ANTITUMOR ACTIVITIES OF BACTERIAL LEUCINE DEHYDROGENASE AND GLUTAMINASE A

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1. Introduction

Current observations on the inhibition of tumor growth by enzymes such as asparaginase [1, 2], glutaminase [3], arginase [4, 5] and phenylalanine ammonia-lyase [6], which catalyze the essentially irreversible degradation of amino acids, have stimulated the search for the antineoplastic activity of other microbial enzymes related to amino acid metabolism. The antitumor activities of a folate-cleaving bacterial enzyme, carboxypeptidase G_1 [7, 8], and jack bean urease [9], and the inhibition of growth and DNA synthesis of tumor cells by ascorbic acid oxidase [10] also have been recently reported.

In the present communication we describe some studies on the antitumor activities of several bacterial enzymes, especially leucine dehydrogenase and isozyme A of glutaminase, which catalyze the reversible oxidative deamination of L-leucine and some other aliphatic amino acids in the presence of NAD [11], and the deamidation of glutamine and asparagine [12], respectively.

2. Materials and methods

2.1. *Enzyme preparations*

The following enzymes were isolated and purified to homogeneity from the cell-free extracts of bacteria according to the procedures given in the literature: leucine dehydrogenase (L-leucine: NAD oxidoreductase (deaminating) EC class 1.4.1) from *Bacillus sphaericus* IFO 3525 [11], alanine dehydrogenase (L-alanine:NAD oxidoreductase (deaminating) EC 1.4.1.1) from *B. sphaericus* IFO 3525 [13], isozymes A and B of glutaminase (L-glutamine amidohydrolase EC 3.5.1.2) from *Pseudomonas aeruginosa* IFO 3080 [11], amino acid racemase with low substrate specificity (EC class 5.1.1) from *Pseudomonas striata* [14], D-amino acid aminotransferase (D-aspartate: 2-oxoglutarate aminotransferase EC 2.6.1.10) from *B. sphaericus* IFO 3525 [15] and L-lysine: α ketoglutarate ϵ-aminotransferase (EC class 2.6.1) from *Achromobacter liquidum* IFO 3084 [16]. The enzymes, except alanine dehydrogenase and glutaminase B, were obtained in their crystalline forms. Glutamate dehydrogenase (L-glutamate: NAD(P) oxidoreductase (deaminating) EC 1.4.1.3) of bovine liver (Type II) was purchased from Boehringer Mannheim GmbH.

2.2. *Methods*

Amino acid racemase with low substrate specificity, D-amino acid aminotransferase and L-lysine: α-ketoglutarate ϵ-aminotransferase were dialyzed overnight at 4°C against about 500 vol of 0.01 M sodium phosphate buffer (pH 7.6) containing 0.9% NaCl and 10^{-5} M pyridoxal 5'-phosphate. The other enzymes

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Table 1
Effect of highly purified enzymes on growth of Ehrlich ascites carcinoma in mice.

Experiment no.	Enzymes	Dose X days (mg/kg/day)	Body weight increased on 14th day (g)		Median survival day of mice (range)		T/C (%)	Treated 60 days survivors	
			Control	Treated	Control	Treated		Control	Treated
1	Leucine dehydrogenase (S.A.: 100)†	1 X 14	+ 8.3	+ 4.2	13.6 (10–20)	29.8 (29–41+)	219	0/5	4/5
		4 X 14	+ 8.3	+ 4.2	13.6 (10–20)	41 (41+)	300	0/5	4/5
2	Leucine dehydrogenase (S.A.: 200)	2 X 10	+11.5	+10.0	17.9 (12–21)	24.4 (14–54+)	136	0/10	1/5
		4 X 10	+11.5	+ 5.7	17.9 (12–21)	42.4 (14–54+)	236	0/10	3/5
		8 X 10	+11.5	+ 5.6	17.9 (12–21)	32.2 (17–54+)	180	0/10	2/5
3	Leucine dehydrogenase (S.A.: 130)	1 X 14	+14.1	+ 7.3	15.3 (11–17)	26.0 (18–46+)	170	0/10	1/5
		2 X 10	+14.1	+ 5.0	15.3 (11–17)	29.2 (18–46+)	191	0/10	2/5
	Glutaminase A (S.A.: 25 IU/mg protein)	50 X 10*	+14.1	+ 3.7	15.3 (11–17)	30.8 (18–46+)	201	0/10	2/5
		100 X 9*	+14.1	+ 2.1	15.3 (11–17)	24.6 (20–29)	161	0/10	0/5

The mice were inoculated intraperitoneally with 2×10^6 cells/mouse of Ehrlich ascites carcinoma, and intraperitoneal treatment with purified enzymes was carried out for 9 to 14 days consecutively starting 24 hr later.

* IU/kg/day.

† S.A. = *specific activity, in μmoles per min per mg protein.*

were dialyzed against the buffer lacking pyridoxal 5'-phosphate.

Ehrlich ascites carcinoma cells were inoculated by intraperitoneal injection of 0.5 ml aliquots containing 2×10^6 cells in 20 to 22 g DD mice. The enzymes were injected intraperitoneally once daily for 6 to 14 days consecutively, starting 24 hr after tumor inoculation. Antitumor activity was evaluated by the increase in life span and weight gain from tumor growth. Increase in life span (T/C %) was considered significant at the point of three times longer (300%) of a median survival day of controls, and survivors were observed for 60 days.

3. Results and discussion

L-leucine dehydrogenase and glutaminase A were shown to be highly inhibitory to Ehrlich ascites carcinoma *in vivo* (table 1). Tumor-bearing mice treated with leucine dehydrogenase showed a progressive increase in life span with increasing doses of the enzyme. The dosage of 4 mg/kg X 10 to 14 days produced 236 to 300% increase in median survival time over controls, and smaller doses still provided increased median survival time: 219 to 170% at 1 mg/kg X 14 days, and 136 and 191% at 2 mg/kg X 10 and 14 days. Furthermore, cures (60-day survivors) were observed in 5 out of 10 mice at 1 mg/kg X 14 days, 2 out of 10 mice at 2 mg/kg X 10 to 14 days, and 7 out of 10 mice at 4 mg/kg X 10 to 14 days of treatment with leucine dehydrogenase. Neither α-ketoisocaproate, which is a product of the oxidative deamination of L-leucine, glutamate dehydrogenase, nor alanine dehydrogenase inhibited the growth of Ehrlich ascites carcinoma cells *in vivo*.

An isozyme of glutaminase from *Ps. aeruginosa*, glutaminase A, resulted in significant prolongation of survival time (210%) and complete regression in 40% of tumor-bearing mice at 50 IU/kg X 10 days, but higher dose, 100 IU/kg X 9 days, was associated with a transient loss of about 40% body weight and showed 161% increase in survival time of mice, while glutaminase B did not significantly change life span (106% at 25 IU/kg X 14 days and 120% at 50 IU/kg X 14 days). This discrepancy may result from a difference in requirement by Ehrlich ascites carcinoma for asparagine or in affinity of the enzymes for glutamine, because glutaminase A hydrolyzes both L-glutamine (K_m = 1.2×10^{-4} M) and L-asparagine (K_m = 6.8×10^{-5} M), but glutaminase B has essentially no asparaginase

activity and the K_m value for glutamine is 1.8×10^{-4} M [12]. Further experiments are in progress to elucidate the relationship between the physicochemical and catalytic properties of the enzymes, and antitumor activity. Other purified bacterial enzymes, aminotransferases and racemase, showed substantially no antitumor activity.

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