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COMPARISON OF GLYCINE METABOLISM IN MOUSE LYMPHOMA CELLS EITHER SENSITIVE OR RESISTANT TO L-ASPARAGINASE

JAMES F. KEEFER, DAVID A. MORAGA and SHELDON M. SCHUSTER*
Department of Chemistry, and School of Biological Sciences, University of Nebraska, Lincoln, Nebraska 68588-0304, U.S.A.

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Abstract—Previous work suggested a relationship between glycine metabolism and the effect of L-asparaginase upon tumor cells. Therefore, L5178Y (sensitive) or L5178Y/L-ASE (resistant) ascites lymphoma cells were incubated with ^{14}C-labeled glyoxylate, glycine, serine, or asparagine, and the metabolism to other amino acids was measured by high performance liquid chromatography. Metabolic differences between the two cells lines were found. Under control conditions, the interconversion rate of glycine and serine via serine hydroxymethyltransferase (SHMT) was higher in sensitive than in resistant cells. The transformation rate of glyoxylate to serine was also higher in sensitive cells. These results may indicate a difference in the activity of SHMT. An alternate explanation would be that transport or diffusion of serine and glycine into sensitive cells is greater than into resistant cells. Several crucial metabolic differences were observed between the two cell types when L-asparaginase was added. A key difference is the decrease of glycine synthesis from glyoxylate observed in the sensitive cells compared to resistant cells which show no change. This suggests that asparagine is used for transamination of glyoxylate. Also, only sensitive cells appear to compensate for L-asparaginase-induced loss of glycine formation from glyoxylate by increasing glycine synthesis from serine. Alterations in sensitive tumor glycine metabolism may be an important function of L-asparaginase anticancer activity.

L-Asparaginase is an effective antitumor agent against some leukemias. *Escherichia coli* L-asparaginase alone caused complete remission of acute lymphoblastic leukemia in 40% of the cases observed [1]. Although L-asparaginase is known to catalyze the deamidation of asparagine to form aspartate, it is not clear how L-asparaginase causes cell death. L-Asparaginase treatment of both resistant and sensitive cells causes severe decreases in asparagine levels [2–4] so that a lower concentration of asparagine *per se* is apparently not the direct cause of death.

There is controversy concerning the biochemical basis for sensitivity to L-asparaginase. Several investigators have found that asparagine synthetase levels are higher in resistant than sensitive cells [5–8]. However, asparagine synthetase levels did not correlate with L-asparaginase sensitivity in clinical tests [9]. Also, Waye and Stanners [10] found sensitive cells with asparagine synthetase levels as high as the resistant cells. Others suggest a relationship between asparaginyl-tRNA synthetase and asparagine synthesis but experimentally rule out the possibility that regulation of asparagine synthesis is based solely on the extent of amino-acylation of asparaginyl-tRNA [11]. Control of asparagine synthetase by product inhibition [12–15] or a heat labile inhibitor [16, 17] has also been proposed. Ryan and colleagues [18, 19] suggested that asparagine may be needed in L-asparaginase sensitive cells for a metabolic process that resistant cells can carry out by other means. In 6C3HED lymphosarcoma, L-asparaginase caused the concentration of glycine to decrease in only the sensitive variants while the concentrations of the other amino acids changed in the sensitive cells the same as in the resistant cells. Also, the intraperitoneal injection of 0.8 mmole of glycine per day (in four doses) for a period of 5 days into 20 g mice with sensitive tumors countered the antitumor effect of L-asparaginase. This led to the hypothesis that an important pathway in glycine synthesis could include transamination of glyoxylate by asparagine. Therefore, we have attempted to study glycine and asparagine metabolism in tumor cells by monitoring the fate of radioactive compounds including glyoxylate, glycine and asparagine. Serine was also used because serine is a substrate for the major glycine synthetic pathway in the liver. Metabolic relationships between asparagine, glycine and serine are shown in Fig. 1. A major effort was expended to compare the amino acid metabolism of L-asparaginase sensitive and resistant tumor cells. It was hoped that a thorough study of cellular glycine metabolism might indicate whether alterations in glycine metabolism are a primary or secondary action of L-asparaginase antitumor activity. This is of special interest because lymphomas in general have higher glycine levels relative to other cells [20].

EXPERIMENTAL

Ortho-phthaldialdehyde (OPA) amino acid derivatives were separated and detected by high performance liquid chromatography (HPLC) and

* Author to whom all correspondence should be addressed.

