a matrix extract. When this mixture of matrix extract and added H-protein and L-protein activities was fractionated on a XM 300 membrane, over 90% of the added activity passed through the membrane (Table III). Although it was not possible to determine if the added subunits failed to join the glycine decarboxylase complex in the matrix extract or joined the complex and displaced equal

Table I. Ultrafiltration of the Component Enzymes of the Glycine Decarboxylase Complex

The indicated amount of the component enzymes were individually forced through a XM 300 (for P- and L-proteins) or YM 100 (for H- and T-proteins) membrane and the protein that passed through the membrane was determined.

Protein	Monomer Molecular Mass	Amount Loaded	Amount Recovered	Passed
	<i>kD</i>	<i>mg protein</i>		<i>%</i>
H-Protein	13.7	0.91	0.86	95
T-Protein	45	0.49	0.45	92
L-Protein	59	0.61	0.56	91
P-Protein	100	1.42	1.15	81

Table 2. Ultrafiltration of Mitochondrial Extracts

The crude matrix extract was forced through a XM 300 membrane and the amount of glycine decarboxylase activity as well as the activity of the P-, H-, and L-proteins determined before (loaded) and after (retained) ultrafiltration (experiment 1). In experiment 2, the crude matrix extract was adjusted to the NaCl concentrations, pH, or temperature noted, the ultrafiltration performed, and the glycine decarboxylase activity determined before (loaded) and after (retained) ultrafiltration. All reactions were run at 4°C unless otherwise indicated. The initial protein concentration was 1.2 mg/mL.

Condition	Amount of Enzyme Activity	
	Loaded	Retained
	<i>μmol/min</i>	
Experiment 1, matrix extract	2.76	2.43
H-protein	1.24	1.18
L-protein	3.63	3.25
P-protein	0.29	0.27
Experiment 2, matrix extract		
+ 0.2 M NaCl	1.12	1.23
pH 6.5	1.08	1.05
pH 7.8	1.32	1.01
20°C	1.28	0.98

amounts of the same component protein, it is clear that the native complex must have had a fixed subunit stoichiometry and was not just held together by random associations between matrix proteins.

The fractionation experiments, therefore, suggested that the component proteins of the glycine decarboxylase complex interacted very weakly while the filtration experiments indicated that the complex was specific and fairly stable under a range of environmental conditions. This discrepancy was at least partially resolved by the experiment described in Figure 2. The matrix extract was diluted to a range of 0.01 to 0.25 mg protein/mL and subjected to ultrafiltration through a YM 100 membrane. The amount of H-protein that passed through the filter was then measured to determine how much of the complex had dissociated. When the protein concentration was at 0.01 mg/mL, nearly 90% of the H-protein passed through the filter. As the concentration of protein was increased a larger portion of the H-protein was retained by the filter until at a concentration of 0.25 mg/mL about 90% of the H-protein was retained. These results suggested that the stability of the complex was highly dependent on the concentration of the component proteins. At higher concentrations the complex was sufficiently stable to be completely retained on an ultrafiltration membrane. The separation of subunits which occurred during fractionation by gel filtration or ultracentrifugation may have reflected the combination of dynamic association-dissociation between the subunits and the fractionation of the dissociated subunits. Assuming that the interaction of the subunits is not reversed above the concentrations of protein measurable by this technique, the component proteins of glycine decarboxylase clearly formed a specific complex at the protein levels found within the mitochondrial matrix.

Table III. Ultrafiltration of Additional Subunits Added to the Matrix Extract

The indicated amounts of purified H-protein and L-protein were added to 10 mg of matrix extract (total volume 5 mL). This mixture was then fractionated on an Amicon XM 300 membrane and the amount of both activities that passed through the filters determined.

Subunit Added	Activity Added	Activity Passed	Passed
	$\mu\text{mol}/\text{min}$		%
+H-protein	1.63	1.47	90
+L-protein	4.13	3.78	92

Subunit Stoichiometry of the Glycine Decarboxylase Complex

In order to better understand the physical structure of the glycine decarboxylase complex *in situ*, the ratio of protein in crude mitochondrial matrix extract was determined. The amounts of P-, H-, L-, and T-proteins were measured by quantitative ELISA assays using the purified subunits as standards. The amount of each subunit in the matrix extract is shown in Table IV.

Given that the P- and L-protein were dimers and H- and T-protein were monomers (see below), the subunit ratio calculated for the complete complex was approximately 1 L-protein dimer:2 P-protein dimers:27 H-protein monomers:9 T-protein monomers.

