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Glycine Formation *via* Threonine and Serine Aldolase

Its Interrelation with the Pyruvate Formate Lyase Pathway of One-carbon Unit Synthesis in *Clostridium kluyveri*

Kurt A. JUNGERMANN, Wilhelm SCHMIDT, F. Hans KIRCHNIAWY, Eckhard H. RUPPRECHT, and Rudolf K. THAUER

Biochemisches Institut der Universität, Freiburg i. Br.

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^{14}C-labeling experiments with growing *Clostridium kluyveri* have shown that two pathways each are operative for glycine and one-carbon unit synthesis: about two-thirds of the cell's glycine is formed from threonine and one third from serine; serine simultaneously yields about a quarter of the cell's C_1-units, while the remaining three-quarters are derived from CO_2 *via* the pyruvate carboxyl group.

The key enzymes, threonine aldolase and pyruvate formate lyase as well as serine aldolase interrelating glycine and C_1-unit synthesis, could be demonstrated in cell-free lysates in activities sufficient to account for the anabolic requirements during growth. The substrate specificity, cofactor requirements and K_m values indicate that threonine aldolase and serine aldolase are two separate, constitutive and pyridoxal phosphate dependent enzymes.

In *Clostridium kluyveri* C_1-units are predominantly synthesized from the pyruvate carboxyl group *via* pyruvate formate lyase and only to a minor degree from the serine hydroxymethyl group *via* serine aldolase [1—3]. Two reasons for the predominance of the pyruvate pathway may be envisaged: (a) the organism has a much larger requirement for C_1-units than for glycine, and/or (b) glycine is mainly synthesized from a metabolite other than serine.

Both explanations are valid for *C. kluyveri*; for the first explanation evidence has already been presented [2]; in this paper it will be reported that glycine is mainly formed from threonine *via* threonine aldolase and only to a smaller extent from serine *via* a separate serine aldolase.

MATERIALS AND METHODS

Chemicals and Enzymes

All chemicals were reagent grade. Enzymes and coenzymes were obtained from Boehringer Mannheim GmbH (Mannheim, Germany). ^{14}C-labeled substrates were purchased from the Radiochemical Centre (Amersham).

Enzymes. Lysozyme (EC 3.2.1.17); deoxyribonuclease or DNase (EC 3.1.4.5); L-serine hydroxymethyltransferase or serine aldolase (EC 2.1.2.1); L-threonine acetaldehydelyase or threonine aldolase (EC 4.1.2.5); pyruvate formate lyase; formyltetrahydrofolate synthetase (EC 6.3.4.3); alcohol dehydrogenase (EC 1.1.1.1); phosphate acetyltransferase or phosphotransacetylase (EC 2.3.1.8); lactate dehydrogenase (EC 1.1.1.27); acetaldehyde dehydrogenase, CoA acylating (EC 1.2.1.10); L-serine dehydratase (EC 4.2.1.13); NADH oxidase (EC 1.6.99).

d,l-Tetrahydrofolic acid was prepared from *d,l*-folic acid as described by Hatefi *et al.* [4]. L-[3-^{14}C]-Phosphoserine was synthesized by modifying the method of Neuhaus and Korkes [5] to microscale: 0.1 mC L-[3-^{14}C]serine (23.4 μmoles) and 21 mg L-serine (200 μmoles) were dissolved in 4.4 ml water. 2.2 ml of this 50 mM solution were taken to dryness in a pear shaped flask at a rotatory evaporator. Then 0.1 ml of freshly prepared monochlorophosphoric acid [5] (780 μmoles) was added to the serine crystals; the mixture was stirred with a glass rod. Then another 0.1 ml monochlorophosphoric acid (780 μmoles) was added and the mixture was heated in a waterbath at 60°. On occasional stirring with the glass rod gas bubbles (HCl) were evolved. After 4 h 0.5 ml 1 N HCl was added; the solution was heated in a boiling water bath for 20 min in order to hydrolyze the byproduct polyphosphate. This reaction mixture was cooled to room temperature, applied to a Dowex-50 W-X 8-H^+ column (35 cm long, 1 cm diameter) and chromatographed with 1 N HCl at a flow rate of 20 ml/h. 2.5 ml fractions were collected: inorganic phosphate was eluted in fractions 12—14, L-[3-^{14}C]phosphoserine in fractions 17—20 and L-[3-^{14}C]serine in fractions 35—40. Phosphoserine was obtained in 75 % yield (radioactivity), serine was recovered in 23 % yield. The phosphoserine and serine fractions were evaporated to dryness and dissolved in water to give 50 mM solutions. The purity of phosphoserine was checked by electrophoresis (40 mM ammonium acetate pH 8.0; 700 V/35 cm; 20 mA; 90 min) and paperchromatography (*n*-butanol—acetic acid—water, 35:10:25).