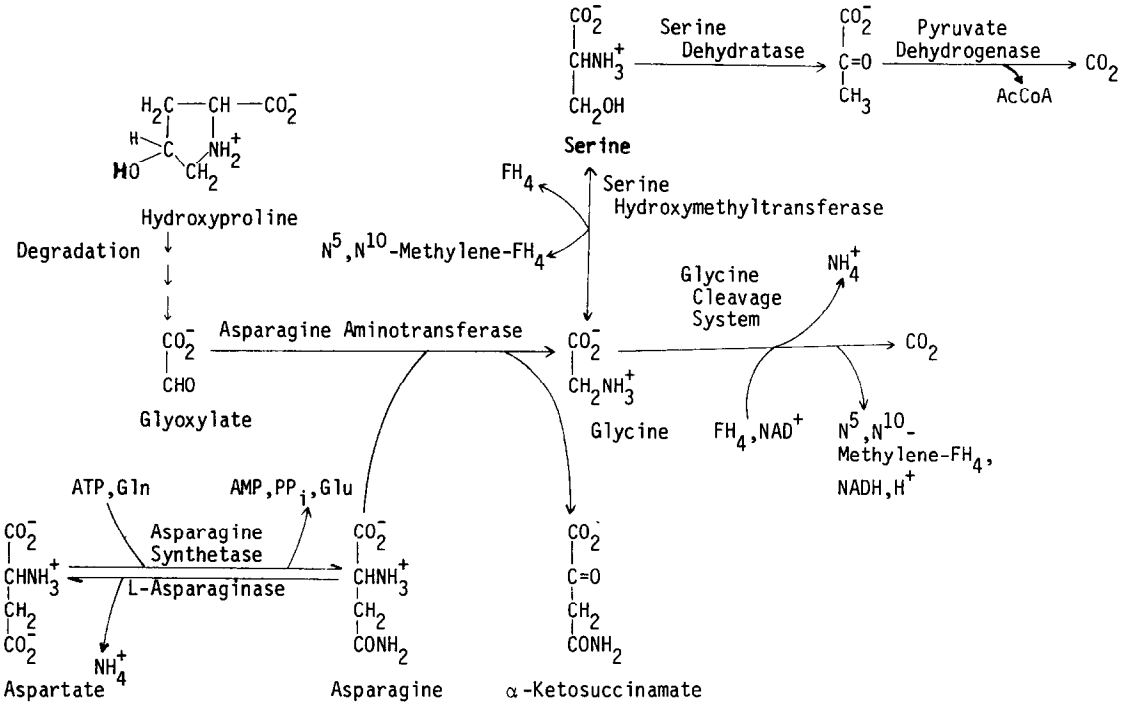

Fig. 1. Metabolic pathways involved in asparagine and glycine metabolism. Abbreviations: FH_4, tetrahydrofolate; gln, glutamine; glu, glutamate; and PP_i, inorganic pyrophosphate.

fluorescence respectively. A binary HPLC system was used. It included two 112 pumps (Beckman), a 421 controller and an Altex 210 injector with a 100 μl loop. Derivatized amino acids were detected using an 836 DuPont fluorescence detector with a Corning CS 7–60 350 nm excitation filter and a 3–74 400 nm emission filter. The analytical columns used were either an Ultrasphere-ODS C-18 150 × 4.6 mm i.d., 5 μm particle size (Beckman), or a Microsorb C-18 100 × 4.6 mm i.d., 3 μm particle size (Rainin, Woburn, MA) preceded by a 50 × 4.6 mm guard column packed with CO:Pell ODS, 30–38 μm particles (Whatman Inc., Clifton, NJ).

Solvents. Deionized water and commercial grade methanol were distilled in glass and were as good as HPLC grade solvents for our system. HPLC grade tetrahydrofuran (Burdick-Jackson) was placed in 0.5% (w/v) cuprous chloride under nitrogen and refluxed for 1 hr before distillation [21]. This treatment removes peroxides that may damage the HPLC column particles. Sodium acetate buffer (50 mM, pH 5.9) was made by adding 2.87 ml reagent grade glacial acetic acid to water, adjusting the pH with 5 N sodium hydroxide, and adding enough water to make 1 liter. Solution A contained 30 ml tetrahydrofuran and 970 ml sodium acetate. Solution B was 100% methanol. Tetrahydrofuran and proper sodium acetate pH were necessary for optimum resolution of the glycine–threonine and serine–histidine pairs. The sodium acetate solution was filtered daily through a 0.45 μm nylon-66 filter (Whatman) and degassed by bubbling with helium before mixing with tetrahydrofan.