Support for the biological significance of this subunit ratio came from experiments where the complex was reconstituted from different ratios of the purified subunits. The reaction was reconstituted with 0.1 nmol of P-protein dimer, 0.4 nmol of T-protein monomer, and 0.4 nmol of L-protein dimer and glycine-dependent NAD^+ reduction was measured with 0 to 2.5 nmol of H-protein monomer. Under these conditions, maximum glycine decarboxylase activity was achieved with approximately 1.5 nmol of H-protein (Fig. 3A). This represents a P-protein to H-protein ratio of 1:15. Decreasing the amount of P-protein to 0.05 nmol decreased the H-protein requirement to 0.75 nmol, again a 1:15 ratio (Fig. 3A). When

equivalent experiments were done holding three of the component proteins fixed and independently varying the amount of T-protein and L-protein, maximum activity was measured at a ratio of 4 T-protein monomers to 1 P-protein dimer (Fig. 3B) and 1 L-protein dimer to 1 P-protein dimer (Fig. 3C). The 1 L:1 P:15 H:4 T ratio determined to cause optimum activity was very close to the 1 L:2 P:27 H:9 T ratio measured by the ELISA assays. The 1 L:2 P:27 H:9 T ratio would suggest that the complete complex had a molecular mass of approximately 1,300 kD.

Role of the H-Protein in the Complex

The 13.9 kD H-protein serves a unique role in the complex in that its bound lipoamide acts as a substrate for the P-, T-, and L-proteins. It is a cosubstrate with glycine for the P-protein reactions, a cosubstrate with THF for the T-protein, and a cosubstrate with NAD for the L-protein. The glycine decarboxylation reaction was reconstituted from the purified P-, T-, and L-proteins and the activity measured with increasing concentrations of H-protein (Fig. 4). The ratio of P-, T-, and L-proteins was maintained constant at 2 P-protein dimers:8 T-protein monomers:1 L-protein dimer and the P-protein dimer concentration was varied from 0.0025 nM to 0.1 nM (note that these protein concentrations were much lower than those used in Fig. 3 and under these conditions the complex was dissociated). As the H-protein concentration in the reaction was increased from 0 to 0.5 μM , the resulting reaction rate showed typical saturation kinetics. At 0.0025 nM P-protein dimer the K_m for H-protein was 0.154 μM and the K_{cat} was 0.59 nmol/min, at 0.01 nM the K_m was 0.144 μM and the K_{cat} 2.15 nmol/min, and at 0.1 nM the K_m for H-protein was 0.255 μM and the K_{cat} was 6.99 nmol/min.

At the low concentrations of enzyme used in this experiment the complex had dissociated into its individual component proteins (see above) and the H-protein acted as a true substrate with an apparent K_m that was nearly independent of the concentration of the P-, T-, and L-proteins. The K_m values presented are complicated terms which are comprised of the K_m values for the interaction between the H-protein

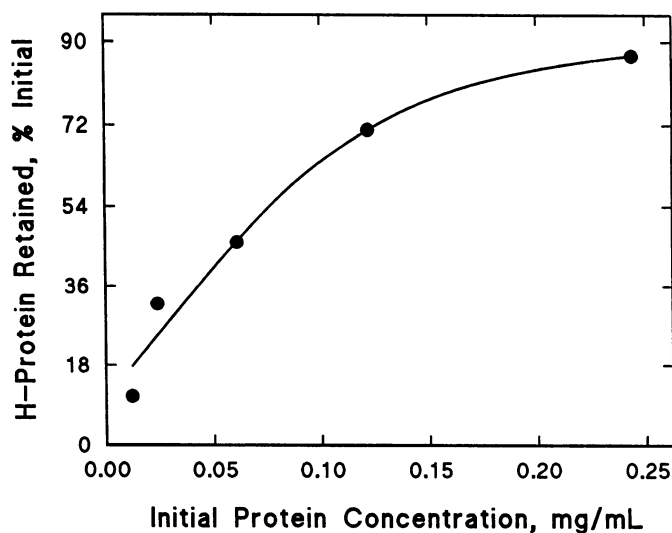


Figure 2. Effect of matrix extract concentration on the ability of H-protein to pass through a YM 100 ultrafiltration membrane. Crude matrix extract was diluted in assay buffer to the concentration indicated before 1 mL was filtered through a YM 100 membrane in a Amicon Centricon-100. The retentate (about 30 μL) was then assayed for H-protein activity.

