EVIDENCE FOR THE PRESENCE OF A PROTEIN-BOUND INTERMEDIATE IN THE CLEAVAGE AND THE SYNTHESIS OF GLYCINE*

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An intermediate bound to a protein called hydrogen carrier protein was isolated from an incubation of the glycine metabolizing system with glycine or methylene tetrahydrofolate followed by filtration on Sephadex G-100. The possible mechanism of the metabolism of glycine was discussed.

Extracts of acetone-dried rat liver mitochondria or cell free extracts of Arthrobacter globiformis grown on glycine catalyzed the synthesis and the cleavage of glycine which may be represented by the following single equation (1-3):

glycine + H_4folate $\rightleftharpoons$ 5,10-CH_2-H_4folate + NH_3 + CO_2 + 2H

The extracts also catalyzed the exchange of the glycine carboxyl group with bicarbonate which was regarded as a partial reaction of the glycine cleavage (2,3).

Two protein components, tentatively called "carboxylation enzyme" and "hydrogen carrier protein", respectively, have been obtained from the mitochondrial extracts which, when combined, catalyzed the above three reactions under proper conditions (4).

Abbreviation: NEM, N-ethylmaleimide.

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Carboxylation enzyme fraction exhibited at least the following three enzyme activities (4): 1) a pyridoxal phosphate-containing enzyme, 2) a lipoamide dehydrogenase-like enzyme, and 3) serine hydroxymethylase. Hydrogen carrier protein was found to be homogeneous on disc electrophoresis, and its principal role was considered to be the transfer of electrons between nucleotides and glycine, acting as hydrogen donor in the glycine synthesis and receiving hydrogen in the glycine cleavage (4).

This investigation is concerned with the further study of the properties of hydrogen carrier protein in an attempt to elucidate the mechanism of the glycine metabolism.

MATERIALS AND METHODS

Carboxylation enzyme and hydrogen carrier protein were isolated from the extracts of acetone-dried rat liver mitochondria as described previously (4).

The protein components were incubated either with ^{14}C-glycine (10 mM, 10^6 cpm/μmole), pyridoxal phosphate (0.25 mM), GSH (10 mM), and Tris-HCl buffer, pH 8.0 (50 mM) in air or with ^{14}C-formaldehyde (2 mM, 10^6 cpm/μmole), H_4folate (2 mM), NH_4Cl (10 mM), hydroxylamine (4×10^{-5} M), and Tris-HCl buffer, pH 8.0 (50 mM) under the atmosphere of nitrogen at 37° for 30 min. These reaction mixtures were designed to facilitate the accumulation of intermediates, preventing the over-all reaction from proceeding to completion by depleting H_4folate (in the cleavage of glycine) or adding hydroxylamine (in the synthesis of glycine) (cf. Ref. 1, 4). The reaction mixture (1 ml) was then chilled in an ice bath and applied to a column of Sephadex G-100 (1.5 x 40 cm) previously equilibrated with 0.02 M Tris-HCl buffer, pH 8.0. Elution was carried out with the same buffer at 4° and 2-ml fractions were collected. A 0.5-ml sample from each fraction was taken onto a

planchet, evaporated to dryness with an infrared lamp and the radioactivity was counted by a windowless gas flow counter. Protein was estimated by the method of Lowry et al. (5).

RESULTS

When glycine-2-^{14}C was incubated with the protein components, two protein fractions associated with radioactivity were obtained by gel filtration of the reaction mixture on Sephadex G-100. As shown in Fig. 1A, the first and the second radioactive peaks were well separated from the larger third peak that contained the bulk

