UPTAKE AND UTILIZATION OF L-GLUTAMINE BY HUMAN LYMPHOID CELLS; RELATIONSHIP TO γ-GLUTAMYL TRANSPEPTIDASE ACTIVITY

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Received July 18, 1977

SUMMARY

Initial rates of glutamine uptake were studied in human lymphoid cell lines whose γ-glutamyl transpeptidase activities vary from 93 to 11,300 units/mg. In general, glutamine was transported at lower rates than other amino acids (met, phe, leu) in all cell lines studied. A cell line with very high transpeptidase activity exhibited an increased rate of glutamine uptake as compared to other amino acids, and a markedly decreased intracellular concentration of glutamine. In all cell lines transported glutamine was extensively (>80%) converted to glutamate. Treatment of cells with 6-diazo-5-oxo-L-norleucine (DON) decreased transpeptidase and conversion of transported glutamine to glutamate by about 80%. Inhibition of glutamine transport was less pronounced (0-20%). The findings indicate that transported glutamine does not equilibrate with glutamine in the intracellular pool, but may enter a separate pool in which it is rapidly converted to glutamate.

Previous studies in this laboratory showed that γ-glutamyl transpeptidase, which catalyzes transfer of the γ-glutamyl moiety of glutathione and other γ-glutamyl compounds to amino acid and peptide acceptors, is present in a wide variety of human and rat lymphoid cells and that it is located on the cell-surface (1). The transpeptidase activity of human lymphoblastic lines varies considerably; cell lines from patients with lymphoproliferative disease have 60-300 units per mg of protein, whereas higher activities (1,000-1,300 units/mg) were found in B cell lines from normal subjects. The highest activity (11,300 units/mg) was found in a cell line derived from a patient with multiple myeloma (1). The catalytic properties of γ-glutamyl transpeptidase of lymphoid cells closely resemble those of γ-glutamyl transpeptidase obtained from other mammalian sources. Thus, glutamine is

Abbreviations: DON, 6-diazo-5-oxo-L-norleucine; PBS, phosphate buffered saline (pH 7.2); S-methyl-GSH, S-methylglutathione.

ISSN 0006-291X

an excellent acceptor substrate of the γ-glutamyl group, and it can also serve as a γ-glutamyl donor. Previous publications from this laboratory have dealt with the hypothesis that γ-glutamyl transpeptidase, a component of the γ-glutamyl cycle, may function as one of the systems that mediates the transport of amino acids (for review, see (2)). In the present work we studied the uptake and utilization of glutamine by a number of lymphoid cell lines whose levels of γ-glutamyl transpeptidase activity vary considerably.

MATERIALS AND METHODS

The human lymphoid cell lines were obtained and maintained as described (1). 6-Diazo-5-oxo-L-norleucine (DON) was kindly supplied by Dr. R.E. Handschumacher. Generally labelled L-[^{14}C]glutamine, L-[^{14}C]phenylalanine, L-[^{14}C]methionine, and L-[^{14}C]leucine were obtained from New England Nuclear Corp.

In the amino acid uptake studies, lymphoid cells were harvested by centrifugation from suspension cultures containing a medium (RPMI 1640) supplemented with 20% fetal calf serum, penicillin (100 μg/ml), and streptomycin (100 μg/ml) (GIBCO). The cells were resuspended at 4° in phosphate buffered saline (PBS; pH 7.2 (3)) lacking Ca^{++} and Mg^{++} (GIBCO), centrifuged, and then suspended in complete PBS supplemented with glucose (1 mg/ml) to achieve a concentration of 10^7 cells/ml. Viability was checked by dye exclusion with Trypan blue (0.15%). Protein was determined as described (4). [14C]Amino acid (20 μl of 0.2 mM; 10 μCi/ml) was added to 0.2 ml of cell suspension and shaken at 37°. Aliquots (50 μl) were removed after 0-4 min. and added to Microfuge tubes containing 0.1 ml of a 1:4 mixture of mineral oil and dibutyl phthalate. After centrifugation in a Beckman Microfuge for 0.3 min., the tip of the tube containing the cells was cut off with a razor and the pellet was resuspended in 0.9 ml of 0.9% NaCl. The cells were treated with 0.1 ml of 1 M NaOH and 10 ml of Bray's solution (5); radioactivity was determined by scintillation counting.

