[CANCER RESEARCH 33, 1109-1112, May 1973]

Orotate Phosphoribosyl Transferase and Orotidylic Acid Decarboxylase Activities in Liver and Morris Hepatomas

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SUMMARY

The activities of orotate phosphoribosyl transferase (OATase) and orotidine 5'-monophosphate decarboxylase (OMPase) in the 3683, 3924A, 7288C, 5123D, and 7800 Morris hepatomas were higher than those of normal liver. However, neither of these enzyme activities correlated with the growth rates of the hepatomas.

OATase and OMPase activities in 24-hr regenerating livers were 149 to 169% (and after 48 hr were 218 to 244%) those of livers from sham-operated rats. OATase and OMPase activities in livers from sham-operated rats were 94 to 125% those of normal liver. Fasting, or fasting and refeeding had no effect on either enzyme.

The K_m's for OATase in liver and hepatomas were 1.8 to 3.6 $\times$ 10^{-6} M for orotic acid, and the K_m's for OMPase were 6.7 to 8.8 $\times$ 10^{-7} M for orotidine monophosphate. The pH for maximum activity was 7.1 for OATase and 6.2 for OMPase in liver or the hepatomas.

INTRODUCTION

We have reported previously on the activities of carbamylaspartate transferase, dihydroorotase, and dihydroorotate dehydrogenase in Morris hepatomas (11, 12). To ascertain the importance of each enzyme in the *de novo* formation of pyrimidines, we have compared the activities of these enzymes in relation to the growth rates of the hepatomas. Those enzyme activities that correlate with growth rate may be rate-limiting steps, if increased growth necessitates increased enzymatic activity.

In this study we report on OATase[1] (EC 2.4.2.10) and OMPase (EC 4.1.1.23) activities, the last 2 enzymes in the *de novo* synthesis of UMP from aspartic acid.

MATERIALS AND METHODS

The Morris hepatomas studied were the slow-growing 7800 and 5123D, the intermediate 7288C, and the rapidly growing 3924A and 3683. Their growth rates are listed in Table 1. Buffalo rats weighing 150 to 200 g were the hosts for the 7800, 5123D, and 7288C hepatomas. ACI rats were hosts for the 3924A and 3683 hepatomas. All hepatomas were maintained and transferred at The Lilly Laboratories (Indianapolis, Ind.) by s.c. trocar implantation of tumor fragments into the axillary region of the rats. All animals received food and water *ad libitum* unless fasting was indicated. Tumors were excised when they were 1 to 2 cm in diameter, and the tissue was scraped from the capsule, separated from necrotic and hemorrhagic debris, and chilled until homogenized. A 10% homogenate of liver or hepatoma was prepared in H_2O, with a Teflon pestle at 600 rpm for 90 sec. The method of Higgins and Anderson (7) was used for partial hepatectomy.

Assay Procedures. OATase activity was measured by the production of $^{14}CO_2$ from the 2-step conversion of orotic acid-7-^{14}C to UMP. The incubation mixture contained 1.2 ml of water; 0.4 ml of phosphate buffer, 0.1 M, pH 7.1; 0.2 ml of $MgCl_2$, 0.06 M; 0.5 ml of PRPP 6 mM; 0.5 ml of orotate-7-^{14}C, 0.3 mM, 0.42 mCi/mmole; and 0.2 ml of a 10% tissue homogenate.[2] The reaction mixture was incubated for 20 min at 37° and was stopped by the addition of 0.2 ml of 11 N perchloric acid. The released $^{14}CO_2$ was collected in disposable center wells containing NCS (Nuclear-Chicago solution). A unit of OATase activity is defined as the formation of 1 μmole of CO_2 per g of tissue in 1 hr at 37°.

OMPase activity was measured by the release of $^{14}CO_2$ from OMP-7-^{14}C. The incubation mixture contained 0.3 ml of water; 0.1 ml phosphate buffer, 0.1 M, pH 6.2; 0.5 ml OMP-7-^{14}C, 0.155 mM, 0.645 mCi/mmole; and 0.1 ml of a 10% homogenate. The mixture was incubated for 5 min at 37°, and $^{14}CO_2$ was collected as described in the OATase assay. A unit of OMPase activity is defined as the formation of 1 μmole of CO_2 per g of tissue in 1 hr at 37°.

RESULTS

Enzyme Kinetics. There were no biologically significant differences between the K_m's (orotic acid) of OATase for the ACI rat liver (3.4 $\times$ 10^{-6} M), the Buffalo rat liver (2.8 $\times$ 10^{-6} M), or the Morris hepatomas [3683 (3.6 $\times$ 10^{-6} M), 3924A (1.8 $\times$ 10^{-6} M), 7288C (3.6 $\times$ 10^{-6} M), 5123D (2.5 $\times$ 10^{-6} M), and the 7800 (2.5 $\times$ 10^{-6} M)]. Also, there were no

[1] The abbreviations used are: OATase, orotate phosphoribosyl transferase; OMPase, orotidine 5'-monophosphate decarboxylase; PRPP, 5-phosphoribosyl-1-pyrophosphate; OMP, orotidine 5'-monophosphate.