^{14}C-Labeling Techniques

The following methods used in this study have been described previously: (a) the growth of radioactive cultures of *Clostridium kluyveri* [2], (b) the determination of the concentration [6] and the specific radioactivities [2] of the growth substrates bicarbonate and acetate, (c) the measurement of the incorporation of ^{14}C-labeled substrates into whole cells [2], (d) the isolation and the determination of the specific radioactivities [2,7] of the purines, the methionine-*S*-methyl group and the protein amino acids.

The concentration of the respective substrate amino acid was followed during growth by taking 2 ml samples from the medium at the desired time intervals: the cells were separated by centrifugation; the supernatant was made alkaline with 5 N KOH (50 µl/1 ml), evaporated to dryness and taken up in the original volume of water; the supplemented amino acid, from which no other amino acids were formed in the medium, was then assayed with the ninhydrin method [8].

For the determination of the specific radioactivities of the supplemented amino acids 10 ml of medium was acidified with 1 ml 50 % trichloroacetic acid and passed over a 1.5 g moist weight Dowex-2-formate column; from the effluent the amino acids were isolated by paper electrophoresis (1700 V/35 cm, 35 mA, 2 h; $HCOOH-CH_3COOH-H_2O$, 25:75:1000) and assayed as described [7].

Radioactivities were determined in a Tri Carb Liquid Scintillation Spectrometer 3380 using Bray scintillator [9] for neutral and acidic and a 4 % (w/v) Aerosil Bray scintillator for alkaline solutions [10].

Enzyme Assays

Cell-free lysates were prepared from ethanol-acetate-bicarbonate grown [6] cells by incubation with lysozyme and DNase at 37° under H_2 for 30 min and centrifugation at $40000 \times g$ [11]. Dowex-2-acetate treated lysates were obtained as reported [3]. All enzyme assays were carried out at 37°.

Threonine aldolase was assayed anaerobically by coupling with alcohol dehydrogenase: 3 ml cuvettes, lightpath 1 cm, were filled with the assay mixture, closed with a soft rubber stopper, evacuated and filled with the desired gas. After lifting the stopper the lysates were added against a stream of argon. The cuvettes were again closed, evacuated and filled with gas. The reaction was started by injection of 50—100 µl of an evacuated substrate solution. The change in absorbance was measured with a recording Photometer Eppendorf.

Serine aldolase was tested with L-[3-^{14}C]serine as the substrate according to Taylor and Weissbach [12].

Pyruvate formate lyase was tested with [1-^{14}C]-pyruvate as the substrate; the product [^{14}C]formate was separated by isoionic exchange chromatography [13] as described earlier [3].

Formyltetrahydrofolate synthetase was measured with the method of Rabinowitz and Pricer [14].

RESULTS

The object of this investigation was to study the synthesis of glycine and its interrelation to one-carbon unit formation in *Clostridium kluyveri*. The organism was grown on standard ethanol-acetate-bicarbonate [6] media and on standard media supplemented with glycine, L-serine, L-threonine, L-serine plus formate, and glycine plus formate, respectively. Each of the various carbon substrates was labeled in turn.