In studies in which glutamine and glutamate were determined, the suspensions (2-3 ml) containing cells and [^{14}C]glutamine were shaken at 37° for 0-5 min. After centrifugation over oil (see above), the pellet was suspended in 0.6 ml of 0.9% NaCl; 2.4 ml of 1% picric acid was added and the mixture was centrifuged at 750 x g for 5 min. The supernatant solution was collected and applied to a Dowex 2 column (1.5 x 0.5 cm) which was eluted with 5 ml of 0.01 M HCl; the eluate was evaporated to dryness. The residue was suspended in 40 μl of water and spotted on Whatman No. 3 chromatography paper. Chromatography was performed in a solvent containing phenol (80 ml), water (20 ml), and EDTA (50 mg); a beaker containing NH_4OH was placed in the chromatography chamber. Glutamate and glutamine are effectively separated; the respective R_f values were 0.15-0.24 and 0.41-0.60. Glutamate and glutamine were located by spraying with ninhydrin, and the papers were cut into 1 cm strips, and the radioactivity present was determined.

In studies in which cells were treated with DON, suspensions containing 10^7 cells/ml in PBS supplemented with glucose were incubated for 30 min. at 37° with shaking in the presence of 0 to 2 mM DON. Where indicated, S-methyl-GSH was added at a final concentration of 10 mM. The cells were

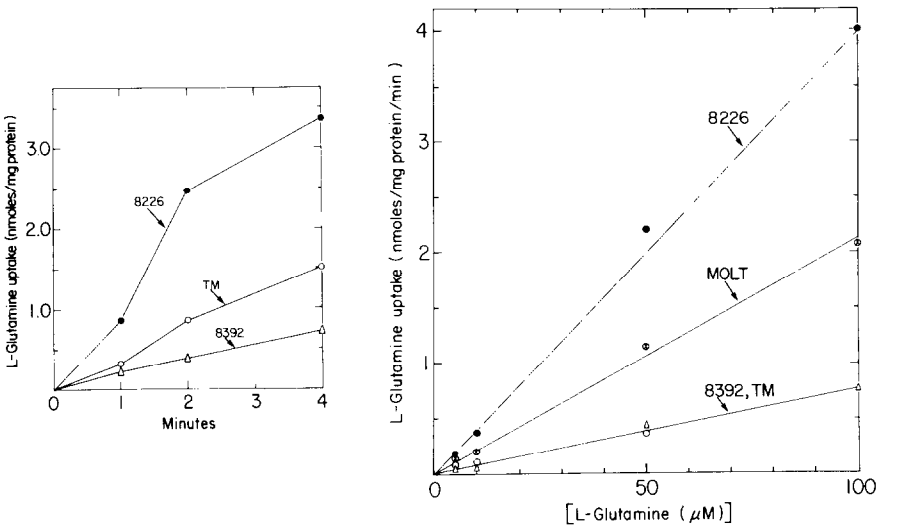

Figure 1. Uptake of Glutamine by Several Human Lymphoid Cell Lines. Left: The concentration of L-[^{14}C]glutamine was 20 μM. Right: Initial rates of uptake. The γ-glutamyl transpeptidase (units/mg) of the cell lines was 11,300 (RPMI 8226), 1,120 (TM), 105 (8392) and 93 (MOLT) (1).

then cooled and washed twice at 4°. Viability was checked by dye exclusion prior to the experiments.

