GLYCINE SYNTHESIS BY EXTRACTS OF ACETONE POWDER OF RAT-LIVER MITOCHONDRIA*

Takao Sato, Yutaro Motokawa, Hideo Kochi and Goro Kikuchi

Department of Biochemistry, Tohoku University School of Medicine
Sendai, Japan

Received June 29, 1967

Kawasaki, Sato and Kikuchi (1966) reported that liver mitochondria catalyzed the following new reaction: L-serine + CO_2 + NH_3 (+2H) $\longrightarrow$ 2 glycine. β-Carbon of serine and bicarbonate carbon were incorporated specifically into the α-carbon and the carboxyl carbon of glycine, respectively, at a stoichiometric ratio of one. We observed that the activity was widely distributed in various species of mammals and birds, not only in liver but also in kidney, although the activity in kidney was far lower (about 15%) than in liver. The enzymes catalyzing the overall reaction have been solubilized from acetone powder of rat-liver mitochondria.

The mitochondrial acetone powder was extracted with 10 times the weight of 0.02 M Tris-HCl buffer of pH 8.0. The extracts were usually dialyzed for 3 hrs against the same buffer; the buffer being renewed every hr. Longer dialysis was avoided because of the instability of enzymes. Other experimental methods

Abbreviation: THFA, tetrahydrofolic acid.

* This investigation was supported in part by U.S. Public Health Service Research Grant AM-08016-03, from the National Institute of Arthritis and Metabolic Diseases (G.K).

Table I. Requirements for glycine synthesis from L-serine, $NaH^{14}CO_3$ and NH_4Cl

Reaction system	^{14}C-Amino acids (cpm)
Complete system	12,500
Minus THFA	590
Minus dithiothreitol	5,460
Minus THFA and dithiothreitol	245
Minus pyridoxal phosphate	8,340
Minus NH_4Cl	1,140

Complete system contained, in 3.0 ml: 2.3 mg protein N of dialyzed enzyme preparation, 150 µmoles of Tris-HCl buffer (pH 8.0), 20 µmoles of L-serine, 20 µmoles of NH_4Cl, 60 µmoles of $NaH^{14}CO_3$ (0.02 mc/mmole), 5 µmoles of $MgCl_2$, 1 µmole of THFA, 0.5 µmole of pyridoxal phosphate and 30 umoles of dithiothreitol. Reactions were carried out for 1 hr at 37° in N_2 gas.

were similar to those described previously (Kawasaki, Sato and Kikuchi, 1966).

With the 3 hrs dialyzed enzyme preparation, the ^{14}C-glycine synthesis from serine, $NaH^{14}CO_3$ and NH_4Cl required the addition of THFA (Table I). Other ^{14}C-amino acids than glycine and serine were not detected in the ^{14}C-products and more than 90% of the total ^{14}C fixed into amino acids were recovered in glycine. The glycine synthesis was increased more than three-fold by the addition of dithiothreitol. Mercaptoethanol, cysteine and glutathione failed to increase the activity. The glycine synthesis was inhibited by very low concentrations of sodium arsenite and the inhibition was completely reversed by the addition of dithiothreitol. The yield of glycine was increased by about 30% by the addition of pyridoxal phosphate. The reaction was inhibited about 90% by 3 mM cycloserine, and almost completely by 1 mM hydroxylamine or semicarbazide. Without

NH_4Cl, the yield of ^{14}C-glycine was very small; NH_4Cl could not be replaced by glutamine, asparagine, glutamate, aspartate and other amino acids tested. ATP had no effect on the reaction; rather a slight inhibition was observed in some cases after the addition of ATP. Under the employed reaction conditions, practically no ^{14}C-glycine was obtained when serine was replaced by other amino acids, including glycine (cf. Table IV).