^{14}C-LABELLED EXPERIMENTS

The Determination of n-*Values*

If the carbon pools are distinct, non-mixing and have constant specific radioactivities and if the cell mass has multiplied about one hundred times during the growth experiment, the number of carbon atoms in a cell component or the percentage in any position derived from a given ^{14}C-labeled precursor is indicated by the *n*-values:

$$n = \frac{\text{specific radioactivity of the cell constituent}}{\text{specific radioactivity of the substrate}}$$

Condition 1, distinct carbon pools with constant specific radioactivities, was met in all experiments (Table 1); there was practically no transfer of $^{14}CO_2$ to the acetate pool or to any one of the added amino acids, nor could any significant conversion of ^{14}C-labeled amino acids to CO_2 or acetate be detected. Of the supplemented carbon substrates L-serine and L-threonine were degraded by growing cultures while glycine and formate were not (Table 1). Degradation of L-serine must have occurred *via* serine dehydratase to pyruvate as indicated by the assimilation of L-[3-^{14}C]serine into pyruvate (alanine, threonine) (Table 2, 3-S) and the dilution of $^{14}CO_2$-fixation into pyruvate by L-serine (Table 2, C_S).

The Synthesis of Glycine

The labeling pattern [7,15] of glycine ($C_1 = CO_2$; C_2 = acetate-C_1) is compatible with its formation both from serine ($C_1 = CO_2$; C_2 = acetate-C_1; C_3 = acetate-C_2) *via* serine aldolase and from threonine (C_1 and $C_4 = CO_2$; C_2 = acetate$-C_1$; C_3 = acetate-C_2) *via* threonine aldolase.

When *Clostridium kluyveri* was grown on $^{14}CO_2$ labeled media glycine was found to have an *n*-value of 0.98 (Table 2, C). If L-serine were the sole precursor the *n*-value of glycine should approach zero in cells grown in serine supplemented cultures. However, the glycine *n*-value was decreased to only 0.67 (Table 2,

Table 1. *Specific radioactivities and concentrations of growth substrates at the start (S) of the subculture and at the end (E) of the main culture*

The label is designated with C for $^{14}CO_2$, 2-EA for [2-^{14}C]ethanol/acetate, 3-S for L-[3-^{14}C]serine and 2-G for [2-^{14}C]glycine; the unlabeled supplement is denoted by subscripts with G for glycine, S for L-serine, T for L-threonine and F for formate

Expt. No.	Labeled substrate/ supplement to standard medium	Specific radioactivities (Concentrations)									
		Bicarbonate		Glycine		Serine		Threonine		Acetate	Formate
		S	E	S	E	S	E	S	E	E	E
		decompositions × min^{-1} × μmoles^{-1} (mM)									
C	$^{14}CO_2$/ —		84000 (22.6)	—	—	—	—	—	—	150 (63.5)	—
C_G	$^{14}CO_2$/glycine	86600 (30.0)	85000 (23.5)	— (1.98)	<100 (1.92)	—	—	—	—	120	—
C_S	$^{14}CO_2$/L-serine		83500	—	—	— (5.00)	<100 (2.70)	—	—	95	—
C_T	$^{14}CO_2$/L-threonine		82000	—	—	—	—	— (4.95)	<100 (3.45)	140	—
3-S	L-[3-^{14}C]serine	—	0	—	—	29600 (5.00)	28600 (2.80)	—	—	515	—
3-S_F	L-[3-^{14}C]serine + formate	—	0	—	—		28800 (2.64)	—	—	545	750 (1.96)
2-G	[2-^{14}C]glycine	—	0	106000 (1.98)	100000 (1.89)	—	—	—	—	<100	—
2-G_F	[2-^{14}C]glycine + formate	—	0		102000 (1.93)	—	—	—	—	<100	2100 (1.92)

Table 2. *The synthesis of glycine and one-carbon units in growing* Clostridium kluyveri