γ-Glutamyl transpeptidase activity was determined on twice-washed cell suspensions in PBS containing 10^7 cells/ml. The assay solution (1.0 ml) contained 0.1 ml of cell suspension, 2.5 mM L-γ-glutamyl-p-nitroanilide, 30 mM glycylglycine (pH 8.0), 50 mM Tris-HCl (pH 8.0) and 75 mM NaCl. The suspension was shaken at 37° for 30 min. and then cooled to 4°; after removal of cells by centrifugation, the absorbance of the supernatant was determined at 410 nm. A unit of enzyme activity is defined as the amount of enzyme which releases one nmole of p-nitroaniline per hr.

Amino acid analyses were performed on cell suspensions deproteinized by adding sulfosalicylic acid (final conc. 5%). After centrifugation, the pH of the supernatant was adjusted to 2.2 by adding 4M NaOH and the amino acids were determined with a Durrum Model D-500 amino acid analyzer.

RESULTS

Figure 1 gives data on the uptake of glutamine by several human lymphoid cell lines which exhibit γ-glutamyl transpeptidase activities that vary by 2 orders of magnitude. Cells of line RPMI 8226, which exhibit very high transpeptidase activity (11,300 units/mg), are the most active in glutamine uptake. Several cell lines were examined with respect to uptake of glutamine, methionine, phenylalanine, and leucine (Table I). In general, the rate of

Table I: Uptake of Glutamine and Other Amino Acids by Lymphoid Cells

Cell Line	Transpeptidase (units/mg)	Uptake (nmoles/mg protein/min.)			
		Glutamine	Methionine	Phenylalanine	Leucine
8392	105	1.1	2.7 [0.40]*	5.7 [0.19]	4.3 [0.26]
TM	1,120	0.75	5.2 [0.14]	11.0 [0.07]	5.5 [0.14]
RPMI 8226	11,300	1.9	3.1 [0.61]	2.1 [0.90]	2.6 [0.73]

*Ratio of glutamine uptake to amino acid uptake.

Table II: Content of Glutamine and Glutamate in Lymphoid Cell Lines*

Cell line	$\frac{\text{Glutamine}}{\text{Glutamine + Glutamate}} \times 100$	
	Intracellular Pool+	Transported‡
RPMI 8226	20;27	6;4;5
MOLT	40;78;72	2;5;5;7;12;13
8392	64;76	10;12
TM	45;65	6

*The experimental details are given in the text; values for separate determinations are given. +The ratio of glutamine to glutamate was 1.5-5.0 (average, 2.7) for 12 cell lines (MOLT, 8392, TM, RPMI 1788, RPMI 6410, RPMI 6237, SB, 8402, K562, CL, CEM, HSB: see (1) for origin and characterization); the ratio for RPMI 8226 was 0.3. ‡Based on determinations of [^{14}C]glutamine and [^{14}C]glutamate.

glutamine uptake was lower than the rates of uptake of other amino acids, but when glutamine uptake was compared to that of other amino acids, a preference for glutamine transport was shown by cell line RPMI 8226. These findings were pursued by comparing the intracellular concentrations of glutamine and glutamate of this cell with other lymphoid cells (Table II). Study of 12

cell lines with transpeptidase activities <1200 units/mg revealed that the intracellular concentrations of glutamine were in the range 1.5-2.5 μmol per g (wet weight), and that of glutamate was 0.5-1 μmol/g; when expressed as % glutamine of the total glutamine plus glutamate present, values of 33-84% are obtained. In contrast, cell line RPMI 8226 had an intracellular glutamine concentration of 0.3 μmol/g (or about one-fifth of the lowest glutamine level found in the other cell lines) and 1.2 μmol/g of glutamate (or about 20% higher than the highest level found in the other cells); the glutamine content, as percent of total glutamine plus glutamate, was about 20%.

Studies were carried out on several cell lines in which the intracellular concentrations of [^{14}C]glutamine and [^{14}C]glutamate were determined after incubation of the cells with [^{14}C]glutamine. As indicated in Table II, values for intracellular [^{14}C]glutamine were very low indicating rapid conversion of the transported glutamine to glutamate. Comparison with data on the composition of the intracellular pool show that the transported glutamine does not equilibrate with the glutamine of the intracellular pool.

