INHIBITION OF THYMIDINE KINASE ACTIVITY IN LIVER AND HEPATOMAS BY TTP and d-CTP [1]

Edward Bresnick*, U. B. Thompson*, H. P. Morris** and A. G. Liebelt***

*Department of Pharmacology, ***Department of Anatomy, Baylor University College of Medicine, Houston, Texas, and **National Cancer Institute, Bethesda, Maryland

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Thymidine kinase possesses many of the attributes of a rate-determining enzyme for DNA[2] biosynthesis. Enzymatic activity is markedly enhanced in regenerating liver (1) and in other tissues undergoing rapid cellular proliferation (2-5), in livers of rats sustained on a high protein diet (6), and in cultured mammalian cells after viral infection (7,8). Furthermore, thymidine kinase activity is inhibited by its distal product, TTP, as reported by Maley and Maley (9) in chick embryo, Ives et al (10) in the Novikoff hepatoma, Breitman (11) in regenerating rat liver and by this laboratory (12) in human leukocytes. In the latter study, enzymatic activity was equally inhibited by TTP and d-CTP. Accordingly, we have investigated the comparable ability of these nucleotides to regulate thymidine kinase in liver and in a series of hepatomas. The results suggest a relationship between the source of the enzyme and the requirements for inhibition.

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[2] Abbreviations: DNA, deoxyribonucleic acid; TMP, thymidine-5'-monophosphate; TTP, thymidine-5'-triphosphate; d-CTP, deoxycytidine-5'-triphosphate; ATP, adenosine-5'-triphosphate; MC, methylcholanthrene; MDAB, 3-methyl-4'-dimethylaminoazobenzene.

Experimental - The livers and the solid hepatomas were homogenized in cold 0.25M sucrose in a Potter-Elvehjem homogenizer and were centrifuged at 100,000xg for 60 minutes to yield a soluble extract containing the activity. The Novikoff ascites cells (grown 7 days in vivo) were washed twice with 0.25M sucrose, suspended in 0.25M sucrose and were subjected to ultrasonic oscillation for 1.5 min at 4°C. The sonicated material was centrifuged at 100,000xg for 30 minutes. The soluble extracts in all cases were brought to pH 4.5 with 1N acetic acid; the precipitate was removed by centrifugation. The pH of the extract was adjusted to 7.5, and ammonium sulfate was added to 30% saturation. The enzyme was redissolved in cold 0.05M tris buffer, pH 8.0 and thymidine kinase activity was assayed as described in the legends. The protein concentration of the extracts was determined by the Lowry method (13) with bovine serum albumin as the reference standard.

Results - The effects of d-CTP and TTP upon thymidine kinase which was partially purified from adult rat liver, regenerating rat liver[3] and 17 day embryonic rat liver are compared in Table 1. Both d-CTP and TTP inhibited enzymatic activity of normal liver by approximately 40-50%; d-CTP was slightly more effective at this concentration. In contrast, d-CTP had little or no effect upon the partially purified enzyme from either embryonic liver or regenerating liver. The inhibition, however, by TTP was more pronounced, i.e., 70-80%.

The effects of various concentrations of d-CTP and TTP upon thymidine kinase from adult liver and embryonic liver are presented in Table II. These data reaffirm the lack of inhibition of enzymatic activity by d-CTP and the pronounced effect of TTP on embryonic liver.

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[3] The partial hepatectomies were kindly performed by Dr. H. Mavioglu of the Department of Pharmacology.

TABLE I

INHIBITION OF THYMIDINE KINASE FROM ADULT RAT LIVER, REGENERATING RAT LIVER AND EMBRYONIC RAT LIVER

Source of Enzyme	Protein ug.	Additions:	TMP (μμmoles) None	d-CTP	TTP
Adult Liver	16		1.4	0.7	0.8
	40		3.1	1.6	1.9
	80		5.9	2.7	3.9
Embryonic Liver	20		70	61	7.7
	50		202	200	28
Regenerating Liver	26		17	16	5.0
	65		45	39	11

Reaction mixture: Thymidine-2-C^{14} (0.5μc/17 μmoles/0.05ml), 0.05ml; ATP, 5 m$\underline{M}$; $MgCl_2$, 5 m$\underline{M}$; enzyme; inhibitors, 0.08 m$\underline{M}$; 0.2$\underline{M}$ tris buffer, pH 8.0, to 0.25 ml. The test tubes were incubated at 37° for 15 min and then the reaction was stopped by immersing the tubes in ice. Aliquots of 25 μl were withdrawn and placed on DEAE-cellulose paper discs (2cm in diameter) and the discs were washed three times with 0.001$\underline{M}$ ammonium formate, water and finally with methanol. The dried discs were placed in counting vials containing 6 ml of a toluene-phosphor mixture (12) and counted in a Packard scintillation spectrometer with an efficiency of 33%. Thymidine kinase was concentrated from the livers of adult male Holtzman rats approximately 250 g, in wt; from livers obtained from 17 day fetuses and from 24 h regenerating rat liver.

