A NEW REACTION FOR GLYCINE BIOSYNTHESIS*

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It was reported that the α-carbon of glycine was converted to β-carbon of serine in rat liver (Nakada et al., 1955), avian liver (Richert et al., 1962), plants (McConnell, 1964; Sinha and Cossins, 1964) and in bacteria (cf. Morris, 1965). The conversion was shown also by a feeding experiment with rats (Arnstein and Neuberger, 1956). Richert et al. (1962), on the basis of analysis of the reaction products with avian-liver preparations, suggested the following equation:

$$2 \text{ glycine} + H_2O \longrightarrow CO_2 + NH_3 + \text{serine} + 2H$$

In the course of studies to elucidate the detailed mechanism of the reaction, we have found evidence for the reverse reaction in rat liver homogenates and in mitochondria as well, although further experiments are needed to estabish the mechanism of the synthetic process.

Rat liver mitochondria were prepared according to the method of Schneider and Hogeboom (1950). Reactions were carried out in Warburg manometer flasks in air or in N_2 gas. When necessary, keto- and organic acids were analyzed for radioactivity as

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described previously (Okuyama et al., 1965). Amino acids in the deproteinized solutions were collected by a column of Dowex 50(H^+), then eluted with 2N NH_4OH, concentrated to dryness, and subjected to successive one dimensional paper chromatography, first with tert.-butanol-methylethylketone-water-dimethylamine (40:40:20:4, by vol.), then with methanol-water-pyridine (80:20:4, by vol.) (Redfield, 1953), locating each amino acid by radioautography and with the ninhydrin reagent. Methods of Kruger (1949) and of Frisell et al. (1954) were used to determine the amounts of glycine and serine, respectively. Degradation of ^{14}C-glycine by ninhydrin was performed in citrate buffer; CO_2 liberated was trapped in 10% KOH and converted to $BaCO_3$, and formaldehyde formed was isolated with carrier as dimedon derivative. Radioactivities of reaction products were expressed in terms of counts per min at infinite thinness, after appropriate corrections. The gas-flow counter used counted approx. 10,000 counts per min per μmole of ^{14}C-sample of 0.01 mc/mmole.

As shown in Table I, when intact mitochondria were incubated with L-serine, NH_4Cl and $NaH^{14}CO_3$, radioactive carbon was incorporated into glycine and serine under aerobic and anaerobic conditions. Other radioactive amino acids found consisted mainly of aspartic and glutamic acids; and yields of these acidic amino acids were larger when reactions were run aerobically. Keto- and organic acid fractions did not contain appreciable radioactivities. Boiling of the mitochondria resulted in complete loss of the activity.

In the standard systems in Table I, the amounts of glycine found after the reaction were 0.47 and 0.87 μmole in aerobic and anaerobic conditions, respectively, while the amount of endogenous glycine was 0.15 μmole. Decrease in serine was small, and speci-

Table I. Radioactive Amino Acids formed from L-Serine, NH_4Cl and $NaH^{14}CO_3$

Gas phase	Reaction system	Amino acids (counts/min)		
		Glycine	Serine	Others
Air	Standard	3,156	3,500	3,327
	Minus NH_4Cl	1,401	1,212	1,103
	Minus serine	210	192	109
	Minus NAD^+ and pyridoxal phosphate	2,283	2,930	2,651
N_2	Standard	5,103	10,159	1,068
	Minus NH_4Cl	2,544	5,091	539
	Minus serine	700	604	194
	Minus NAD^+ and pyridoxal phosphate	3,180	6,583	574

Standard systems contained, in 3.0 ml: 1 ml of mitochondrial suspension (5.2 mg protein nitrogen), 125 µmoles of phosphate buffer (pH 7.4), 20 µmoles of L-serine, 20 µmoles of NH_4Cl, 30 µmoles of $NaH^{14}CO_3$ (0.02 mc/mmole), 5 µmoles of $MgCl_2$, 0.5 µmole of NAD^+, 0.5 µmole of pyridoxal phosphate, and 0.25 M sucrose. Reactions were carried out for 1 hr at 37°.

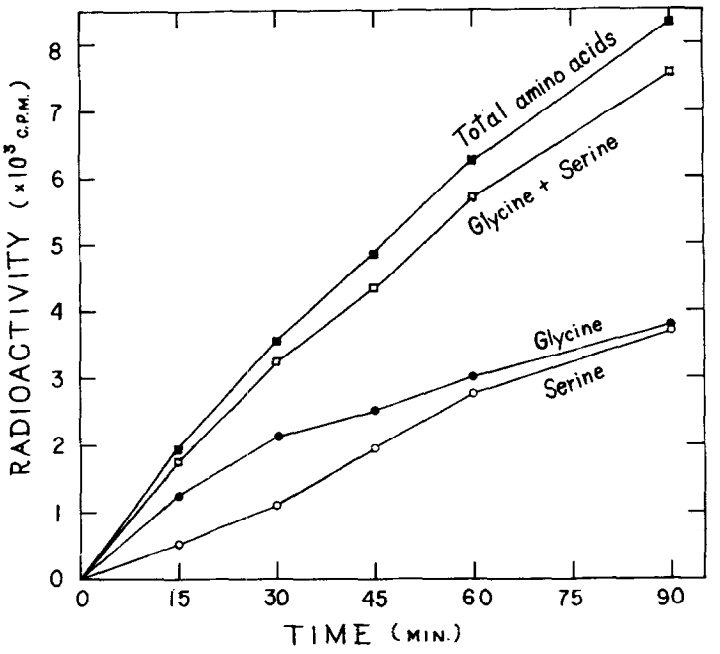

Fig. 1. Incorporation of the radioactive carbon from $NaH^{14}CO_3$ into amino acids. Reaction system was same as the "standard system minus NAD^+ and pyridoxal phosphate" in Table I, except that 5.9 mg protein nitrogen of mitochondria were used. Reactions were carried out at 37° in N_2 gas.