DON, a compound known to be an effective glutamine antagonist, was recently shown to irreversibly inhibit rat kidney γ-glutamyl transpeptidase by attaching to the γ-glutamyl site of this enzyme (6,7). We carried out similar studies with lymphoid cell γ-glutamyl transpeptidase and found that DON also inhibits this enzyme irreversibly. S-Methylglutathione, an impermeable substrate, prevents inhibition of transpeptidase by DON in intact cells, a finding consistent with our previous observations that the enzyme is located on the cell surface (1). Studies in which RPMI 1788 and 8226 cell lines were treated with DON (Table III) showed that both transpeptidase activity and conversion of transported glutamine to glutamate were inhibited >80%, whereas the effect on the transport of glutamine was less pronounced (0-20% inhibition). S-Methyl-GSH protected transpeptidase against DON, but did not affect the inhibition by DON of the conversion of transported glutamine to glutamate.

Table III: Effect of DON on Glutamine Transport, Utilization, and γ-Glutamyl Transpeptidase Activity[1]

Exp. No.	DON	S-Methyl-GSH	Glutamine Uptake[2]	γ-Glutamyl Transpeptidase[3]	$\frac{[^{14}C]Gln \times 100}{[^{14}C]Gln + [^{14}C]Glu}$
1	0	0	1,010	803	5
	1 mM	0	1,040	145	81
	0	10 mM	978	777	10
	1 mM	10 mM	1,150	711	84
2	0	0	939	826	<5
	2 mM	0	730	164	>95

[1] Cell line RPMI 1788; similar effects of DON were observed in studies on cell line RPMI 8226. [2] pmoles/mg/min. [3] nmoles/mg/h.

DISCUSSION

The data (Fig. 1; Table I) show a preference for glutamine transport by a cell line (RPMI 8226) which exhibits very high γ-glutamyl transpeptidase activity. The high transpeptidase activity of this cell line is associated also with relatively decreased intracellular concentrations of glutamine. In all cell lines there was extensive conversion of the transported glutamine to glutamate; thus, 80-98% of the transported glutamine was found as intracellular glutamate. The data (Table II) indicate that the transported glutamine does not equilibrate with intracellular glutamine and suggest that glutamine is transported into a cell compartment (separate from that which contains most of the intracellular glutamine) and is rapidly converted to glutamate.

The mechanism responsible for conversion of glutamine to glutamate is not yet clear. This conversion is inhibited by DON, which also inhibits γ-glutamyl transpeptidase, as well as glutaminases, and other glutamine-utilizing

enzymes (6-8). It is notable that S-methyl-GSH did not prevent inhibition by DON of glutamine conversion to glutamate, but it effectively protected against DON-inactivation of transpeptidase. This suggests that the conversion of transported glutamine to glutamate is not catalyzed by transpeptidase. Another observation that must be considered is the finding (9) that incubation of mouse and rat lymphocytes (which had been stimulated by concanavalin A) with [^{14}C]glutamine led to a similar rapid conversion of transported glutamine to glutamate. The rat cells have about 60 times more transpeptidase than the mouse cells (1). Thus, while the present studies do not show a direct correlation between transpeptidase activity and the transport and utilization of transported glutamine, more detailed information is needed to elucidate the relationship between these reactions in lymphoid cells. In particular the finding that a cell line with high transpeptidase activity has low intracellular glutamine to glutamate ratios and shows a preference for glutamine transport requires further study. The cell surface localization of γ-glutamyl transpeptidase in lymphoid cells and thus its accessibility to externally supplied substrate (1) must also be considered in relation to the fact that glutathione, presumably a physiological substrate of the enzyme, is found almost exclusively intracellularly. Thus, measurement of the enzyme activity of intact cells in a system in which substrate is supplied extracellularly may not reflect the activity of the enzyme *in vivo*. Studies on the relationship between the γ-glutamyl transpeptidase activity of lymphoid cells and the utilization of intracellular glutathione are currently in progress.

We acknowledge the skillful technical assistance of Mr. Robert Nehring. This research was supported in part by a grant from The American Cancer Society.

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