Received November 22, 1972; accepted February 15, 1973.

[2] Purified yeast OMPase (Sigma Chemical Co., St. Louis, Mo.), added at an amount equal to 10 to 15 times endogeneous decarboxylase activity, as measured under OATase conditions, did not significantly increase the OATase results in initial studies. Therefore, yeast OMPase was not used in the determination of data presented in this paper.

Table 1

Tissue sources	Generations/mo.[a]	Relative growth rate (%)
3683	2	100
3924A	1.67	84
7288C	0.67	34
5123D	0.45	23
7800	0.32	16
Liver	0.33	17

[a] Calculated from values presented by Morris (9) with the exception of the value of the liver. During the growth period of the rat, the liver also increases in size and the growth rate is comparable to that of the 7800 hepatoma.

biologically significant differences between the K_m's (OMP) of OMPase for the ACI rat liver (7.0×10^{-7} M), the Buffalo rat liver (8.0×10^{-7} M), or the Morris hepatomas [3683 (6.9×10^{-7} M), 3924A (6.7×10^{-7} M), 7288C (8.8×10^{-7} M), 5123D (7.3×10^{-7} M), and the 7800 (7.8×10^{-7} M)]. A representative K_m plot for OMPase is given for Buffalo rat liver (Chart 1). Maximum enzyme activities were attained at 25 μM orotate and 5 μM OMP as determined by concentration curves. A final concentration of 1 mM PRPP and 4 mM Mg^{++} gave maximum OATase activity and was used for all experiments (Chart 2).

There were no differences between the pH optimum of either OATase (pH 7.1) or OMPase (pH 6.2) from liver or hepatomas, and both enzyme activities were linear for at least 30 min.

OATase and OMPase Activities in Fasted and Regenerating Liver. The OATase activity in the livers of normal ACI and Buffalo rats fasted 24, 48, or 72 hr (or fasted 72 hr and refed 24 hr) were 103 to 117% those of normal liver, and OMPase activities were 95 to 110% those of normal liver.

In 24-hr regenerating liver, the OATase and OMPase activities were 149 to 169% those of liver from 24-hr sham-operated rats, and in 48-hr regenerating liver they were 218 to 244% those of the liver from 48-hr sham-operated rats (Table 2). The OATase and OMPase activities of liver from sham-operated rats were 94 to 125% those of normal liver.

OATase and OMPase Activities in Host Livers and Hepatomas. The OATase and OMPase activities in the host livers from rats bearing the 3683 hepatoma were 174 and 162% those of normal liver. OATase activities in the 7288C and 7800 host liver were 122% of normal liver. Both enzyme activities in the other host livers were comparable to those of normal liver (Table 3).

In the Morris hepatomas, both OATase and OMPase activities were significantly higher than the activities of normal liver. OATase activity in the hepatomas was 4.29 to 11.31 units compared with 3.45 to 3.74 units for normal liver; OMPase activity was 9.67 to 23.51 units compared with 7.18 to 7.55 for normal liver. Individually, hepatoma 3683 OATase was 328% of normal and OMPase was 327% of normal. Compared with that for normal liver, the OATase and OMPase activities in hepatoma 3924A were 124 and 135%, respectively; for hepatoma 7288C, they were 149 and 174%, respectively; for hepatoma 5123D, they were 242 and 232% respectively; and for hepatoma 7800, they were 268 and 269%, respectively (Table 3).

DISCUSSION

In the Morris hepatomas, the activities of OATase and OMPase were significantly different from those of normal

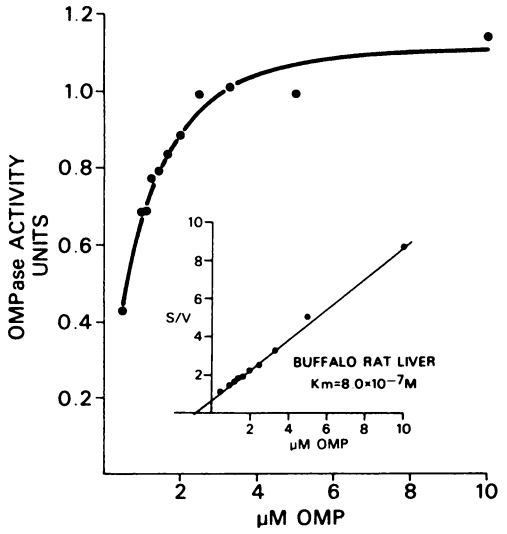

Chart 1. A concentration curve representative of those of the ACI rat liver and the 5 Morris hepatomas for OMPase. The time of incubation was 1 min with a 5% homogenate of Buffalo rat liver. These are the only exceptions to the procedure as described in "Materials and Methods." The shorter reaction time and lower enzyme concentration were necessary to prevent the complete utilization of the substrate at the lowest concentrations. *Inset*, K_m calculated with the use of a standard programmed *s versus s/v* plot.