The designation of the experiments is described in Table 1

Expt.	Supplement to standard medium (Concn.)		Labeled substrate	Glycine	Serine	Threonine	Alanine (pyruvate)	Purines $C_2 = C_8$	Methionine *S*-methyl-
		mM		n = dis. × min^{-1} × μmole cell constituent^{-1} / dis. × min^{-1} × μmole labeled precursor^{-1}					
C	—		$^{14}CO_2$	0.98	1.18	1.92	1.00	0.78	0.54
C_S	L-serine	5	$^{14}CO_2$	0.52 (0.67)[a]	0.06	1.53 (1.70)[a]	0.78 (1.00)[a]	0.55 (0.71)[a]	0.17 (0.22)[a]
C_G	glycine	2	$^{14}CO_2$	0.02	—	1.96	1.08	0.90	0.86
C_T	L-threonine	5	$^{14}CO_2$	0.10	1.08	0.13	0.98	0.94	0.80
2-EA	—		[2-^{14}C]ethanol [2-^{14}C]acetate	—	—	—	—	0.26	0.41
3-S	L-serine	5	[3-^{14}C]serine	0.05	0.95	0.43	0.44	0.27	0.65
3-S_F	L-serine formate	5 2	[3-^{14}C]serine	0.06	0.90	0.40	0.41	0.17	0.62
2-G	glycine	2	[2-^{14}C]glycine	1.00	0.47	0	0	0	0.05
2-G_F	glycine formate	2 2	[2-^{14}C]glycine	1.00	0.69	0	0	0	0.01

[a] As $^{14}CO_2$ is solely assimilated *via* pyruvate, the n-values have been corrected for [^{14}C]-pyruvate dilution by L-serine.

C_S). As serine was taken up by the growing cells, this finding may indicate (a) that the glycine carboxyl group exchanges with CO_2 or (b) that glycine is predominantly synthesized from threonine. A glycine-$^{14}CO_2$ exchange is excluded by the observation that exogenous glycine is incorporated unlabeled into protein (Table 2, C_G). It is concluded therefore that even in the presence of exogenous serine only 33 % of the cell's glycine is synthesized from serine.

Glycine isolated from cells grown on media labeled with $^{14}CO_2$ and supplemented with L-threonine had an n-value of 0.1 (Table 2, C_T) indicating that glycine was solely formed from threonine. This implicates that the serine cleavage to glycine was not operative in the presence of threonine.

On the contrary, formation of serine was observed from glycine and a C_1-unit when [2-^{14}C]glycine was supplied in the medium (Table 2, 2-G and 2-G_F).

The Synthesis of One Carbon Units

For the study of C_1-unit formation the n-values of the $C_2 = C_8$ positions of the purines and of the methionine-*S*-methyl group were determined. L-[3-^{14}C] Serine was found to be a precursor of C_1-units (Table 2, 3-S and 3-S_F). This is regarded as evidence for the earlier assumption [2] that the methyl group of acetate is incorporated into the C_1-units *via* serine (Table 2, 2-EA).

[2-^{14}C]Glycine, a precursor of C_1-units in *Escherichia coli* [16], was not converted to C_1-units in *C. kluyveri* (Table 2, 2-G and 2-G_F).

In the presence of glycine or of threonine serine aldolase was operative in the direction of serine formation. Serine could no longer serve as a C_1-donor, consequently nearly all of the cell's one-carbon units were derived from CO_2 (Table 2, C_G and C_T).

ENZYMATIC STUDIES

The labeling experiments have indicated that glycine is synthesized both from serine and threonine presumably *via* serine and threonine aldolase. These enzymes were to be demonstrated in cell-free extracts.

Threonine Aldolase

Assay. Glycine and acetaldehyde are formed from threonine by the action of threonine aldolase. Its activity was measured by following the oxidation of NADH with the acetaldehyde formed in the presence of alcohol dehydrogenase. In crude extracts of *Clostridium kluyveri*, however, two enzymatic activities interfere with this test: (a) a highly active NADH oxidase and (b) a catabolic CoA dependent acetaldehyde dehydrogenase, which competes for acetaldehyde with the concomitant formation of NADH. These interfering activities were excluded using anaerobic conditions and Dowex-2-acetate treated, *i. e.* CoA free, extracts.