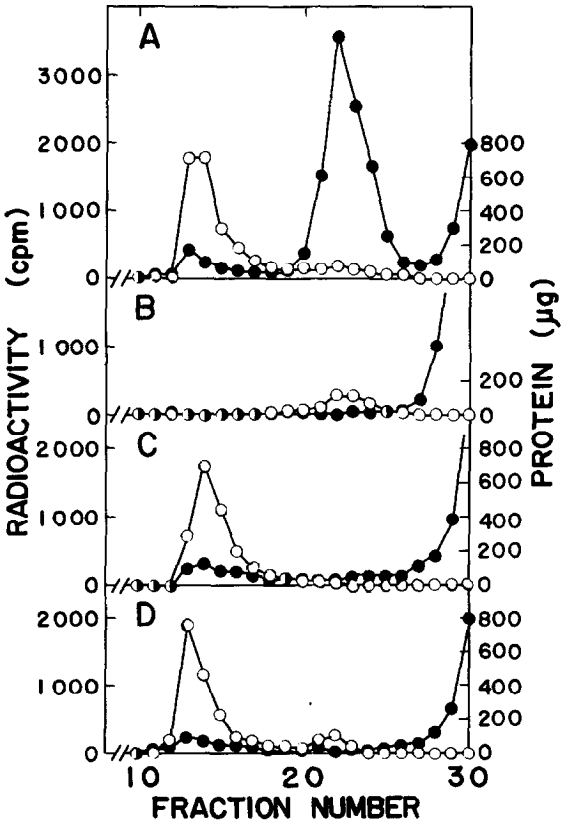


Fig. 1. Isolation of radioactive hydrogen carrier protein complex from ^{14}C-glycine and the protein. ●——●, radioactivity; o——o, protein. A, complete with glycine-2-^{14}C; B, with glycine-2-^{14}C, minus carboxylation enzyme; B, with glycine-2-^{14}C, minus hydrogen carrier protein; D, complete with glycine-1-^{14}C.

of the radioactive glycine. When carboxylation enzyme was omitted from the reaction mixture, no radioactivity associated with the protein fractions could be observed (Fig. 1B). When hydrogen carrier protein was omitted, no radioactive second peak was obtained (Fig. 1C). From the elution patterns depicted in Fig. 1, it is apparent that the first protein peak corresponds to carboxylation enzyme and the second one corresponds to hydrogen carrier protein. When glycine-1-^{14}C was used as substrate instead of glycine-2-^{14}C, there was no radioactive second peak as shown in Fig. 1D. These results demonstrate the complex formation of the glycine α-carbon with hydrogen carrier protein during the cleavage of glycine. The formation of this complex was reduced when H_4folate was added to the reaction mixture (Table I). This is in accord with the observations that H_4folate increased the decarboxylation of glycine whereas decreased the exchange reaction (4). Like P_2 fraction of the glycine cleavage system obtained from Peptococcus glycinophilus (6), hydrogen carrier protein was revealed to contain a functional disulfied bridge per molecule which could be reduced by NADH to a disulfhydryl group by the catalytic action of lipoamide dehydrogenase (4). Masking of this sulfhydryl groups with NEM resulted in yielding no measurable complex as shown in Table II.

TABLE I EFFECT OF TETRAHYDROFOLATE ON THE FORMATION OF RADIOACTIVE HYDROGEN CARRIER PROTEIN WITH GLYCINE-2-^{14}C

System	Complex formed (cpm)
Control	1980
Plus H_4folate (1 mM)	46

TABLE II EFFECT OF NEM ON THE FORMATION OF RADIOACTIVE HYDROGEN CARRIER PROTEIN WITH GLYCINE-2-^{14}C

Hydrogen carrier protein	Complex formed (cpm)
Disulfide form	1308
Disulfhydryl form	0

For the preparation of the NEM-treated disulfhydryl form of hydrogen carrier protein, disulfide form was incubated with pig heart lipoamide dehydrogenase and 3 mM NADH at 37° for 10 min, and then incubated with 5 mM NEM at 37° for 15 min. Excess NEM was removed by dialysis and an aliquot of the protein solution was used for the reaction. The NEM-treated disulfide form of hydrogen carrier protein was prepared as described above but without NADH.

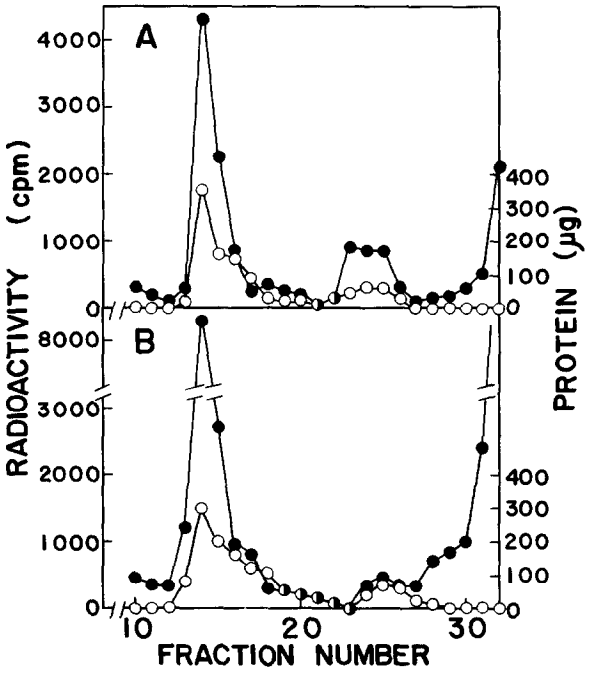


Fig. 2. Isolation of radioactive hydrogen carrier protein from ^{14}C-formaldehyde, H_4folate, NH_4Cl, and the protein. ●——●, radioactivity; o——o, protein. A, complete: B, without NH_4Cl.

