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How methionine and glutamine prevent inhibition of growth by methionine sulfoximine

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SUMMARY

Methionine sulfoximine, a potent inhibitor of glutamine synthetase, inhibits growth of *Chlorella vulgaris*. Growth inhibition is prevented by adding L-glutamine or L-methionine to the growth medium. We show that glutamine and methionine act by blocking methionine sulfoximine uptake into the cell.

Methionine sulfoximine induces epileptogenic convulsions in various mammals[1-3] and inhibits the growth of microorganisms[4,5]. Glutamine as well as methionine prevent the toxic effect of methionine sulfoximine when added to the culture medium of microorganisms or when administered to animals at the same time or shortly before methionine sulfoximine[3,5-8]. Since methionine sulfoximine is a potent inhibitor of glutamine synthetase[9], and the effects of methionine sulfoximine are prevented by glutamine, it has been suggested that this compound acts by lowering cellular glutamine concentrations. This interpretation does not account for the effect of methionine since methionine does not prevent or reverse inhibition of glutamine synthetase activity by methionine sulfoximine *in vitro*[10] and is not obviously related to glutamine metabolism. Lamar and Sellinger[9] have shown that methionine inhibits uptake of [^{14}C]methionine sulfoximine into rat brain suggesting that methionine prevents seizures by blocking the entry of methionine sulfoximine into brain cells. In this report we show that both glutamine and methionine prevent the toxic effects of methionine sulfoximine in the unicellular alga, *Chlorella vulgaris*, by blocking methionine sulfoximine uptake into the cell.

We verified Braun's[5] finding that methionine sulfoximine inhibits growth of *Chlorella*. Methionine sulfoximine at a concentration of $2 \cdot 10^{-5}$ M inhibited growth by

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50%; 10^{-3} M methionine sulfoximine inhibited growth completely. Growth inhibition was decreased by adding glutamine or methionine to the culture medium (Fig. 1). Since the effect of glutamine or methionine could be prevented by increasing the concentration of methionine sulfoximine, we concluded that these amino acids are competitive inhibitors of methionine sulfoximine action.

Experiments were performed to investigate the relationship of methionine sulfoximine uptake to growth inhibition. [^{14}C]Methionine sulfoximine uptake by *Chlorella* followed Michaelis-Menten kinetics suggesting that this amino acid is transported by a carrier mechanism[12] (Fig. 2). The apparent K_m for uptake, $5 \cdot 10^{-5}$ M, was within experimental error equal to the concentration of methionine sulfoximine required for 50% inhibition of growth. These results indicate that methionine sulfoximine uptake can be the limiting step in growth inhibition. Further support for this conclusion was obtained by comparing [^{14}C]methionine sulfoximine uptake in methionine sulfoximine-resistant and -sensitive strains of *Chlorella*. A resistant strain was isolated that is unaffected by 10^{-3} M methionine sulfoximine which completely inhibits growth of the wild type. This strain took up [^{14}C]methionine sulfoximine at 1/10 the rate of the wild type indicating that methionine sulfoximine sensitivity is correlated with ability to take up methionine sulfoximine (Fig. 3).

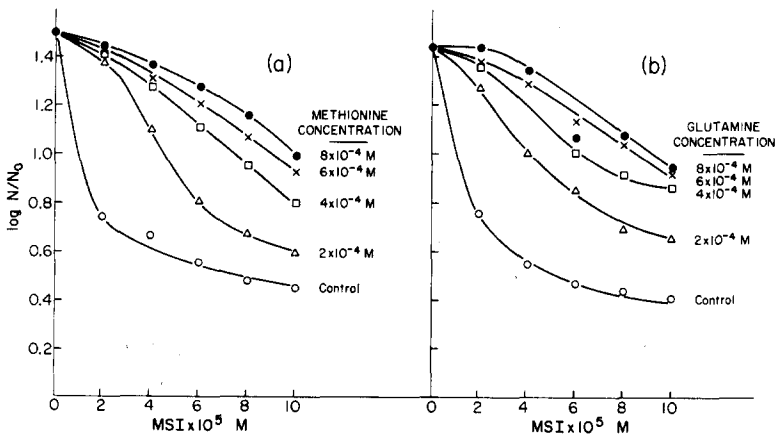

Fig. 1. Prevention of methionine sulfoximine inhibition of growth by methionine and glutamine. Growth experiments followed the method of Braun[11]. Data expressed as the log of cell number at 96 h/initial cell number. Initial cell number = 10^7. a. Increasing concentrations of methionine. b. Increasing concentrations of glutamine. MSI = methionine sulfoximine.

To establish that glutamine and methionine block methionine sulfoximine we compared [^{14}C]methionine sulfoximine uptake in the presence and absence of these amino acids. Both glutamine and methionine inhibited uptake at concentrations that greatly reduce methionine sulfoximine inhibition of growth (Fig. 2). We found that the apparent K_m for uptake was altered, and maximum rate was unchanged indicating that glutamine and methionine are competitive inhibitors.