Reagents, standards and chromatographic techniques. All chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO) or common suppliers unless otherwise indicated. When [1-^{14}C]glyoxylate was added as a substrate, an OPA–2-mercaptoethanol solution was used to obtain amino acid fluorescent derivatives [22]. A 74 mM solution was made by dissolving 250 mg of OPA in 2.5 ml methanol, followed by dilution to 25 ml with 0.4 M boric acid previously adjusted to pH 9.5 with NaOH. One hundred microliters of 2-mercaptoethanol was added, followed by a further 10-μl addition every 3 days. For the experiments in which [1-^{14}C]glycine and [1-^{14}C]serine were the substrates, 3-mercaptopropionate was added to the OPA solution instead of 2-mercaptoethanol. This reagent formed more stable glycine and serine OPA derivatives which resulted in a lower radioactivity background thus allowing a better quantitation of the label flow from the corresponding radioactive substrate [23]. These reagents were stable indefinitely at room temperature.

A slight modification was also needed in the chromatographic conditions (see below) in order to accomplish the appropriate separation when 3-mercaptopropionate was used as the reducing reagent. Additionally, 1-ml fractions of the HPLC eluant were collected in 3-ml disposable cups and transferred without loss into plastic scintillation vials containing 10 ml of 3a70B scintillation fluid (RPI). After mixing thoroughly, the samples were counted in a Beckman LS-8000 scintillation counter for 1 min each. A standard containing 10 μM of each amino

acid was made and stored at −20°. This solution was reprepared periodically from a concentrated stock solution because of the instability of asparagine and especially glutamine upon freezing and thawing.

HPLC analysis conditions. A volume of 120 μl of an acidified sample obtained from incubation cells (below) was neutralized with 40 μl of 1.1 N sodium hydroxide. A volume of 120 μl of OPA solution was then added and 200 μl of this mixture was loaded into the 100 μl loop. The sample was injected exactly 1 min after addition of the OPA solution to prevent variation from short-lived OPA-amino acid derivatives, especially glycine [22]. HPLC gradient conditions are shown in Fig. 2.

Cell preparation and CO_2 trapping assay. Murine lymphoma cells that were either sensitive (L5178Y) or resistant (L5178Y/L-ASE) to L-asparaginase were obtained from the Division of Cancer Treatment, National Cancer Institute (DCT Tumor Bank), Frederick Cancer Research Facility (Frederick, MD), and maintained in 20–25 g DBA-2 male mice (Jackson Laboratories, Bar Habor, ME). These types of tumor variants were used to compare our results with previous observations made by Ryan and his colleagues [18, 19] on solid 6C3HED tumor. Cells (1×10^6) in 0.1 ml of phosphate-buffered saline (PBS), pH 7.4, including 1 g glucose/liter, were injected intraperitoneally into each mouse. On day 3, 0.5 I.U. L-asparaginase was injected into mice with resistant tumors to maintain their resistance. All cells were allowed to grow as an ascites tumor for approximately 9 days, and then the mice were starved for 12 hr before they were killed by ether asphyxiation. Cells were harvested by washing the peritoneal cavity with 4 ml PBS. Typically, a volume of 5 ml was recovered containing about 1×10^8 cells/cc. Occasionally, mice would develop large solid tumors, in which case they were not used. Cells were immediately placed on ice after removal from mice and then centrifuged at 0° for 10 min at 270 *g* (1500 rpm) in a Sorvall Centrifuge (DuPont) equipped with a SS-34 vertical rotor. The cell pellet was resuspended in a small volume of fresh PBS-glucose by repeated suction using a 5-ml plastic tipped pipette (Eppendorf).

Red blood cells were then lysed by diluting to a 1:4 ratio, cell solution: 50 mM Tris–NH_3 buffer, pH 7.6, and setting on ice for 10 min [24]. Next, 4 parts of double concentration PBS-glucose was added, and the cells were centrifuged at 270 *g*, resuspended, and washed two more times with PBS-glucose. Cells were then diluted to give approximately 6×10^7 cells/cc. The viability of the cells was above 90%, as determined by 1% trypan blue dye exclusions, and decreased only slightly throughout the 1-hr experiments for both cell lines whether or not 20 I.U. of L-asparaginase was present.

