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Dihydroorotate Dehydrogenase in Liver and Morris Hepatomas

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SUMMARY

Dihydroorotate dehydrogenase (DHODH) activity in the 3683, 3924A, 7288C, and 5123D Morris hepatomas was one-half that of normal rat liver. However, the DHODH activity in Morris hepatoma 7800 was the same as that of rat liver. No correlation existed between the activities of DHODH and the rates of growth of the hepatomas.

The K_m's of the DHODH of the hepatomas ranged from 2.5 to 11.5×10^{-5} M and were similar to the K_m's of 4.2×10^{-5} for liver from Buffalo rats and 8.25×10^{-5} M for liver from ACI rats. Both rat livers and the five hepatomas had a pH optimum of 8.05 ± 0.1 for DHODH The activity of DHODH from liver was not influenced by fasting, refeeding, or partial hepatectomy in Buffalo and ACI rats, except for an increase in the livers of fasting ACI rats.

INTRODUCTION

Our 1st report on the activities of the enzymes in the pyrimidine pathway showed that both carbamyl aspartate transferase and dihydroorotase activities in the Morris hepatomas correlated with the growth rates of the tumors (7). These studies were initiated to determine the regulatory mechanism of *de novo* pyrimidine biosynthesis in tumors. This paper presents the data on DHODH[1] (3), the 3rd enzyme in the pyrimidine pathway (Chart 1), in liver and in some of the Morris hepatomas.

MATERIALS AND METHODS

Animals and Tissues. ACI rats were hosts for the 2 rapidly growing hepatomas, 3683 and 3924A. The slower growing 7288C and the 2 slowest growing hepatomas in this study, 5123D and 7800, were carried in Buffalo rats. Table 1 shows the relative growth rates of the hepatomas. These hepatomas were originally sent to us by Dr. Harold P. Morris and they have been maintained at The Lilly Research Laboratories for the past several years. We transplanted the hepatomas by a s.c. trocar implant of a tumor fragment in the axillary regions of the rats. Hepatomas used in assays were excised when they reached 1 to 2 cm in diameter. Tumor tissue was scraped from the capsule, separated from hemorrhagic and necrotic debris, and homogenized in water. Partial hepatectomies were performed according to the procedure of Higgins and Anderson (2).

Enzymes. DHODH activity was measured in 10% homogenates of liver or hepatomas with absorbance at 295 nm at pH 1 as a measure of orotate production (8). This method was originally not suitable for crude homogenates until we reduced the high background absorbance by adding NaOH and $ZnSO_4$. The assay mixture was 0.6 ml 0.1 M Tris-HCl buffer, pH 8.0; 0.6 ml 0.01 M $MgCl_2$; 1.0 ml 3 mM dihydroorotate; 0.3 ml 10% w/v homogenate in water; and 0.5 ml H_2O. The mixture was incubated at 37° for 0.5 hr. The reaction was stopped by adding 0.2 ml 0.5 N NaOH and 0.2 ml 10% $ZnSO_4$. The pH was adjusted to 1 with 0.2 ml 11 N percholoric acid, which further reduced the background absorbance. The mixture was spun at 2700 rpm for 5 min in an IEC clinical centrifuge. The supernatant was decanted and the absorbance at 295 nm was measured. Dihydroorotic acid had no UV absorption at 295 nm. A unit of DHODH activity is defined as the formation of 1 μmole of orotic acid in 1 hr by 1 g of tissue at 37°. K_m's in the crude homogenates were calculated by the *s versus s/v* method.

RESULTS

Enzyme Kinetics. The optimum pH for DHODH activity in the homogenates of the liver or hepatomas was 8.0 ± 0.15 in Tris-HCl buffer, 0.02 M final concentration. The pH readings were taken on a duplicate set of tubes before and after incubation. No changes in pH occurred during this time. The reaction was linear for at least 30 min and maximum activity was reached at 0.5 μmoles of dihydroorotate per ml. Only 10% of the initial substrate was utilized by the most active tissues.

All data on K_m values were determined in the 10% homogenate with no further purification. There were no biologically significant differences between the K_m's for the ACI liver (8.2×10^{-5} M); the Buffalo liver (4.2×10^{-5} M); or Morris hepatomas 3683 (4.6×10^{-5} M), 3924A (11.8×10^{-5} M), 7288C (2.5×10^{-5} M), 5123D (3.5×10^{-5} M), and 7800 (6.5×10^{-5} M).

