PROTEIN METHYLASE III OF RAT KIDNEY DURING THE EARLY DEVELOPMENT

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1. Introduction

It is now well established that side chains of lysine, arginine, histidine and dicarboxylic amino acids of certain proteins are enzymatically methylated *in vivo* as well as *in vitro* [1] and that these enzymatic transmethylations between S-adenosyl-L-methionine and protein side chains are highly specific with respect to the side chains of the amino acids involved. However, the biological significance of protein methylation remains clouded. In a variety of proliferating cell systems, such as rapidly growing hepatomas [2], fetal brain [3], regenerating adult rat liver [4], and continuously dividing HeLa S-3 cell culture [5, 6], the activity of the enzyme which methylates the ϵ-NH_2 groups of lysine residues of protein (protein methylase III; S-adenosylmethionine: protein–lysine methyltransferase) was found to be elevated. Through continuing efforts to elucidate the biochemical significance of protein methylase III, we have studied the activity of this enzyme during the early development of rat kidney. Data resulting from this investigation are presented in this paper.

2. Methods and experimental procedures

S-Adenosyl-L-methionine-[^{14}C]methyl (specific activity, 28.9 mCi/mmole in an aqueous solution, pH 3.0) was obtained from Amersham-Searle Corp. Histone type II-A (mixture of various histones from calf thymus) from Sigma Chemical Co. was used as substrate for the assay of protein methylase III. Rats of CFN strain were used, and were fed ad libitum on Ralston Purina rat chow and housed in a room with 12 hr of light daily. The animals were sacrificed at about 10.00 a.m. in order to minimize the diurnal fluctuation. The remaining chemicals were obtained from various local sources.

Kidneys were pooled (number of kidneys for each assay depended on the age of the rats, ranging from 17 fetuses to 2–3 rats) and were homogenized in 9 vol of 0.25 M sucrose plus 3×10^{-3} M $CaCl_2$ with an electrically driven Teflon-glass homogenizer. The homogenate was passed through a double layer of cheese cloth and a portion of the whole homogenate was centrifuged at 800 *g* for 10 min to obtain the crude nuclear fraction. All the detailed experimental procedures for the assay of protein methylase III activity have been previously published [2]. Agreement within 10% of values for duplicates on each run was arbitrarily set as a test of validity.

DNA was isolated from the whole homogenate by the method of Schmidt and Thannhauser [7] and the amount of DNA was determined by the method of Dische [8] using diphenylamine. Finally, the concentration of protein was measured by the method of Lowry et al. [9] using bovine serum albumin as standard.

3. Results

Column 4 and 5 of table 1 show the changes in the amount of DNA and protein in rat kidney through the early developmental phase. The amount of DNA expressed on the basis of 100 mg fresh tissues increases rapidly during the late fetal stage and the first day of life, thereafter, decreasing gradually. It was observed by Winick and Noble that the most rapid changes in

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Table 1
Changes in the amount of DNA, protein, and protein methylase III activity of rat kidney during the early life span.

Age (days)	Body weight (g)	No of rats pooled	μg DNA/ 100 mg fresh tissue	mg protein per 100 μg DNA	Enzyme activity		
					nmoles AMe/min/ 100 μg DNA	nmoles AM/min/ mg protein	
					Whole homog.	Whole homog.	Nuclear
Fetal	1.92	17	227.9	1.79	15.72	8.80	3.10
1	5.27	10	439.6	2.25	12.51	5.58	4.27
3	7.43	7	334.5	3.55	19.19	5.40	5.77
6	12.4	5	309.3	3.27	13.33	4.07	3.79
9	19.8	3	250.9	3.52	11.28	3.21	2.87
14	34.3	2	259.0	4.45	10.30	2.32	1.80
18	29.8*	4	247.0	4.43	6.72	1.24	1.44
24	55.3*	3	213.9	6.33	3.60	0.79	0.32
	300	2				–	0.50
	490	2				0.44	0.59

* Different litters from the rest of the animals.

the amount of DNA in rat kidney occurred during the first 40 days of life span [10]. Since the amount of DNA per cell is constant in the somatic cells of a given animal, the results in the table indicate that the rate of cell proliferation in rat kidney is the highest during the late fetal stage and the first few days of life. Thereafter, the amount of DNA per mg of fresh tissue gradually decreases, indicating more individual cell growth rather than further proliferation. Column 5 of table 1 shows that the amount of protein per cell (per 100 μg of DNA) increases continuously during the early phase of life span.

Table 1 also shows the results obtained on the activity of protein methylase III. It can be seen in the 6th column of the table that protein methylase III activity per cell (nmoles of S-adenosyl-L-methionine used per minute per 100 μg DNA) stays relatively high until the 3rd day of life, and thereafter decreases rather rapidly. When the enzyme activity is expressed on the basis of protein concentration (specific activity, nmoles of S-adenosyl-L-methionine used per minute per mg protein). the enzyme activity in the whole homogenate or partially purified nuclei has a strikingly similar pattern to the activity expressed on the basis of DNA. Therefore, these results indicate that, regardless of how the enzyme activity is expressed, protein methylase III activity parallels the rate of cell proliferation.

4. Discussion

Protein methylase III (S-adenosylmethionine: protein–lysine methyltransferase) methylates the ε-amino group of lysine residues of proteins such as histones, polylysine and pancreatic ribonuclease [11]. Various proteins such as histone, actin, myosin, cytochrome *c*, opsin from bovine retina, flagella protein from *Salmonella typhimurium*, and ribosomal protein from water fungus *Blastocladiella emersonii* are also methylated at the ε-amino group of lysine residues *in vivo* [1]. In the higher organisms, the enzyme is exclusively localized in the nuclei with S-adenosyl-L-methionine serving as methyl donor. In the case of HeLa S-3 cells, evidence indicates that histone is the major substrate for protein methylase III *in vivo* [5].

Protein methylase III activity was found to be elevated wherever increased cell proliferation was noted, for example, fetal brain [3], fast growing Morris and Novikoff hepatomas [2], in regenerating adult rat liver [4], and in continuously dividing HeLa S-3 cell culture [5, 6]. On the other hand, there is no change at all in the enzyme activity in tadpole liver during thyroxine-induced metamorphosis where all the biochemical differentiations proceed in a fixed cell population [12, 13 and unpublished data by W.K. Paik].

There appears, therefore, to be a direct correlation between the extent of methylation of lysine residues

of histone and cell proliferation. However, it is highly unlikely that methylation of histone is the cause of cell proliferation, since histone methylation was found to be a rather late event in the cell cycle where the peak of RNA or DNA synthesis precedes the peak of protein methylase III activity [6, 14]. In light of the evidence gathered thus far, it is our working hypothesis that methylation of lysine residues of histone molecule might be a mechanism to attain proper conformation of chromatin after newly synthesized DNA, histone and non-histone chromosomal protein are conjugated.

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