Table IV. Amount of P-, H-, T-, and L-Protein in the Matrix Extract from Pea Leaf Mitochondria

The amounts of P-, H-, and T-proteins were determined by ELISA assays using the purified proteins as standards. The amount of L-protein was estimated by comparing the specific activity of the purified enzyme with that of the matrix extract. The values were measured three times and are presented as the percentage of the total protein \pm SE. The subunit ratio is calculated assuming that two P-protein dimers are present per complex and other component proteins are on an integral ratio relative to the P-protein. The matrix concentration of each component is calculated assuming a matrix protein concentration of 0.4 g/mL. The predicted subunit ratio is an approximation and any integral multiples of this ratio could comprise the intact complex.

	Component Protein			
	P	H	T	L
% of total protein	9.6 \pm 1.4	9.0 \pm 0.9	10.2 \pm 2.1	3.3 \pm 0.2
Subunit ratio	2	27	9	1
Subunit structure	Dimer	Monomer	Monomer	Dimer
Concentration in matrix, mM	0.19	2.62	0.91	0.11

and all three of the other proteins required for the glycine decarboxylase reactions, the P-, T-, and L-proteins. The limiting interaction in this case may have been between the P-protein and the H-protein. When this interaction was measured by the glycine-bicarbonate exchange reaction which only requires these two subunits, the H-protein again showed apparent K_m values of 0.15 to 0.20 μ M (data not presented). Both of these K_m values were substantially lower than the values estimated earlier for the pea H-protein by Walker and Oliver (21). This earlier estimate used an H-protein preparation that had employed a heat treatment step during the purification, while the H-protein used in this experiment, which was prepared by the technique of Bourguignon *et al.* (2), did not use the heat treatment. Recent evidence has shown that the heat treatment resulted in substantial denaturation of the protein and a decrease in the resulting enzyme activity (data not presented).

The results described above help explain one of the early anomalies noted with the glycine decarboxylase complex, the nonlinear response of the enzyme activity to increasing enzyme concentrations (14, 18). At low enzyme concentrations (less than 50 μ g crude matrix extract/mL or about 33 nM H-protein) the amount of activity was disproportionately low when compared to the rates measured at higher enzyme concentrations. Under the conditions where the activity was lower than predicted by extrapolation, our results show that the complex was dissociated. The addition of excess H-protein (Fig. 5) linearized the rates at the higher level. On the other hand, the addition of excess H-protein to the stable supermolecular complex that is present at the high matrix protein concentration had no effect on the rate of glycine-dependent NAD^+ reduction (2). This suggests that when the complex dissociated the H-protein went from being a catalytic component of a multienzyme complex to a substrate that was no longer present in excess of the amount of the catalytic sub-