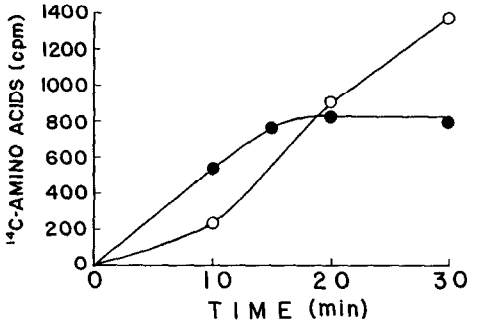

Fig. 1. Time courses of glycine synthesis with methylene-THFA and L-serine as one-carbon donor, respectively. Reaction mixtures in serine series (o——o) contained, in 3.0 ml: 2.9 mg protein N of enzyme, 5 μmoles of L-serine, 5 μmoles of THFA, 30 μmoles of $NaH^{14}CO_3$, and other additions as employed in the complete system of Table I. Reaction mixtures in methylene-THFA series (●——●) were similar to those in serine series except that serine and THFA were replaced by 5 μmoles of methylene-THFA. Reactions were carried out at 37° in N_2 gas.

Chemically synthesized methylene-THFA (cf. Osborn, Talbert and Huennekens, 1960), however, was found to effectively replace serine in the glycine synthesis. The reaction rate was rather higher when methylene-THFA was employed as substrate (Fig. 1). The enzyme preparation was revealed to contain a high activity of serine hydroxymethyltransferase (EC 2.1.2.1). These would

Table II. Effects of various factors on glycine synthesis from methylene-THFA, $NaH^{14}CO_3$ and NH_4Cl

Reaction system	^{14}C-Amino acids (cpm)
Standard system	1,341
Minus dithiothreitol	311
Minus dithiothreitol, plus NADH (0.33 mM)	1,596
Minus dithiothreitol, plus NADH and arsenite (1 mM)	0
Minus pyridoxal phosphate	881

Standard system contained, in 3.0 ml: 3.0 mg protein N of enzyme, 150 μmoles of Tris-HCl buffer (pH 8.0), 20 μmoles of NH_4Cl, 30 μmoles of $NaH^{14}CO_3$ (0.02 mc/mmole), 5 μmoles of $MgCl_2$, 30 μmoles of dithiothreitol, 0.5 μmole of pyridoxal phosphate and 5 μmoles of methylene-THFA. Reactions were carried out for 20 min. at 37° in N_2 gas.

indicate that methylene-THFA acted as the direct one carbon donor for the glycine synthesis. The reaction with methylene-THFA required the addition of NADH or dithiothreitol for the full activity, and the reaction was strongly inhibited by arsenite (Table II). The reaction was stimulated by addition of pyridoxal phosphate, and inhibited by about 30% by 3 mM cycloserine. Without addition of NH_4Cl, the yield of ^{14}C-glycine was negligible. Neither ^{14}C-glycolate nor glyoxylate was obtained after the incubation either in the presence or absence of NH_4Cl.

The enzyme preparation also catalyzed the decarboxylation of glycine (Table III). The decarboxylation occurred significantly only aerobically and the reaction required THFA. The reaction was stimulated by pyridoxal phosphate, and inhibited by hydroxylamine, cycloserine and by arsenite. The Km for glycine was 1.7 mM. The decarboxylation of glycine was accompanied by

Table III. Glycine cleavage

Expt. No.	Reaction system and substrate	$^{14}CO_2$ (cpm)	^{14}C-Serine (cpm)
1	Complete (Glycine-1-^{14}C)	3,280	
	Minus THFA	1,740	
	Minus THFA and dithiothreitol	583	
	Minus pyridoxal phosphate	2,580	
	Minus pyridoxal phosphate, plus hydroxylamine (1 mM)	675	
2	Complete (glycine-1-^{14}C)	3,380	2,870
	Complete (glycine-2-^{14}C)	25	6,000

Complete system in Expt. 1 contained, in 3.0 ml: 2.5 mg protein N of enzyme, 150 µmoles of Tris-HCl buffer (pH 8.0), 20 µmoles of ^{14}C-glycine (0.01 mc/mmole), 5 µmoles of $MgCl_2$, 30 µmoles of dithiothreitol. Complete systems in Expt. 2 were similar to those in Expt. 1, except that 2.3 mg protein N of enzyme were used. Reactions were carried out for 1 hr at 37° in air.