DHODH in Liver. ACI and Buffalo rats were fasted for 24, 48, or 72 hr or fasted for 72 hr and then refed for 24 hr. The stress of fasting had no significant effect on DHODH activity in the Buffalo liver, which was 86 to 97% of normal. However, there were significant increases in DHODH activity after 24, 48, and 72 hr fasting in the ACI liver (126 to 135%, $p < 0.05$). In the livers from ACI rats fasted for 72 hr, then refed for 24 hr, the DHODH activity was 96% of normal (Table 2).

Effect of Partial Hepatectomy. The differences in the DHODH activities in homogenates of 24-, 48-, or 72-hr

[1] The abbreviation used is: DHODH, dihydroorotate dehydrogenase.

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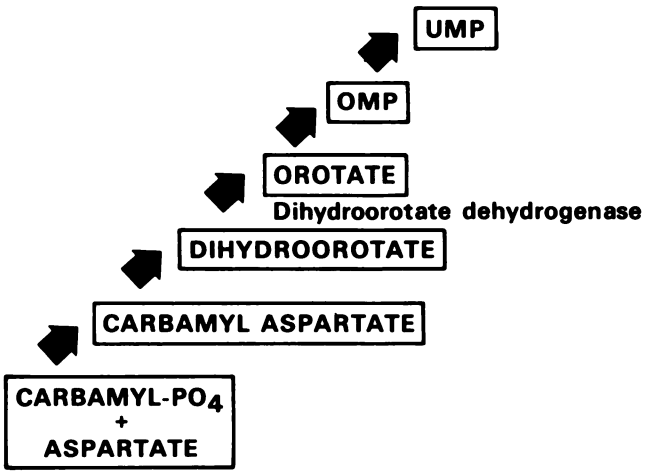

Chart 1. Outline of the biosynthesis of pyrimidines. DHODH mediates the removal of hydrogen from dihydroorotate to form orotate.

Table 1

Relative growth rates of Morris hepatomas

Growth rates were calculated from values presented by Morris (4), with the exception of the values for the liver. During the growth period of the rat, the liver also increases in size and its growth rate is comparable to that of hepatoma 7800.

Source	Av. mo./ generation	Generations/ mo.	Relative growth rate (%)
3683	0.5	2.00	100
3924A	0.6	1.67	84
7288C	1.5	0.67	33
5123D	2.2	0.45	23
7800	3.1	0.32	16
Liver	3.0	0.33	17

Table 2

Activity of DHODH in liver from fed and fasted rats

Liver from	Strain of rat	No. of experiments	DHODH (units[a])	% of normal liver
Normal rat	ACI	8	20.1 ± 0.7[b]	100
	Buffalo	8	20.9 ± 1.5	100
Rats fasted				
24 hr	ACI	3	25.6 ± 0.2	128[c]
	Buffalo	4	19.4 ± 0.3	97
48 hr	ACI	4	25.3 ± 1.4	126[c]
	Buffalo	4	17.8 ± 1.1	89
72 hr	ACI	4	27.0 ± 0.5	135[c]
	Buffalo	4	18.4 ± 1.1	92
Fasted 72 hr, refed 24 hr	ACI	2	19.3 ± 0.4	96
	Buffalo	2	17.2 ± 1.1	86

[a] A unit is 1 μmole of orotic acid formed in 1 hr per g of tissue at 37°.
[b] Mean ± S.E.
[c] $p < 0.05$.

regenerating livers were not significant when compared to the activity, 19.7 to 27.4 units, of liver from sham-operated rats.

DHODH Activity in Host Livers and Morris Hepatomas. The DHODH activity in the host livers from the rats bearing either the 3683 or 3924A hepatomas showed an increase compared to the activity in normal liver, but only the activity between the 3683 host liver and normal liver was significant (129%; $p < 0.05$). In the other 3 hepatoma-bearing rats, the DHODH activity in the host livers showed significant decreases (24 to 47%; $p < 0.05$).

The DHODH activity was measured in homogenates of each of the hepatomas and was compared to values for normal liver. In hepatomas 3683, 3924A, 7288C, and 5123D, the DHODH activities were 44 to 58% of the normal liver values. No significant differences in DHODH activity were found between the 3683, 3924A, 7288C, and 5123D hepatomas. However, the difference in activity between hepatoma 7800 and the other 4 hepatomas was significant, $p < 0.05$. Two rats bearing either the 3924A or 7800 hepatomas were fasted for 3 days. The DHODH activity in these tumors was not altered by the fasting of the hosts (Table 3).