Incubations were done in flat-bottomed 20 ml glass tubes equipped with rubber caps. Each tube contained a 2 × 1 cm piece of filter paper in a small plastic well suspended internally between the cap and the incubation mixture. Two hundred microliters of a 20% (w/v) potassium hydroxide solution was pipetted into these wells in order to trap the $^{14}CO_2$ liberated from the incubation of cells. At the end of the incubation time, the potassium hydroxide containing wells were carefully transferred into plastic scintillation vials containing 10 ml of scintillation fluid, mixed, and counted in a scintillation counter.

The incubation mixture contained 500 μl of cells, 100 μl containing 1×10^6 cpm of radioactive compound without carrier, and 100 μl of L-asparaginase

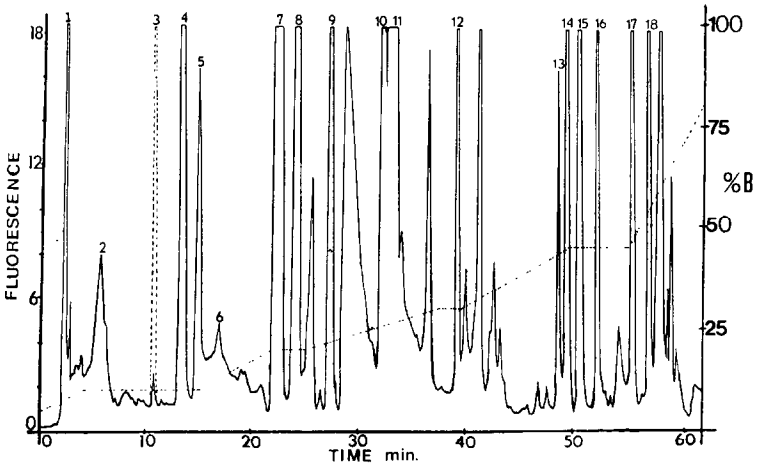

Fig. 2. OPA–2-mercaptoethanol sensitive cell amino acids as separated by a 3 μm C-18 column using high performance liquid chromatography (HPLC), and gradient profile (----) used for qualitative and quantitative analysis. Solution B was 100% methanol. Solution A consisted of a solution of sodium acetate (50 mM, pH 5.9) which had 3% tetrahydrofuran (v/v). The volume injected for analysis was 100 μl. Peaks; aspartate, 1; glutamate, 2; asparagine, 3; serine, 4; histidine, 5; glutamine, 6; glycine, 7; threonine. 8; arginine, 9; alanine, 10; α-aminoisobutyrate, 11; tyrosine, 12, methionine, 13, valine, 14; phenylalanine, 15; isoleucine, 16; leucine, 17; and lysine, 18. The asparagine peak drawn with dashed lines corresponds to the content of this amino acid when sensitive cells are not treated with L-asparaginase. Analytical conditions are described in the Experimental section.

or mannitol in PBS-glucose equal to the amount in the L-asparaginase. It was determined that less than 1% of the radiolabeled substrate was consumed except in the case of asparagine where 6% was used. Therefore, the amount of radioactive substrate in the medium remained approximately unchanged throughout the incubation period. PBS-glucose was a convenient buffer to use because medium with amino acids added would have interfered with the detection of cellular amino acids. *E. coli* L-asparaginase was purchased from Merck Sharp & Dohme (West Point, PA).

Cells were added to the mix to start the incubation. The incubation was done at 37° for 60 min and terminated by injecting 230 μl of 20% (w/v) trichloroacetic acid through the rubber cap. Each sample was transferred to a 1.5 ml plastic centrifuge tube (Sarstedt, Princeton, NJ) and centrifuged for 5 min in an Eppendorf microcentrifuge (No. 5412) at 10,000 *g* to precipitate the debris. The supernatant fraction was then removed and frozen at −20° prior to amino acid analysis.