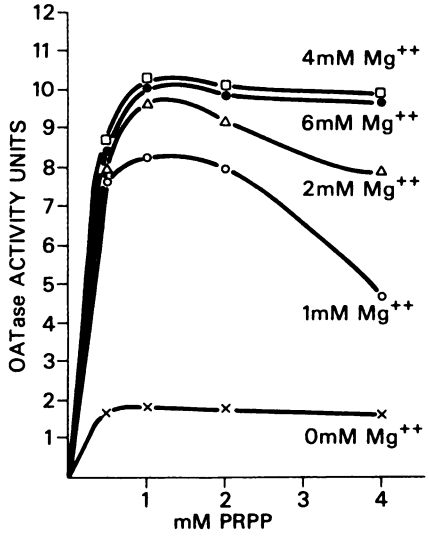

Chart 2. Dependence of OATase on PRPP and Mg^{++} for maximum activity. The assay system was the same as that described in "Materials and Methods" with the exceptions of the concentrations of Mg^{++} and PRPP as shown.

Table 2

OATase and OMPase activities in regenerating liver

Liver from	Rat strain	OATase Units[a]	OATase % of control liver	OMPase Units[b]	OMPase % of control liver
24 hr after sham operation	ACI	4.31 ± 0.38[c]	100	8.24 ± 0.72[c]	100
	Buffalo	3.98 ± 0.26	100	7.59 ± 0.66	100
24-hr regenerating liver	ACI	7.27 ± 0.58	169	13.17 ± 0.58	160
	Buffalo	6.45 ± 0.05	162	11.34 ± 0.02	149
48 hr after sham operation	ACI	3.93 ± 0.00	100	7.73 ± 0.13	100
	Buffalo	3.74 ± 0.21	100	7.08 ± 0.49	100
48-hr regenerating liver	ACI	9.46 ± 0.58	241	16.84 ± 0.62	218
	Buffalo	8.40 ± 0.17	225	17.29 ± 0.40	244

[a] Unit, μmoles orotate (per g of tissue) converted to CO_2 in 1 hr at 37°.
[b] Unit, μmoles OMP (per g of tissue) converted to CO_2 in 1 hr at 37°.
[c] Sham-operated and regenerating liver values are the average of 2 experiments (mean ± S.E.). Both OATase and OMPase activities were measured in the same homogenates.

Table 3

OATase and OMPase activities in host livers and Morris hepatomas

Tissues	Rat strain	OATase Units[a]	OATase % of normal liver	OMPase Units[b]	OMPase % of normal liver
Normal liver[c]	ACI	3.45 ± 0.12[e]	100	7.18 ± 0.22	100
	Buffalo	3.74 ± 0.18	100	7.55 ± 0.31	100
Host liver[d] from rats bearing hepatoma					
3683	ACI	5.99 ± 0.65	174[f]	11.66 ± 0.80	162[f]
3924A	ACI	3.87 ± 0.24	112	7.21 ± 0.43	100
7288C	Buffalo	4.55 ± 0.04	122[f]	8.65 ± 0.31	115
5123D	Buffalo	3.79 ± 0.07	101	7.54 ± 0.10	100
7800	Buffalo	4.55 ± 0.71	122	8.56 ± 0.86	113
Hepatoma[c]					
3683	ACI	11.31 ± 1.00	328[f]	23.51 ± 2.15	327[f]
3924A	ACI	4.29 ± 0.34	124[f]	9.67 ± 0.84	135[f]
7288C	Buffalo	5.58 ± 0.39	149[f]	13.12 ± 0.69	174[f]
5123D	Buffalo	9.06 ± 0.57	242[f]	17.49 ± 0.98	232[f]
7800	Buffalo	10.03 ± 0.34	268[f]	20.31 ± 0.68	269[f]

[a] Unit, μmoles orotate (per g of tissue) converted to CO_2 in 1 hr at 37°.
[b] Unit, μmoles OMP (per g of tissue) converted to CO_2 in 1 hr at 37°.
[c] Normal liver and hepatoma values are the average of 5 to 7 experiments.
[d] Host liver values are the average of 2 experiments.
[e] Mean ± S.E.
[f] Statistically significant difference ($p = < 0.05$) compared to normal liver.

liver. A comparison of enzyme activities with the growth rates of the hepatomas showed no correlation (Chart 3, *A* and *B*). Previously, we reported that the activities of carbamylaspartate transferase and dihydroorotatase correlated with growth rate in the Morris hepatomas, and that they may be the regulators of UMP synthesis (12). The activities of dihydroorotate dehydrogenase (11), OATase, and OMPase do not correlate with growth rate, and their importance in the regulation of pyrimidine biosynthesis is not apparent.