The fact that we observed competitive inhibition of uptake suggested that glutamine,

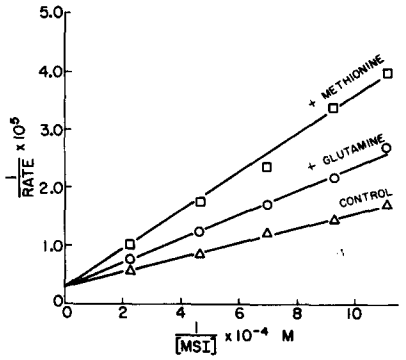

Fig. 2. Effect of methionine and glutamine on uptake of [^{14}C]methionine sulfoximine. Exponentially growing cells were washed twice in an original volume of Braun's[11] medium and incubated for 2 h at 25 °C. Labeling with ^{14}C-labeled amino acids was carried out at 25 °C in 4.0 ml of Braun's medium containing 10^8 washed cells. The incubation mixture in 50-ml Erlenmeyer flasks was shaken at 120 rev./min. Uptake was stopped by filtering the incubation mixture through 0.22 μm pore size membrane filters (Millipore). The filters were then washed with 20 ml of unlabeled amino acid solution (100 mg/l), dried and radioactivity measured in a liquid scintillation spectrometer using a toluene-fluor. L-[^{14}C]Methionine DL-sulfoximine used in uptake experiments was synthesized from L-[^{14}C]methionine using the method of Bentley *et al.*[13]. Initial rates of uptake were measured and expressed as cpm/10^8 cells per 2 h. Data are presented in double-reciprocal plots. △—△, control; ○—○, 10^{-4} M glutamine added; □—□, 10^{-4} M methionine added.

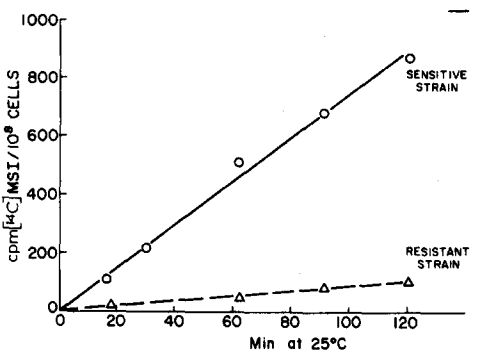

Fig. 3. Comparison of [^{14}C]methionine sulfoximine uptake into resistant and sensitive strains of *Chlorella*. Uptake expressed as cpm [^{14}C]methionine sulfoximine in 10^8 *Chlorella* cells. The concentration of methionine sulfoximine is 10^{-5} M. ○—○, sensitive strain; △– – △, resistant strain.

methionine and methionine sulfoximine compete for a common carrier. To test this hypothesis, uptake of [^{14}C]glutamine, [^{14}C]methionine and [^{14}C]methionine sulfoximine was measured in the presence and absence of the unlabeled amino acids. We found competitive cross-inhibition of uptake which fulfills the major requirement for transport of several solutes by a common carrier[14]. Another indication of a common carrier is that the K_i for cross-inhibition roughly equals K_m for uptake. This relationship was obeyed by the pairs methionine-methionine sulfoximine and methionine-glutamine, but not by glutamine-methionine sulfoximine (Table I).

Inequality of K_m and K_i for a pair of amino acids does not exclude transport by

TABLE I

K_m AND K_i VALUES FOR COMPETITIVE CROSS-INHIBITION OF METHIONINE, GLUTAMINE, AND METHIONINE SULFOXIMINE UPTAKE

K_i for competitive inhibition and K_m values calculated graphically from Lineweaver-Burk plots of uptake data[17]. Initial rates of amino acid uptake were used in calculations.

Amino acid	K_m *uptake* $\times 10^5$ *(M)*	$K_i \times 10^5$ *(M)*		
		Methionine uptake	*Glutamine uptake*	*Methionine sulfoximine uptake*
Methionine	1.7	–	2.2	6.0
Glutamine	2.1	4.8	–	15.0
Methionine sulfoximine	5.0	2.0	21.0	–

a common carrier. This behavior is observed when amino acids are transported by several parallel transport systems, *e.g.* the A and L systems for neutral amino acids in Ehrlich cells[14]. We have found that methionine sulfoximine inhibition of growth reaches a plateau of 73% in the range $8 \cdot 10^{-5}$-$10 \cdot 10^{-5}$ M (Fig. 1). Inhibition is complete at 10^{-3} M suggesting that methionine sulfoximine is transported by two systems, one with high affinity, the other with low affinity. Since methionine and glutamine at high concentrations completely prevent methionine sulfoximine toxicity, it is likely that both carrier systems transport the three amino acids. Therefore, we tentatively conclude that inhibition of methionine sulfoximine uptake results from a competition of methionine, glutamine, and methionine sulfoximine for one or more common carriers.

Our results indicate that: (1) methionine sulfoximine uptake can be the limiting step in growth inhibition; and (2) methionine and glutamine competitively inhibit both methionine sulfoximine toxicity and uptake to about the same extent in the same concentration range. Therefore, we concluded that methionine and glutamine prevent the toxic effects of methionine sulfoximine by blocking uptake into the cell. This conclusion is significant in that it shows that the protective effect of glutamine and methionine results from inhibition of methionine sulfoximine transport and not from reversal of glutamine synthetase inhibition or a metabolic by-pass of this enzyme. This interpretation is consistent with the finding in other systems that methionine and glutamine are not effective when administered after methionine sulfoximine[3,7].

Several studies show that lowering of brain glutamine levels and induction of convulsions are independent effects of methionine sulfoximine[15,16]. This suggests that inhibition of glutamine synthetase is not the cause of convulsions. We have found that methionine sulfoximine is a competitive inhibitor of glutamine uptake. Thus, induction of convulsions in mammals could involve inhibition of glutamine transport.

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