RESULTS

Table 1 shows the amount of the radioactivity in μmoles/g cell protein found in various amino acid products when sensitive cells were incubated with different substrates. Our measurements did not distinguish between extra- or intracellular accumulation of products. When [1-^{14}C]glyoxylate was the substrate, label was found only in glycine and serine, presumably synthesized via a transaminase and serine hydroxymethyltransferase. The label in glycine was 2.5 times the amount of label in serine, suggesting that glyoxylate was converted into glycine which, in turn, was transformed into serine. In the same experiment, when 20 I.U. of L-asparaginase was added per sample, the radioactivity level in glycine and serine decreased at least 20%. This result implies that asparagine was involved in the transamination of glyoxylate. The addition of [1-^{14}C]glycine to sensitive cells caused serine to be the only amino acid labeled. Serine label was 26% higher when L-asparaginase was present. When [1-^{14}C]serine was the substrate, the label was seen only in glycine. This label increased by 79% when L-asparaginase was in the medium.

These same experiments were done on resistant cells (Table 2). The [1-^{14}C]glyoxylate label was found again in glycine and serine. When L-asparaginase was present, the radioactivity in glycine remained unchanged, whereas it increased marginally ($P < 0.1$) in serine. These results are in contrast with those in sensitive cells where the label observed in both amino acids decreased as a result of L-asparaginase addition. The label converted from [1-^{14}C] glycine into serine increased marginally ($P < 0.1$) upon the addition of L-asparaginase. On the other hand, the radioactivity conversion from [1-^{14}C]serine into glycine was not affected. This difference in response to L-asparaginase in the serine to glycine conversion was remarkably different from that in sensitive cells where a much higher percent increase was observed.

It is also interesting to note the difference in label transformation in both cells lines in the absence of L-asparaginase. In every case we observed a similar or higher amount of label in the sensitive versus resistant cells. The largest difference was observed in the direction from glycine into serine which was 2.5 times higher in sensitive than in resistant cells. In the previous experiments, where we measured the conversion from [1-^{14}C]glyoxylate, [1-^{14}C]glycine and [1-^{14}C]serine into amino acids, was also measured 1-$^{14}CO_2$ production. We performed a similar experiment with [U-^{14}C)]asparagine and quantitated the $^{14}CO_2$ liberated. A difference was noted between sensitive cells with or without L-asparaginase when [U-^{14}C]asparagine was added. The amount of $^{14}CO_2$

Table 1. Radioactive label experiments on L5178Y (sensitive) lymphoma cells*

Radioactive compound added per sample of cells†	Radioactive products	Product (μmoles/g cell protein)		Percent change	P
		No asparaginase‡	Plus asparaginase‡		
[1-^{14}C]Glyoxylate (24.49)	Glycine	0.105 ± 0.003	0.084 ± 0.001§	−20	0.01
	Serine	0.042 ± 0.004‖	0.031 ± 0.003	−26	NS¶**
[1-^{14}C]Glycine (2.21)	Serine	0.035 ± 0.001§	0.044 ± 0.002§	+26	0.02
	CO_2	0.0055 ± 0.0003	0.0047 ± 0.0006	−14	NS
[1-^{14}C]Serine (13.47)	Glycine	0.058 ± 0.001	0.104 ± 0.004§	+79	0.01
	CO_2	0.0233 ± 0.0002	0.0236 ± 0.0027	+1	NS
[U-^{14}C]Asparagine (1.46)	CO_2	0.0946 ± 0.0006§	0.0339 ± 0.0032	−64	0.01

* For each sample, 30×10^6 cells were incubated for 60 min in phosphate-buffered saline plus glucose, pH 7.4. One gram of protein was contained per 1×10^{10} cells. Significance of the percent change was determined by using Student's two-tailed *t*-test.

† Amounts of substrate added are reported as μmoles/g cell protein and are indicated in parentheses.

‡ Average above background of three samples ± S.E.M.

§ This content is statistically different from that of resistant cells ($P < 0.05$) (see Table 2).

‖ This content is statistically different from that of resistant cells at $P < 0.1$ (marginally significant).

¶ NS indicates percent change is not significant ($P < 0.05$).