We found increases in activities for both OATase and OMPase in regenerating liver at 24 and 48 hr. Bresnick (3) reported that OATase activity in 24-hr regenerating liver was 375% that of liver from sham-operated rats. He did not find any differences in OMPase activity after up to 24 hr of regeneration. Fausto (5) found that the activity of OATase in liver homogenates was 3 to 4 times normal, 18 hr after partial hepatectomy. Pausch *et al.* (10) reported a similar increase in both OATase and OMPase activities in 5-day regenerating liver.

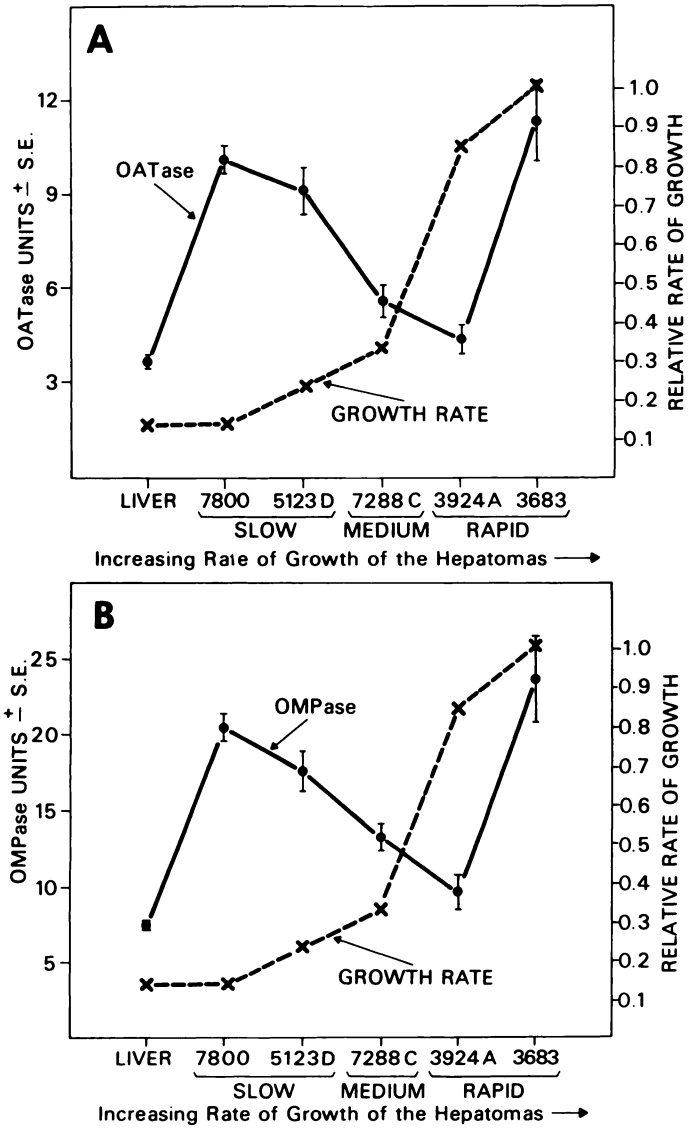

Chart 3. Relationship between the activity of OATase (*A*) and OMPase (*B*) and relative growth rates of the Morris hepatomas. The growth rates for liver and hepatomas are given in Table 1.

The activities of OATase and OMPase parallel each other, indicating that both activities might be regulated by the same enzyme or genome. Beckwith *et al.* (2) found that in *Escherichia coli*, the last 4 enzymes responsible for the *de novo* synthesis of UMP varied coordinately and that the loci determining the structure of the enzymes were closely linked on the same chromosome. However, in calf thymus, OATase and OMPase were shown to be 2 separate enzymes (8).

All K_m's were measured, with the use of 5% homogenates. The apparent K_m for OATase, with the 2-step enzyme reaction, ranged from 1.8 to 3.6 $\times$ 10^{-6} M. The K_m's for OMPase ranged from 6.7 to 8.8 $\times$ 10^{-7} M. These K_m's are not directly comparable to those in literature, since the experimental conditions are different: pH 8.0 *versus* 6.2 and 25° *versus* 37° (1, 4).

The pH maxima in our studies, 7.1 for OATase and 6.2 for OMPase, agree with those reported by others (4, 6). No differences have been reported for the pH optima in any of the enzymes in the pyrimidine pathway between normal rat liver and the Morris hepatomas (11, 12).

ACKNOWLEDGMENTS

The assistance of Joseph Parton in transplanting and maintaining the hepatomas is gratefully appreciated.

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