Specificity. Threonine aldolase was, within the 4 diastereomers, specific for L-threonine; a K_m of 1.2 mM was obtained from Lineweaver-Burk plots. D-Threonine and DL-Allothreonine were not utilized (Table 3). L-Serine seemed to compete with L-threonine, however, no exact measurements could be performed because of interfering serine dehydratase and lactate dehydrogenase activities present in the lysates.

Table 3. *Threonine aldolase activity in cell-free lysates of* Clostridium kluyveri

Complete: Tris acetate pH 7.5, 100 mM; NADH, 0.5 mM; alcohol dehydrogenase, 10 U; pyridoxal phosphate (PLP), 0.2 mM; L-threonine, 12.5 mM; protein, Dowex-2-acetate lysate, 1 mg; water to 2 ml; gas phase: argon. Additions: tetrahydrofolate (FH_4) (+ mercaptoethanol), 0.1 mM (10 mM); potassium, sodium, ammonium acetate, 100 mM; ethylene diaminetetraacetate (EDTA), 1 mM; D-threonine, 12.5 mM; DL-allo-threonine, 12.5 mM

System	CH_3CHO formed
	nmoles/15 min
Complete	193
minus PLP	182
plus FH_4	181
plus K^+	154
plus Na^+	140
plus NH_4^+	158
plus EDTA	180
plus D-Thr	185
plus DL-allo-Thr	181
minus L-Thr, plus DL-allo-Thr	< 2.5
minus L-Thr, plus D-Thr	< 2.5

Cofactor Requirements. Dowex-2-acetate treated extracts were active in the absence of any cofactors. Neither pyridoxal phosphate nor tetrahydrofolic acid had a stimulatory effect (Table 3). Yet, threonine aldolase seems to be a pyridoxal phosphate dependent enzyme. 48 h dialysis against 40 mM cysteine in a 10 mM phosphate buffer containing 20 mM mercaptoethanol resulted in a 70 % loss of activity which could partially (50 %) be restored by preincubation with pyridoxal phosphate and pretreatment of the extract with 0.5 mM hydroxylamine totally inactivated the enzymatic activity.

pH Optimum and Ion Requirement. Threonine aldolase had a broad optimum near pH 7. Monovalent cations, frequently stimulatory with pyridoxal phosphate enzymes, were inhibitory or without effect at 100 mM concentrations. Also divalent cations were not required as EDTA had no effect on the reaction (Table 3).

Activity in Glycine Grown Cells. When the growth medium was supplemented with 2 mM glycine, glycine was not synthesized from threonine nor was threonine synthesized from glycine (Table 2, C_G and 2-G). The finding that threonine aldolase was equally active in cells from standard and from glycine supplemented media indicates that threonine aldolase is not regulated at the genetic level and that it functions *in vivo* only in the direction of glycine synthesis.

Serine Aldolase

Specificity. Neither D-serine nor L-threonine had any effect on the formation of [^{14}C]methylenetetra-

Table 4. *Serine aldolase activity in cell-free lysates of* Clostridium kluyveri

Complete: Tris Cl pH 8.5, 60 mM; pyridoxal phosphate (PLP), 0.2 mM; tetrahydrofolate (FH_4), 0.2 mM; mercaptoethanol, 20 mM; L-[3-^{14}C]serine, 0.2 mM (920000 dis. $\times$ min^{-1} $\times$ μmole^{-1}); protein, crude lysate, 1 mg; water to 0.5 ml; gas phase: air. Additions: KCl, 100 mM; NaCl, 100 mM; NH_4Cl, 100 mM; D-serine, 1 mM; L-threonine, 1 mM; L-[3-^{14}C]phosphoserine (L-*P*Ser), 0.2 mM (920000 dis. $\times$ min^{-1} $\times$ μmole); $MgCl_2$, 1 mM; ethylene diaminetetraacetate (EDTA), 1 mM