fic radioactivity of glycine was more than fifteen times higher than those of serine, indicating that glycine was labelled first. Results of a time study, shown in Fig. 1, also indicate the earlier labelling of glycine. The subsequent formation of radioactive serine under the anaerobic conditions as for Fig. 1 may be the result merely of the exchange reaction between the ^{14}C-glycine formed and the non-labelled serine added, catalyzed by serine hydroxymethyltransferase (EC 2.1.2.1), since we have observed that, when reactions were carried out anaerobically with ^{14}C-glycine as the sole substrate, rates of ^{14}C-serine formation were very low, in agreement with the observation by Richert et al (1962) with avian-liver preparations. When NH_4Cl was omitted from reaction mixtures, the labelling of glycine and serine was reduced significantly (TABLE I), suggesting a possible involvement of an amination or transamination reaction in the observed synthesis of ^{14}C-glycine. Also small amounts of ^{14}C-glycine were formed without added serine. These results may be due to the endogenous supply of substrates for we used relatively large amounts of mitochondria in these experiments.

Klein and Sagers (1965) reported an exchange of glycine-1-^{14}C and bicarbonate catalyzed by components from Peptococcus glycinophilus. In the mitochondrial system, however, the yield of ^{14}C-glycine was far less when serine was replaced by glycine as shown in Table II. Other amino acids tested were also far less effective than serine in yielding ^{14}C-glycine and other ^{14}C-amino acids. These results would eliminate the possibility that the observed ^{14}C-incorporation into glycine is caused by a preliminary cleavage of serine to glycine and a C_1 unit, followed by an exchange between glycine and $^{14}CO_2$, and would indicate a specific role of serine in this reaction.

Table II. Comparison of Effects of Various L-Amino Acids in yielding ^{14}C-glycine from ^{14}C-bicarbonate

Amino acids	^{14}C-amino acids found (counts/min)		
	Total	Glycine	Serine
Serine	17,507	7,350	7,960
Glycine	1,911	1,475	135
Threonine	3,763	2,265	1,010
Histidine	3,383	1,180	930
Glutamic acid	3,654	2,432	958
Aspartic acid	3,083	1,675	1,070

Reaction mixtures contained, in 3 ml: 30 μmoles of $NaH^{14}CO_3$ (0.02 mc/mmole) 5.7 mg protein nitrogen of mitochondria, 20 μ moles of respective L-amino acids, and other additions as for Fig. 1. Reactions were carried out for 1 hr at 37° in N_2 gas.

Serine-3-^{14}C also yielded ^{14}C-glycine as shown in Table III. The results of a combination experiment with the labelled and non-labelled substrates revealed that either combination yielded ^{14}C-glycine of apparently the same specific radioactivity. This would indicate that both of β-carbon of serine and bicarbonate carbon contributed at a stoichiometric ratio of one to the synthesis of glycine in our reaction system. Furthermore, the degradation experiments, shown in Table III, indicated that bicarbonate was incorporated only into the carboxyl carbon of glycine, while the β-carbon of serine was incorporated almost exclusively into the α-carbon of glycine. If we assume that 2 molecules of glycine are formed from 1 molecule of serine and 1 molecule of CO_2, the specific radioactivity of the ^{14}C-glycine formed can be expected to be 0.01 mc/mmole, or 10,000 counts min/μmole, since the specific radioactivity of both the labelled substrates employed was 0.02 mc/mmole, or 20,000 counts/min/μmole. However, the specific radioactivities of ^{14}C-glycine found were approx. 7,800 counts/min; the lower specific radioactivities than the expected are probably due, in part, to the presence of endogeneous non-labelled substrates

Supporting this view, the yield of glycine was not reduced when bicarbonate was omitted from the reaction system, although the specific radioactivity of glycine obtained without addition of bicarbonate was far lower than those obtained in the presence of bicarbonate added. The results in Table III also suggest that considerable

Table III. Formation of Radioactive Glycine from $NaH^{14}CO_3$ or L-Serine-3-^{14}C

Labelled substrate	Glycine found		Distribution of ^{14}C in glycine (%)	
	(μmole)	(counts/min /μmole)	α-carbon	Carboxyl carbon
$NaH^{14}CO_3$	0.83	7,780	2	98
Serine-3-^{14}C	0.68	7,750	94*	6*
Minus $NaHCO_3$	0.82	3,030		

* Glycine samples obtained in both systems with and without $NaHCO_3$ added were combined and degraded.

Reaction mixtures contained, in 3.0 ml: 1 ml of mitochondrial suspension (4.5 mg protein nitrogen), 5 μmoles of $MgCl_2$, 20 μmoles of NH_4Cl, 20 μmoles of labelled or non-labelled L-serine, 30 μ moles of labelled or non-labelled $NaHCO_3$ and 0.25 M sucrose. Specific radioactivity of either ^{14}C-substrates was 0.02 mc/mmole. Reactions were carried out for 1 hr at 37° in N_2 gas.

parts of serine-3-^{14}C were cleaved to give non-labelled glycine and labelled C_1 unit, independently of the glycine synthesis being coupled with CO_2 fixation— the C_1 unit thus formed may be consumed by other ways, including a possible formation of $^{14}CO_2$.

The data presented above would suggest the occurrence in rat liver mitochondria of a new reaction for glycine synthesis which consists in the formation of 2 moles of glycine from one mole each of serine, NH_3 and CO_2, and which may represent a physiological pathway of glycine biosynthesis. Further, the reaction is also unique as a new type of CO_2 fixation reaction.

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