** Percent change is significant at $P < 0.1$ (marginally significant).

Table 2. Radioactive label experiments on L5178Y/L-ASE (resistant) lymphoma cells*

Radioactive compound added per sample of cells†	Radioactive products found	Product‡ (μmoles/g cell protein)		Percent change	P
		No asparaginase‡	Plus asparaginase‡		
[1-^{14}C]Glyoxylate (20.95)	Glycine	0.102 ± 0.003	0.103 ± 0.003§	+1	NS¶
	Serine	0.028 ± 0.001‖	0.039 ± 0.005	+39	NS**
[1-^{14}C]Glycine (2.53)	Serine	0.014 ± 0.001§	0.020 ± 0.002§	+46	NS**
	CO_2	0.0051 ± 0.0006	0.0038 ± 0.0007	−25	NS
[1-^{14}C]Serine (19.43)	Glycine	0.046 ± 0.005	0.052 ± 0.004§	+15	NS
	CO_2	0.0270 ± 0.0006	0.0283 ± 0.0012	+5	NS
[U-^{14}C]Asparagine (1.25)	CO_2	0.0843 ± 0.0003§	0.0306 ± 0.0030	−64	0.01

* For each sample, 30×10^6 cells were incubated for 60 min in phosphate-buffered saline plus glucose, pH 7.4. One gram of protein was contained per 1×10^{10} cells. Significance of the percent change was determined by using Student's two-tailed *t*-test.

† Amounts of substrate added are reported as μmoles/g cell protein and are indicated in parentheses.

‡ Average above background of three samples ± S.E.M.

§ This content is statistically different from that of sensitive cells ($P < 0.05$) (see Table 1).

‖ This content is statistically different from that of sensitive cells at $P < 0.1$ (marginally significant).

¶ NS indicates percent change is not significant ($P < 0.05$).

** Percent change is significant at $P < 0.1$ (marginally significant).

produced from asparagine decreased by 64% when the cells were incubated with L-asparaginase. A similar result was observed in the resistant cells. From Table 3 we observed that treatment of either cell line with L-asparaginase caused a similar (69%) decrease in the content of asparagine (unlabeled) after incubation. No other significant differences were seen in the $^{14}CO_2$ formed by either of the cell lines, whether or not they were incubated with L-asparaginase. In every case, much more $^{14}CO_2$ was detected when the substrate was [1-^{14}C]serine than when [1-^{14}C]glycine was added. This can be explained by considering alternative ways of obtaining CO_2 from serine other than via glycine. In fact, it is known that in rat liver the principal route of serine catabolism is reductive deamination to pyruvate which can be decarboxylated to acetyl CoA [25]. $^{14}CO_2$ was detected over the background in every case except when [1-^{14}C]glyoxylate was the substrate.

Table 3 shows how incubation with or without L-asparaginase changes the asparagine concentration in tumor cells. Upon incubation in the absence of L-asparaginase, the content of asparagine in resistant and sensitive cells rose to the same level. In the presence of L-asparaginase, asparagine decreased by 69% in both cell lines. However, the ratio of this amino acid in resistant to sensitive cells is approximately 1.5.

The radioactive product was less than 1% of the unlabeled amino acid (serine or glycine) present in the cell. Therefore, the production of radioactive compounds should not have affected the rate of product formation in these experiments.

DISCUSSION

Various biomolecular mechanisms can be suggested for the development of drug resistance. These mechanisms include decreased cellular uptake [26, 27], modification of the way the drug is metabolized [28], changes in the intracellular levels of target enzymes (i.e. changes in either protein breakdown or synthesis rate) [29–33], structural alterations of the target enzyme leading to modified kinetic properties [34, 35], and, as more recently described, selective gene amplification [36, 37]. There are sev-

Table 3. Asparagine concentration in L5178Y tumor cells*

Cell type	Asparagine (μmoles/g cell protein)		
	0 min	No L-asparaginase 60 min	Plus 20 I.U. L-asparaginase 60 min
Sensitive	0.51 ± 0.09 (6)	3.60 ± 0.40 (9)	0.16 ± 0.02 (6)
Resistant	0.77 ± 0.17 (6)	3.52 ± 0.71 (9)	0.24 ± 0.02 (6)

* Each sample contained 30×10^6 cells in phosphate-buffered saline plus glucose, pH 7.5. No radioactive compound was added. Numbers are reported as the mean ± the average error of the mean. Numbers in parentheses indicate the number of samples averaged.

eral reports that L-asparaginase sensitive cells have considerably less asparagine synthetase activity than their resistant counterparts [5–8]. It has therefore been suggested that inability of some cells to maintain adequate levels of intracellular asparagine is the determining factor of their susceptibility to L-asparaginase. Other authors, working with CHO cells [11], have reported an inverse correlation between the degree of amino acylation of many t-RNAs (asparagine, leucine, etc.) and asparagine synthetase activity. Contrary to other reports [38], these workers found no significant differences in the kinetic parameters or thermal stability of the enzyme from the cells that had either higher or lower asparagine synthetase levels.