System	$CH_2 = FH_4$ formed
	nmoles/15 min
Complete	13.6
minus PLP	< 0.1
minus FH_4	< 0.1
plus K^+	28.0
plus Na^+	33.6
plus NH_4^+	27.6
plus EDTA	13.8
plus Mg^{2+}	13.2
plus D-Ser	12.8
plus L-Thr	13.6
minus L-Ser, plus L-*P*Ser	1.6
minus L-Ser, plus L-*P*Ser, plus Mg^{2+}	5.6
minus L-Ser, plus L-*P*Ser, plus EDTA	< 0.1

hydrofolate from L-[3-^{14}C]serine (Table 4). It thus appears that serine aldolase was specific for L-serine. Half maximum velocity was obtained with 72 μM L-serine.

The finding that L-serine was not the only precursor of glycine (Table 2, C_S) could also be explained if L-phosphoserine instead of L-serine was a direct precursor of methylene-tetrahydrofolate and glycine. This hypothesis was envisaged because methylene-tetrahydrofolate formation should theoretically be facilitated from phosphoserine as compared with serine, because the phosphate group should be the better leaving group. Methylenetetrahydrofolate was indeed formed from phosphoserine; however, the activity was stimulated by Mg^{2+} and inhibited by EDTA; this indicated that phosphoserine was converted to glycine and methylene-tetrahydrofolate only after hydrolysis to serine rather than directly (Table 4).

Cofactor Requirements. Crude extracts were without activity if pyridoxal phosphate was omitted from the incubation mixture. In contrast to threonine aldolase, serine aldolase appears to belong to the group of pyridoxal phosphate dependent enzymes which readily dissociate into the co- and apoenzyme (Table 4).

pH Optimum and Ion Requirements. The pH optimum was found to be between pH 8.5 and 9. Activity was not influenced by anions, but monovalent cations had a remarkably stimulatory effect, which was not saturated at 100 mM cation concentration. No preference for either sodium, potassium or ammonium ions was observed (Table 4).

Table 5. *Activities of the enzymes catalyzing the synthesis of glycine and one-carbon units in* Clostridium kluyveri

Assays: serine aldolase, as in Table 4, 1 mg crude lysate protein; threonine aldolase, as in Table 3, 1 mg Dowex-2-acetate lysate protein; pyruvate formate lyase: Tris acetate pH 7.5, 100 mM; glutathione red, 2.5 mM; coenzyme A, 0.5 mM; sodium arsenate, 5 mM; sodium glyoxylate, 2 mM; [1-^{14}C]pyruvate, 2 mM (200000 dis. $\times$ min^{-1} $\times$ μmole^{-1}); phosphotransacetylase, 1 U; 8 mg crude lysate protein; water to 1 ml; gas phase: hydrogen; formyltetrahydrofolate synthetase: Tris acetate pH 7.5, 100 mM; ATP, 5 mM; $MgCl_2$, 6 mM; *d,l*-tetrahydrofolate (FH_4), 2 mM; sodium formate, 20 mM; 2-mercaptoethanol, 20 mM; 0.2 mg crude lysate protein; water to 1 ml; gas phase: argon

Enzymes	Activities
	U/g protein[a]
Serine aldolase	1.4
Threonine aldolase	15.3
Pyruvate formate lyase	6.9
Formyltetrahydrofolate synthetase	33.9

[a] 1 U = 1 μmole/min.

Activity in Glycine Grown Cells. Serine aldolase could be demonstrated with equal activity both in cells from standard and in glycine supplemented cultures, indicating that the activity is constitutive. This is in agreement with the finding that in glycine grown cells serine was synthesized from exogenous glycine and C_1-units (Table 2, 2-G).

Differentiation of Serine from Threonine Aldolase. Pyridoxal phosphate was firmly bound to threonine aldolase, while it could be readily dissociated from serine aldolase. Serine aldolase activity was not inhibited by L-threonine, indicating that L-threonine did not compete with L-serine at the active site. Serine aldolase was stimulated by monovalent cations, whereas threonine aldolase was not. The pH-optima were different. These differences are taken as evidence that two separate enzymes are responsible for the serine aldolase and threonine aldolase activities.