the concomitant synthesis of serine, and the data obtained appeared to be consistent with the following equation: 2 glycine $\longrightarrow$ serine + CO_2 + NH_3 + 2H. This type of reaction had been reported to occur in avian and rat-liver (Richert et al., 1962), plants (McConnell, 1964; Sinha and Cossins, 1964; Cossins and Sinha, 1966) and in Peptococcus glycinophilus (Sagers and Gunsalus, 1961) and other microorganisms (cf. Morris, 1963). The enzyme preparation from Peptococcus glycinophilus was also shown to strongly catalyze the exchange reaction between glycine and CO_2. A pyridoxal phosphate enzyme and possibly a dithiol enzyme were revealed to be responsible to this exchange, but THFA was not required for the exchange reaction (Klein and Sagers, 1966, 1966a, 1967, 1967a; Baginsky and Huennekens, 1966). In similarity to the bacterial enzyme, our mitochondrial enzyme preparation also catalyzed the exchange of glycine and $^{14}CO_2$

Table IV. ^{14}C incorporation from $NaH^{14}CO_3$ into glycine under various reaction conditions

Non-labelled substrate	Omission	^{14}C-Amino acids (cpm)	
		In air	In N_2 gas
Glycine	THFA	13,900	756
Glycine	None	1,740	94
L-Serine	None	11,000	12,350

2.5 mg protein N of enzyme, 60 μmoles of $NaH^{14}CO_3$ (0.02 mc/mmole) and 20 μmoles of glycine or serine were employed. Also 20 μmoles of NH_4Cl were added in the serine system. Other additions were similar to those for Expt. 1 in Table III. Reactions were carried out for 1 hr at 37° in air or N_2 gas.

(Table IV). The significant exchange, however, was observed only in air, probably because the reaction mixtures contained excess amounts of dithiothreitol. The rate of exchange was greatly reduced when THFA was added. These data strongly suggest that the mechanism by which the α-carbon of glycine is converted to one carbon unit in liver mitochondria may be essentially similar to the mechanism of glycine cleavage in Peptococcus glycinophilus (cf. Baginsky and Huennekens, 1966). The rate of exchange between glycine and $^{14}CO_2$ in the absence of THFA was found to be very close to that of the synthesis of ^{14}C-glycine from serine, $NaH^{14}CO_3$ and NH_4Cl.

The experimental results so far obtained point to the possible reversibility of the glycine synthesis and the glycine cleavage in liver mitochondria. It is noteworthy that, while the glycine synthesis with methylene-THFA as substrate should involve the CO_2 fixation and the ammonia fixation, apparently no exogenous supply of energy was necessary for the synthesis.

A pyridoxal phosphate enzyme may participate in some step of the reaction and possibly a dithiol enzyme is responsible to the reduction of an intermediate complex. NADH may be the physiological H-donor for reducing the suspected dithiol enzyme. It seems likely that the ammonia fixation precedes the CO_2 fixation in the reaction sequence of glycine synthesis when we consider that the exchange of glycine and $^{14}CO_2$ was suppressed by the addition of THFA, while THFA was required for the $^{14}CO_2$ formation from glycine-1-^{14}C in the absence of bicarbonate added. Thus the whole figure of the glycine synthesis appears to be accounted for by assuming the reverse of the cleavage mechanism, although the final conclusion must await further investigations.

The authors are grateful to Mr. Narikazu Sato for his skillful technical assistance.

REFERENCES

Baginsky, M.L. and Huennekens, F.M., Biochem. Biophys. Res. Commun., 23, 600 (1966).
Cossins, E.A. and Sinha, S.K., Biochem. J., 101, 542 (1966).
Kawasaki, H., Sato, T. and Kikuchi, G., Biochem. Biophys. Res. Commun., 23, 227 (1966).
Klein, S.M. and Sagers, R.D., J. Biol. Chem., 241, 197 (1966).
Klein, S.M. and Sagers, R.D., J. Biol. Chem., 241, 206 (1966a).
Klein, S.M. and Sagers, R.D., J. Biol. Chem., 242, 297 (1967).
Klein, S.M. and Sagers, R.D., J. Biol. Chem., 242, 301 (1967a).
McConnell, W.B., Can. J. Biochem., 42, 1293 (1964).
Morris, J.G., J. gen. Microbiol., 32, 167 (1963).
Osborn, M.J., Talbert, P.T., and Huennekens, F.M., J. Am. Chem. Soc., 82, 4921 (1960).
Richert, D.A., Amberg, R. and Wilson, M., J. Biol. Chem., 237, 99 (1962).
Sagers, R.D. and Gunsalus, I.C., J. Bacteriol., 81, 541 (1961).
Sinha, S.K. and Cossins, E.A., Biochem. J., 93, 27 (1964).