DISCUSSION

The DHODH activity in the Morris hepatomas studied was one-half that of normal liver with the exception of the slow-growing 7800 hepatoma. There was no significant difference between the DHODH activities of hepatomas 5123D, a slow grower, and 3683, the fastest growing hepatoma. The comparison between DHODH activity and growth rate shown in Chart 2 indicates no correlation between these 2 parameters. This is in contrast to the correlations that we reported for carbamyl aspartate transferase and dihydroorotase activities with the growth rates of the same Morris hepatomas (7). The lack of increased DHODH activity in regenerating liver also discounts its role in the regulation of

Table 3

Activity of DHODH in host livers and Morris hepatomas

Liver or hepatoma	Strain of rat	No. of experiments	DHODH (units[a])	% of normal liver
Normal liver	ACI	8	20.1 ± 0.7[b]	100
	Buffalo	8	20.9 ± 1.5	100
Liver from rat bearing hepatoma				
3683	ACI	5	25.8 ± 1.5	129[c]
3924A	ACI	6	23.4 ± 1.9	117
7288C	Buffalo	6	15.8 ± 0.5	76[c]
5123D	Buffalo	5	11.1 ± 0.9	53[c]
7800	Buffalo	7	11.4 ± 0.6	55[c]
Hepatoma				
3683	ACI	7	9.0 ± 0.8	45[c]
3924A	ACI	8	8.7 ± 0.7	44[c]
7288C	Buffalo	8	11.6 ± 0.7	58[c]
5123D	Buffalo	7	9.1 ± 0.7	46[c]
7800	Buffalo	8	21.3 ± 1.4	107[d]
Hepatomas from 72-hr fasted rat				
3924A	ACI	2	10.5	50[c]
7800	Buffalo	2	23.8	115

[a] A unit is 1 μmole of orotic acid formed in 1 hr per g of tissue at 37°
[b] Mean ± S.E.
[c] $p < 0.05$.
[d] $p < 0.05$, statistically significant difference between the activities of hepatomas 7800 and 5123D.

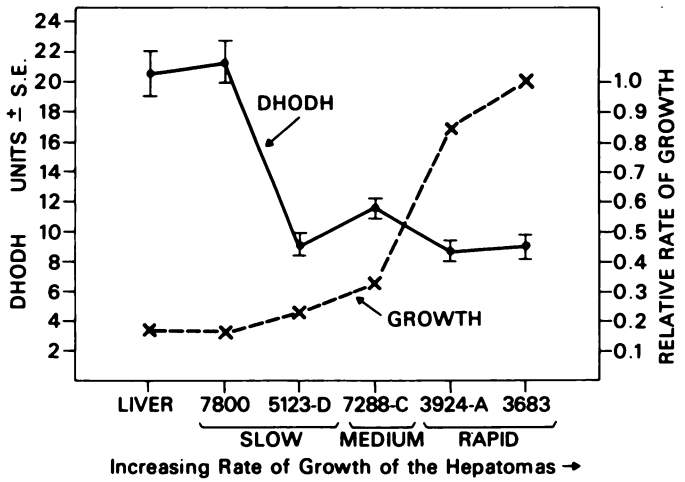

Chart 2. Relationship between the activity of DHODH and the relative rates of growth of the Morris hepatomas.

pyrimidine biosynthesis. This agrees with the conclusion of Bresnick (1), who measured the combined dihyroorotase-DHODH activity in regenerating liver.

This is the 1st report of DHODH activity in the Morris hepatomas. It is also the 1st direct measurement of DHODH in mammalian systems. Smith *et al.* (5, 6) have reported the measurement of DHODH by isotopic dilution methods in human lymphocytes and leukemic cells. They found elevated DHODH activities in the leukemic cells.

Although DPN^+ or TPN^+ is a cofactor in DHODH in bacteria, we could not demonstrate such a dependency in these hepatomas or in liver. Smith *et al.* (5) were not able to show a DPN^+ or TPN^+ dependence for DHODH in human lymphocytes. Further purification of the DHODH may reveal a cofactor requirement.

The K_m of 10^{-5} M for DHODH of rat liver and the hepatomas was lower than the 10^{-4} M reported by Smith *et al.* (5) for human lymphocytes and 2 logs less than the K_m's for carbamyl aspartate transferase and dihydroorotase (7).

We are completing studies on the next 2 enzymes in the pyrimidine pathway, orotic acid phosphoribosyltransferase and orotidylic acid decarboxylase. The data on the 5 enzymes and their interaction will lead to a better understanding of the biosynthesis of pyrimidines in normal and neoplastic tissues.

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