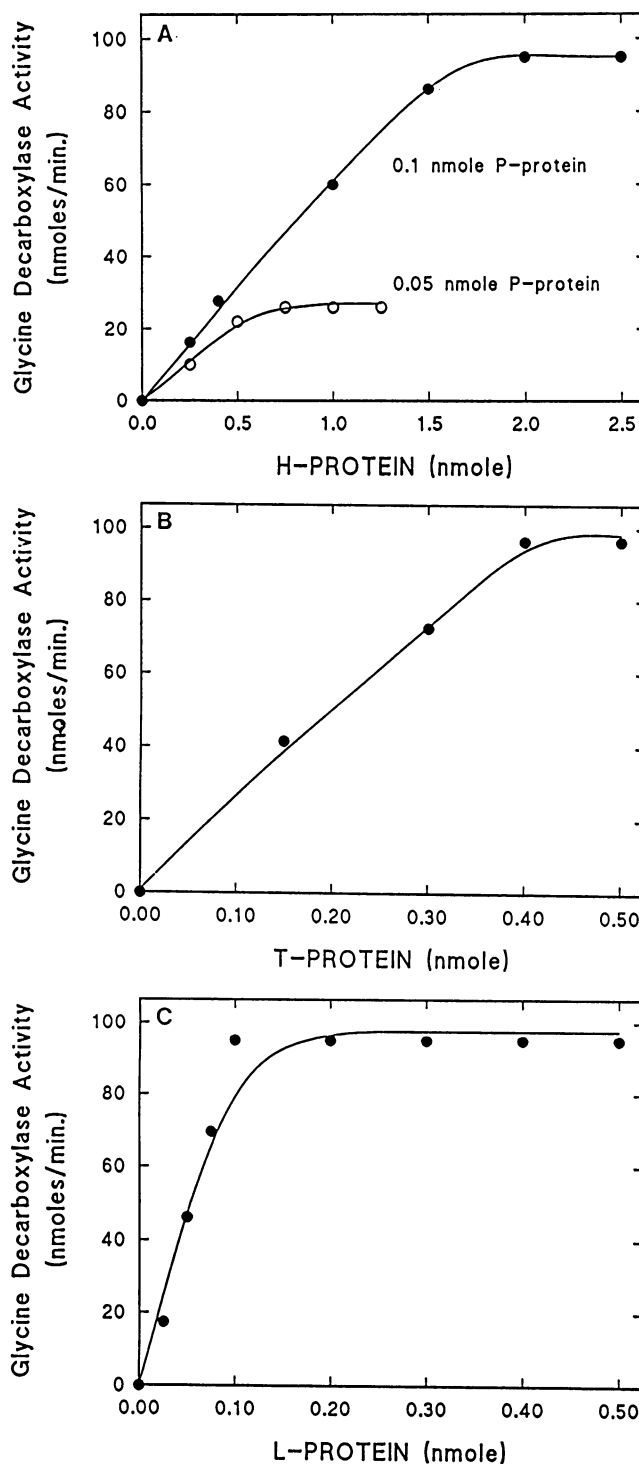


Figure 3. *In vitro* reconstitution of the glycine decarboxylase complex from different ratios of the purified component proteins. Glycine decarboxylase activity was measured as glycine-dependent $NADH$ formation in the presence of different amounts of the four component proteins. In each case three of the proteins were held constant at 0.1 nmol of P-protein dimers (solid circles in 3A) or 0.05 nmol P-protein dimers (open circles, A), 0.4 nmol of T-protein monomers, 0.4 nmol of L-protein dimers, and 1.5 nmol of H-protein monomers in a reaction volume of 0.4 mL and the fourth protein was varied. A, Amount of H-protein was varied from 0 to 2.5 nmol; B, T-protein varied from 0 to 0.5 nmol; C, L-protein was varied from 0 to 0.5 nmol.

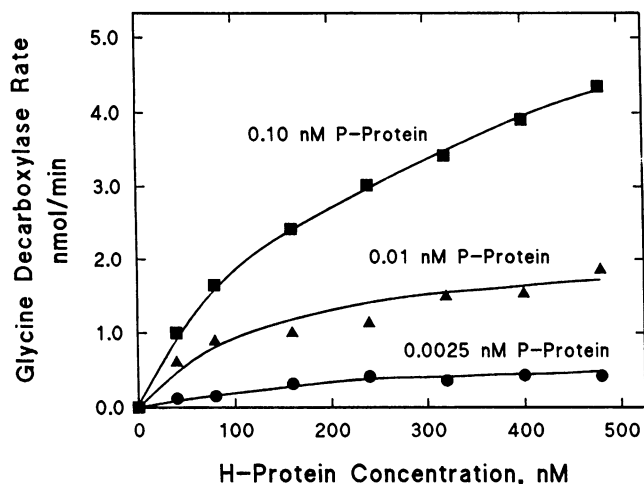


Figure 4. Effect of increasing concentrations of P-, L-, and T-proteins on the apparent K_m for H-protein. Purified P-, T-, and L-proteins were mixed at a ratio of 2 P-protein dimers:8 T-protein monomers:1 L-protein dimer. Concentrations of the mixture containing 0.0025 to 0.1 nM P-protein dimers were then titrated with increasing concentrations of H-protein and the resulting glycine decarboxylase rate measured.

units. The addition of exogenous H-protein elevated its concentration to substrate levels where it could efficiently act to shuttle reaction intermediates between the other three component proteins.

An alternative explanation, that the additional H-protein forced the reassociation of the intact complex was disproved by measuring the ability of the diluted complex to pass through the Amicon XM 300 ultrafiltration membrane. With matrix extract diluted to 25 $\mu\text{g}/\text{mL}$, 33% of the glycine decarboxylase activity was retained after filtration. When the same experiment was repeated with 1.0 μM H-protein (14 $\mu\text{g}/\text{mL}$), 37% of the glycine decarboxylase activity was retained by the filter. The presence of excess H-protein, therefore, did not cause the reassociation of the component enzymes back into a multienzyme complex.

