One Evidence Supporting that Thymidylate Synthetase and Thymidine Kinase Are

the Rate-determining Enzymes of DNA Synthesis in Regenerating Rat Liver

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The integration of the activities of thymidylate synthetase

and thymidine kinase is shown to correlate closely with the amount

of DNA synthesis. Based on the result, these enzymes are suggested

to be the rate-determining enzymes of DNA synthesis in regenerating

rat liver.

Although metabolic pathways of DNA synthesis are well established,[1] it is a

very difficult problem to point out the rate-determining step with a reasonable

evidence. This may be due to the following reasons; 1) The quantity of DNA

synthesis, which is the most basic parameter, is not always a sensitive indicator,

2) A suitable model system, where the rate of DNA synthesis can be changed in a

wide range to allow us a quantitative evaluation, is not available, 3) It is

difficult to estimate the effective concentration of low-molecular-weight pre-

cursors, since the volume occupied by these compounds in the cell is not known and

the turnover rate of the substrate is very high in a proliferative phase, further-

more, the compartmentation of the cell causes a complicated situation. For

chemical understanding of the metabolism consisting of a series of enzymatic

reactions, informations on the rate-determining step are most basic and invaluable.

Thymidylate synthetase (TS : E.C.2.1.1.45) and thymidine kinase (TK : E.C.2.7.

1.21) catalyze the formation of thymidylate via the de novo and salvage pathways,

respectively. The activity of these enzymes increases dramatically in rapidly

proliferative cells such as regenerating liver and cancer cells. Therefore, the

production of thymidylate by the enzymes is believed to be rate limiting for DNA

replication.[1] For the reason, these enzymes have been important targets for the development of their inhibitors, which are assumed to be promizing anti-cancer drugs. However, there has been no quantitative study to show the correlation between the enzymatic activities and the amount of DNA synthesized.

As an initial approach to the chemical study on the material flow in DNA synthesis, we have noticed rat liver regeneration, which takes place after two-thirds partial hepatectomy (P.H.).[2-4] The liver cell possesses high capacity for proliferation and thus liver regeneration has been employed as an excellent model to investigate the regulatory mechanism of cellular proliferation in vivo. In liver regeneration, the quantity of DNA synthesis is very large and therefore the DNA content becomes a sensitive and reliable parameter. Besides, activities of the enzymes and amount of DNA synthesis in regenerating liver can be affected in various ways by surgical operation (thyroparathyroidectomy)[4] and drugs.[2,3] These observations allow us direct comparison of the enzymatic activities with the amount of DNA synthesis. In the present paper, we wish to present one method to evaluate the rate-determining step on the basis of the relationship between the enzymatic activity and DNA synthesis. By applying the method on our results,[2,4] we will show that TS and TK satisfy one of the requirements to be rate-determining enzymes in DNA synthesis in regenerating rat liver.

In control rats, activities of hepatic TS and TK started increasing at about 12th hour (h) and peaked at 24-48 h after P.H.[2,5] as shown in Table 1. Liver DNA content which was obtained according to the literature[2,4] was also shown. If TS and TK are the rate-determining enzymes (i.e., activities of the enzymes determine the amount of DNA synthesis), ΔDNA (amount of DNA synthesized between time t_1 and t_2) can be formulated as follows:

$$\Delta DNA = C_1 \int_{t_1}^{t_2} \{TS(t) + TK(t)\} \, Protein(t) \, dt \qquad (1)$$

Here, C_1 is a constant. The specific activities of TS and TK and the protein content (determined by the method of Lowry et al.,[6]) of 36000 g supernatant (of the liver homogenata) where TS and TK localize are all functions of time. Total activity of the enzyme in the liver is given by the product of the specific activity and the protein content of 36000 g supernatant. The integration between

Table 1.   The relationship between the capacity of TS and TK and the quantity of
DNA synthesis in regenerating rat liver. Each value is a mean of 6-21
animals