The data presented in Table III show that the hepatomas fall into 2 classes a) those which possess a thymidine kinase sensitive to TTP, like regenerating and embryonic liver; b) those which possess a thymidine kinase which is sensitive to both d-CTP and TTP, like adult liver. The 'minimal-deviation' hepatomas without exception gave results similar to adult liver, while the enzymes concentrated from the Novikoff and Dunning[4] hepatomas were markedly

[4] The authors are indebted to Dr. W. F. Dunning for the gifts of the Dunning LC 18 hepatomas.

TABLE II

INHIBITION OF THYMIDINE KINASE BY d-CTP AND TTP

Source of Enzyme	Additions		Thymidine Kinase Activity mμmoles TMP/mg protein
Adult Liver	None		0.087
	d-CTP,	0.08mM	0.059
		0.16mM	0.048
		0.32mM	0.039
	TTP,	0.08mM	0.060
		0.16mM	0.045
		0.32mM	0.025
Embryonic Liver	None		35
	d-CTP,	0.32mM	31
	TTP,	0.08mM	18
		0.16mM	3

See Table I for the components of the reaction mixture. Each value represents an average obtained from 2-5 different purified preparations.

sensitive to TTP but resistant to d-CTP. The primary hepatoma, the McCoy MDAB, was also sensitive to both nucleotides. A group of very slow-growing mouse hepatomas, the MC-induced 2182,T8, a urethan-induced, and the spontaneous hepatomas of the YBR x AKR strain also fall into the first category.

The thymidine kinase prepared from the livers of most of these hepatoma-bearing animals exhibited patterns similar to normal adult liver, i.e., sensitive to both d-CTP and TTP.

The question arises whether the Novikoff and Dunning hepatoma preparations catabolize d-CTP more readily than the other hepatoma extracts and in this manner render the enzyme insensitive to the nucleotide? Preliminary

TABLE III

INHIBITION OF THYMIDINE KINASE OF HEPATOMAS

Source of Enzyme	Additions: None	Thymidine Kinase (mμmoles TMP/mg protein) TTP	d-CTP
7288-C (5)*	2.1 ± 0.2**	1.0 ± 0.2	0.9 ± 0.1
7800 (5)	3.2 ± 0.6	2.1 ± 0.5	1.5 ± 0.4
7316-A (1)	4.0	2.1	2.0
5123-D (2)***	5.5	2.7	2.1
7793 (1)	4.6	2.1	1.9
7787 (1)	4.0	2.3	1.5
3924-A (1)	3.0	1.2	2.4
7794-A (1)	2.3	1.0	1.5
7795 (1)	3.6	3.2	2.5
3683 (1)	1.8	1.1	1.2
H-35 (1)	1.7	1.2	0.9
McCoy MDAB (5)	4.2 ± 0.7	2.0 ± 0.4	1.4 ± 0.3
MC-induced 2182,T8 (1)	5.6	3.9	2.0
Urethan-induced	0.5	0.2	0.3
YBR x AKR spontaneous (1)	0.6	0.4	0.4
Novikoff ascites (3)	24.3 ± 1.5	5.6 ± 1.4	24.3 ± 1.2
Dunning LC18 (3)	2.3 ± 0.6	1.3 ± 0.4	2.7 ± 0.2

See Table I for the composition of the reaction mixture. The concentration of the inhibitors was 0.16 mM. The MDAB primary hepatomas were kindly supplied by Dr. D. Kizer of the Samuel Roberts Noble Foundation, Ardmore, Oklahoma.

* Number of individual determinations.

** Mean ± standard error.

*** Similar results were obtained with the A and B sublines of the 5123 hepatoma.

results with ^{3}H-dCTP [5] have indicated that during the 15 minute incubation period, little d-CTP is either deaminated or dephosphorylated in any of the tumor preparations tested.

[5] Kindly supplied by Dr. N. B. Furlong of the M. D. Anderson Tumor Hospital, Houston.

Discussion - The relative sensitivity of thymidine kinase to inhibition by d-CTP appears related to the growth rate of the tissue. In the Novikoff and Dunning hepatomas, which are both rapidly-proliferating tissues, thymidine kinase is inhibited by TTP and not by d-CTP. The enzymatic activity is sensitive to both TTP and d-CTP in the 'minimal-deviation' hepatomas, some of which have been growing for almost 1 year, as well as in the induced-mouse hepatomas, which also have been growing for 12-18 months, and in the spontaneous mouse hepatoma. Bukowsky and Roth (14) have recently reported feedback inhibition by TTP of the 5123, MDAB, and Novikoff hepatomas although no mention was made of the inhibitory efficacy of d-CTP.

Embryonic and regenerating liver which also represent a class of tissue undergoing rapid cellular proliferation more closely resembled the Novikoff and Dunning tumors in regard to the type of thymidine kinase. It is interesting that the thymidine kinase of the rapidly-growing bacteria, E. coli, (15) and of the vaccinia-infected L-cells, in culture, [6] is not affected by d-CTP although the enzyme is markedly sensitive to TTP

The data in the present report are suggestive of thymidine kinase enzymes, one of which is sensitive to TTP, while the other is inhibited by both TTP and d-CTP. We are presently purifying the Novikoff and liver enzymes so that we may definitely establish the nature of the inhibitory requirements of these enzymes.

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[6] Private communication from Dr. S. Kit of the Division of Biochemical Virology, Baylor University College of Medicine, Houston.

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