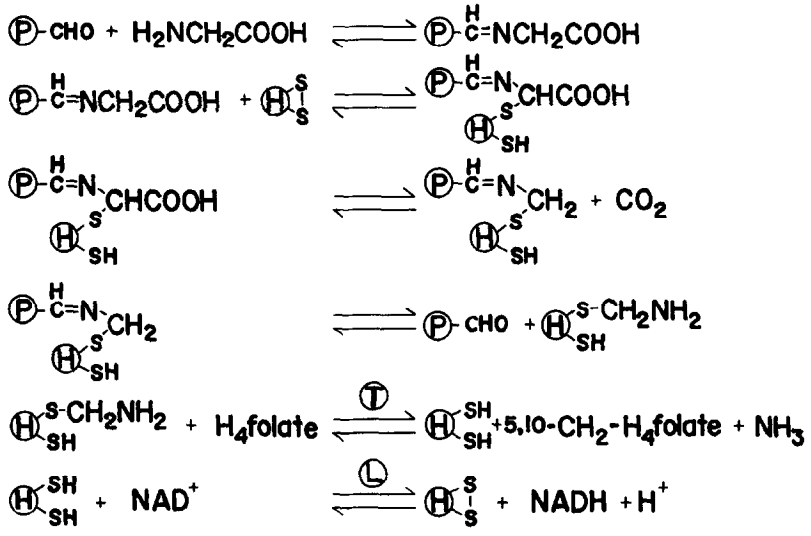
The intermediary complex was also found to be present in the reverse reaction of the glycine cleavage, i.e., the glycine syn-

thesis from 5,10-CH_2-H_4folate, NH_4^+, and CO_2. As shown in Fig. 2A, radioactive hydrogen carrier protein was detected when ^{14}C-formaldehyde, H_4folate, and NH_4^+ were used as substrates. Omittion of carboxylation enzyme from the reaction mixture produced no radioactive complex. When NH_4^+ was omitted, the amount of radioactivity associated with hydrogen carrier protein was reduced as shown in Fig. 2B.

DISCUSSION

The present studies have demonstrated that in the glycine cleavage reaction, the glycine α-carbon is combined with a protein called hydrogen carrier protein before conversion to 5,10-CH_2-H_4folate and that the complex can be isolated under the condition where the reaction could not proceed to completion. The formation of the complex was inhibited by the treatment of the disulfhydryl form of hydrogen carrier protein with NEM. This finding would support the view that the glycine α-carbon is attached to a -SH group of hydrogen carrier protein as in the pyruvate dehydrogenase system where pyruvate reacts with a -SH group of lipoic acid after decarboxylation (7).

In the previous works, we discussed the reversibility of the glycine cleavage (2,4). The formation of the intermediary complex from 5,10-CH_2-H_4folate gives another evidence for the reversibility of the reaction. The formation of the complex from 5,10-CH_2-H_4folate seemed to require the addition of NH_4^+ as shown in Fig. 2. This would indicate that the material attached to hydrogen carrier protein is in the form of $-CH_2NH_2$ and that carboxylation enzyme exhibits activity of an enzyme which catalyzes bond formation between the C and N atoms. Perhaps this activity corresponds to that of P_4 fraction obtained from Peptococcus glycinophilus (8).



Scheme I. A tentative reaction scheme for the cleavage and the synthesis of glycine. Ⓟ -CHO, pyridoxal phosphate-requiring protein; Ⓗ, hydrogen carrier protein; Ⓛ, lipoamide dehydrogenase-like protein; Ⓣ, H_4folate-requiring protein.

On the basis of the data presented in this and previous communications (1-4, 9) as well as the results obtained from the studies with Peptococcus glycinophilus (5,8,10), the hypothetical reaction scheme as shown in Scheme I would explain the metabolism of glycine. In the glycine cleavage reaction, glycine first forms a Shiff base with the protein-bound pyridoxal phosphate. The first radioactive peak shown in Fig. 1 possibly represents this complex. Next, the disulfide form of hydrogen carrier protein combines with this complex, and in this step the glycine carboxyl group is released as CO_2. The pyridoxal phosphate-containing protein is then detached from the complex and the resulting complex of hydrogen carrier protein with the glycine α-carbon having amino group liberates 5,10-CH_2-H_4folate and ammonia under the action of another enzyme which requires H_4-folate as cofactor. Finally, the resulting disulfhydryl form of

hydrogen carrier protein is oxidized back to the disulfide form by lipoamide dehydrogenase-like enzyme and hydrogen is transferred to NAD^+. The glycine synthesis from 5,10-CH_2-H_4folate, ammonia, and CO_2 may follow the reverse of this reaction process.

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