Treatment	Time after P.H. h	TS ((pmol/mg protein)/min)	TK	Total protein of 36000 g sup. (mg)	Capacity of TS and TK (µmol/24 h)	DNA (mg)	ΔDNA (mg/24 h)	C_2 (mg/mmol)
Control (P.H. only)	24	$64.78^{2)}$	$201.15^{2)}$	253.07		6.87		
					114.18		3.60	31.8
	48	$75.58^{2)}$	$163.00^{2)}$	375.60		10.47		
					126.02		4.37	34.7
	72	$56.40^{2)}$	$111.21^{2)}$	486.22		14.84		
					112.71		3.95	35.1
	96	$34.42^{2)}$	$81.44^{2)}$	618.22		18.79		
TPTX	24	$22.0^{4)}$	$45.5^{4)}$	245.19		$5.16^{4)}$		
					75.54		2.72	36.5
	48	$77.2^{4)}$	$211.0^{4)}$	336.94		$7.88^{4)}$		
					131.72		4.12	32.3
	72	$58.4^{4)}$	$138.5^{4)}$	417.29		$12.00^{4)}$		
Reserpine	24	$37.04^{2)}$	$64.45^{2)}$	263.12		6.33		
					73.45		2.64	35.9
	48	$89.08^{2)}$	$129.19^{2)}$	374.96		8.97		
					118.08		3.80	32.2
	72	$58.69^{2)}$	$90.08^{2)}$	518.63		12.77		
Phenoxy-benzamine	48	$82.58^{2)}$	$76.33^{2)}$	384.78		8.62		
					115.44		3.90	33.8
	72	$54.52^{2)}$	$124.86^{2)}$	563.13		12.52		
					130.81		4.07	31.1
	96	$42.14^{2)}$	$87.57^{2)}$	612.42		16.59		

time t_1 and t_2 $(=t_1 + 24)$ affords the capacity of the enzyme to produce

thymidylate for a given 24 h. For the first approximation, Eq. 1 becomes

$$\Delta DNA \doteqdot C_2 \{ [TS]_{mean} + [TK]_{mean} \} \times [Protein]_{mean} \times 1440 \ min \ (i.e., \ 24 \ h)$$

C_2 is a constant. This is converted into Eq. 2.

$$C_2 = \Delta DNA / \{ ([TS]_{mean} + [TK]_{mean}) \times [Protein]_{mean} \times 1440 \} \tag{2}$$

The denominator of the right side is a capacity of TS and TK to synthesize

thymidylate for a given 24 h. C_2 values are calculated for each 24 h after P.H. These values are shown in Table 1.

In TPTX (thyroparathyroidectomized) rats, DNA synthesis after P.H. is reduced with a concomitant decrease of TS and TK activities.[4] C_2 values for these studies are evaluated and included in Table 1. C_2 values estimated from the experiments utilizing α-blockers[2] (phenoxybenzamine and reserpine), which prevent liver regeneration, are also cited in Table 1.

Since all C_2 values are found to be nearly constant (33.7 ± 1.94 (S.D.)), it is concluded that the integration of activities of TS and TK closely correlates with DNA synthesis (correlation coefficient was 0.93). This result suggests that the activities of these enzymes determine the quantity of DNA synthesis and these enzymes satisfy the requisit to be rate-determining in DNA synthesis in regenerating rat liver. The dimension of C_2 is mg DNA formed/mmol of the capacity for producing thymidylate. $C_2 = 33.7$; it means that 33.7 mg of DNA is produced when TS and TK have a capacity to generate 1 mmol of thymidylate.

We have presented here a reasonable evidence supporting that TS and TK are rate-determining enzymes of DNA synthesis in liver regeneration. The regulatory mechanism of the enzymes seems to be closely linked with that of liver regeneration.

References

1) A. Kornberg, "DNA Replication," Freeman, San Francisco, U.S.A. (1980).

2) R. Nakata, I. Tsukamoto, M. Nanme, S. Makino, M. Miyoshi, and S. Kojo, European J. Pharmacol., 114, 355 (1985).

3) R. Nakata, I. Tsukamoto, M. Miyoshi, and S. Kojo, Biochem. Pharmacol., 35, 865 (1986).

4) R. Nakata, I. Tsukamoto, M. Miyoshi, and S. Kojo, Clin. Sci., 72, 455 (1987).

5) R. Labow, G. F. Maley, and F. Maley, Cancer Res., 29, 366 (1969).

6) O. H. Lowry, N. H. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).