In any event, treatment with L-asparaginase results in severe depletion of cellular asparagine whether the cells are sensitive or resistant [2–4]. It was, therefore, proposed that an important metabolite formed by the participation of asparagine was also depleted and that the sensitive cells could not compensate. Ryan and his colleagues observed that, whereas in resistant cells only asparagine was depleted upon L-asparaginase treatment, both asparagine and glycine decreased in susceptible cells [18]. Cooper [39] described an asparagine-glyoxylate aminotransferase which catalyzes the amino transfer from asparagine to glyoxylate producing α-ketosuccinamate and glycine. The α-ketosuccinamate is presumably hydrolyzed by an ω-amidase to form oxaloacetate and ammonia in mammals [40, 41]. The subcellular distribution of these enzymes and their possible roles in tumor or normal cell metabolism have not been investigated yet.

Uncovering the source of glyoxylate carbon has also posed important questions. Since mammals are not thought to have any isocitrate lyase activity, it is likely that the breakdown of hydroxyproline is a major source of glyoxylate [42]. The enzymes involved in this process have been described by Adams [43], Adams and Goldstone [44–46] and Dekker and his coworkers [47–50]. Nonetheless, glyoxylate is not expected to exist in high concentrations in living cells due to its great chemical reactivity and many possible metabolic fates. In such case, the hydroxyproline serum and cell levels as well as catabolism may be the crucial factor in the production of glyoxylate.

We have found that the total conversion of label from [1-^{14}C]glyoxylate into glycine was similar and that the conversion into serine was marginally higher in L-asparaginase sensitive cells than in resistance cells when untreated. This amount of label decreased in sensitive cells and slightly increased in resistant cells when L-asparaginase was added. This may be related to the lower concentration of asparagine in sensitive than in resistant cells after incubation with L-asparaginase (see Table 3). The reduced level of asparagine in sensitive cells may be rate limiting for the glyoxylate-asparagine transaminase. For example, when sensitive cells are incubated with L-asparaginase, glycine production by the transaminase (0.084 μmole/g cell protein) may require half the asparagine available (0.16 μmole/g cell protein). When [1-^{14}C]glycine was added to the incubated cells, the amount of radioactivity found in serine was about 2.5 times higher in sensitive than in resistant cells in the absence of L-asparaginase.

A comparison of the percent changes due to administration of L-asparaginase (Tables 1 and 2) shows that the amount of label transformed from [1-^{14}C]serine into glycine was higher in sensitive cells in the presence than in the absence of L-asparaginase. When treated with L-asparaginase, this level remained nearly unchanged in resistant but almost doubled in sensitive cells.

Differences in the amounts of labeled products obtained from radioactive substrates can be explained in several ways: (1) differences in the amount of enzyme present; (2) differences in enzyme K_m where a lower $K_m$causes a higher label conversion at low substrate concentrations; and (3) differences in substrate transport where higher transport causes higher label flow. Our results may be explained, for example, by a higher serine hydroxymethyltransferase activity in sensitive cells, or by an asparagine-glyoxylate transaminase with a higher K_m for asparagine in sensitive cells. Under normal conditions the concentration of intracellular asparagine in both cell lines may be sufficiently far above the K_m values so that no difference is observable unless an additional stress is imposed. It can further be suggested that the serine hydroxymethyltransferase is probably regulated differently in both cell lines as shown by the effect on the conversion of label from [1-^{14}C]-serine into glycine when L-asparaginase was applied. The possibility of differential membrane permeability or relatively deficient sensitive cell glycine metabolism involving products other than those analyzed (e.g. nucleic acids) could provide alternate explanations. Our studies using radioactive labeled substrates as probes have nevertheless been useful in providing us with a rationale for a more specific approach in the studies of the enzymes closely related to asparagine metabolism and their possible roles in tumor metabolism. We are presently comparing the asparagine-glyoxylate transaminase, and the serine hydroxymethyltransferase as well as the asparagine synthetase enzymes isolated from tumor cells.

The reasons for the differences in label conversion found are not immediately evident. However, we have shown that L-asparaginase sensitive and resistant cells do utilize glycine differently when subjected to L-asparaginase treatment.

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