The subunit structure of the H-protein is still undetermined. Gel filtration in high salt on Superose 12, Ultragel AcA 44, and Sephadex G-100 all showed that H-protein eluted with an apparent molecular mass equivalent to 30 kD. Similarly, the protein was retained on an Amicon YM 30 ultrafiltration membrane with a 30 kD cutoff. These data suggest that under nondenaturing conditions the H-protein existed as a dimer. Under low salt conditions using the same technique the H-protein ran as a tetramer; however, this could have resulted from interactions between the protein and chromatography matrix at low ionic strength. When the protein was analyzed on a nondenaturing polyacrylamide gradient gel (high salt conditions), the molecular mass was estimated at 15 kD, suggesting that the monomeric form was predominant. Cross-linking experiments have supported the idea that the H-protein is a monomer by failing to show any evidence for specific cross-linking between H-protein subunits.

CONCLUSIONS

Glycine decarboxylase is a true multienzyme complex. At lower protein concentrations the complex dissociates into its

four component enzymes. Given the concentration of the P-protein, H-protein, T-protein, and L-protein, within the mitochondrial matrix (Table IV), however, the enzyme clearly exists as a complex in its normal state. The enzymes of the glycine decarboxylase complex comprise about 32% of the total soluble protein in the mitochondria. Given a matrix protein concentration of 400 mg/mL (4), the concentration of the glycine decarboxylase complex within the mitochondrial matrix was about 130 mg/mL . This is much higher than the concentrations of protein required in Figure 2 for reassociation of the complex. The enzyme should also form a complex at the much lower concentrations present in the mitochondria from sources other than C_3 plant leaves.

The dissociated form of the complex that occurs at low protein concentrations has enzymological properties that are very different from those of the intact complex. Because the H-protein, which contains the lipoamide cofactor that acts to shuttle reaction intermediates between the P-, T-, and L-proteins, was no longer in intimate contact with the three larger subunits following dissociation, the H-protein must commute between these three proteins and in its different chemical forms acts as mobile cosubstrates for the reactions catalyzed by the larger proteins. As a result, at low concentrations of the glycine decarboxylase complex the reaction rate was much lower (limited by the rate of H-protein diffusion) and was stimulated by the addition of extra H-protein. At higher concentrations of glycine decarboxylase, under conditions where the enzyme remained in a complex, the H-protein did not need to shuttle reaction intermediates between active sites and additional H-protein did not stimulate the reaction rate. This rapid reversible association between subunits is unusual for multienzyme complexes and makes glycine decarboxylase an interesting model system for studying protein-protein interactions.

The enzyme complex from animal mitochondria is also unstable and during normal isolation procedures it dissociates

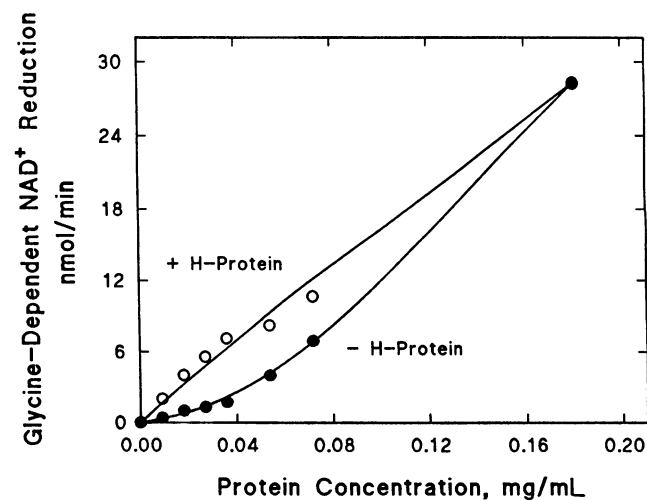


Figure 5. Effect of added H-protein on the glycine decarboxylase reaction. The rate of glycine decarboxylase reaction at increasing enzyme concentrations was measured in the presence and absence of 1.0 μM H-protein.

into the four component enzymes. Hiraga and Kikuchi (7) have shown that the P-protein and H-protein can form a complex that is stable through column chromatography. A similar complex has been observed with the plant enzyme (21). The T-protein from animal mitochondria can also bind to the H-protein and this complex has been isolated (15). The L-protein from pea mitochondria has been reported to immunoprecipitate with the H-protein suggesting that some interaction occurs between these two proteins (22). This information suggests a possible structure for the complex. The central core of the complex would be comprised of the 27 polypeptide chains of H-protein. The larger subunits, two dimers of the P-protein, nine monomers of T-protein, and one dimer of L-protein would bind to the H-protein core and thus form the intact complex. No direct interactions between the P-, T-, and L-proteins have yet been noted so it is likely that they are held to the complex solely by their interaction with the H-protein. Due to our current inability to isolate this complex in an intact form new techniques are necessary before this model can be tested.

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