CONCLUSION

The ^{14}C-labeling experiments have shown that in *Clostridium kluyveri* two pathways each are operative for glycine and for C_1-unit synthesis (Fig. 1). 67 % of the cell's glycine is formed from threonine and 33 % from serine (Table 2, C_S). The cells contain per 100 mg dry weight [2] 84 μmoles C_1-units derived from CO_2 and 64 μmoles glycine, of which 21 μmoles are derived from serine. The ratio of total C_1-units to glycine is thus calculated to be 105:64. It follows from this ratio that about 25 % of the cell's C_1 units are synthesized from serine and the remaining 75 % from CO_2. Final evidence has been obtained by the demonstration that the key enzymes for all the pathways were present in the lysates in activities sufficient to account for the anabolic requirements during growth (Table 5).

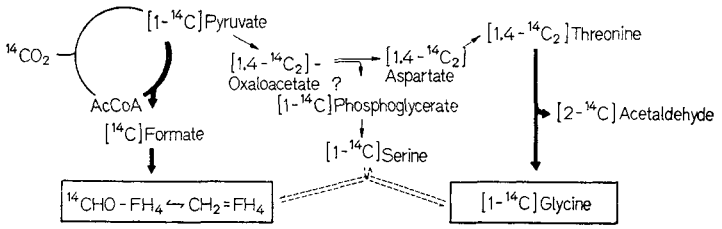

Fig. 1. *Interrelation of glycine and one-carbon unit synthesis.* FH_4 = tetrahydrofolate

DISCUSSION

Serine is thought to be the ubiquitous major precursor of both glycine and C_1-units. In *C. kluyveri*, however, serine is neither the major precursor of C_1-units nor of glycine: C_1-units are mainly synthesized from pyruvate *via* pyruvate formate lyase, while glycine is predominantly formed from threonine *via* threonine aldolase (Fig. 1).

Also in *C. pasteurianum* glycine has very recently been shown to be derived from threonine [17]; in addition threonine aldolase activity has been demonstrated in several anaerobes [17,18], while it does not appear to be well developed in aerobic bacteria [19,20]. It is therefore suggested that in *Clostridia* glycine is predominantly formed from threonine.

Glycine synthesis from threonine necessitates C_1-unit formation from a precursor other than serine; as pyruvate formate lyase, the key enzyme of one-carbon unit synthesis in *C. kluyveri* [3], has long been known to be present also in other anaerobes [21—23], it is most likely that in *Clostridia* C_1-units are predominantly synthesized from pyruvate.

In *C. kluyveri* this metabolic situation, pyruvate formate lyase catalyzing C_1-unit formation and threonine aldolase catalyzing glycine synthesis, is further complicated in that serine aldolase also appears to be operative. Such an enzyme outfit may be regarded from two viewpoints. (a) If serine alone were the precursor for both glycine and C_1-units, the larger requirement of the cell for C_1-units than for glycine would make the second pathway of one-carbon compound formation *via* pyruvate obligatory. The necessity of the second pathway of glycine synthesis *via* threonine, however, is not readily apparent. (b) If threonine were the precursor of glycine and pyruvate that of C_1-units, no interrelation between glycine and C_1-unit synthesis would be imposed by the different requirement of the cell for the two compounds and the necessity of the serine pathway is not readily understood.

However, the serine pathway to glycine and the aspartate pathway to threonine and thence to glycine may be under interrelated feedback control such that neither way alone can meet the requirements of the cell. The necessity and exact role of the two aldolases for the over-all economy of the organism remain to be defined.

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K. A. Jungermann, W. Schmidt, F. H. Kirchniawy,
E. H. Rupprecht and R. K. Thauer
Biochemisches Institut der Universität
BRD-7800 Freiburg i. Br., Hermann Herder-Straße 7,
Germany