ω-N-METHYLARGININE IN HISTONES

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Summary

The amount of ω-N-methylarginine in various proteins was determined. All the histones isolated from rat liver nuclei contained the amino acid. On the other hand, histones from calf thymus contained only a neglegible amount. Among the histones from rat liver, slightly lysine-rich histone had the highest amount. The cytosol fraction of rat liver appeared to be lacking this amino acid.

When isolated calf thymus nuclei were incubated with S-adenosyl-L-methionine-methyl-^{14}C, nucleic acids and phospholipids were subsequently removed, and its acid-hydrolyzate analyzed by the Beckman automatic amino acid analyzer, there appeared four radioactivity peaks in the basic region (1), two of which coincided with the ninhydrin peaks of ε-N-methyllysine and ε-N-dimethyllysine. One of the remaining peaks before arginine was designated as guanidino-methylarginine and the other as α-N-methyl,guanidino-methylarginine. Subsequent chemical as well as radiochemical analysis established the structure of guanidino-methylarginine to be ω-N-methylarginine (2-4). Furthermore, an enzyme protein methylase I was identified (5), and was shown to methylate exogenous histones added to the reaction mixture to give rise to the formation of ω-N-methylarginine on acid-hydrolysis of the methylated histone (6). Since methylation of proteins might play a role in various functions (7-9) and the amount of ω-N-methylarginine in nature was so small as to remain undetected until now, it was felt to be extremely important to devise some method to detect as well as quantitate it. In the present paper, we report a method of quantitative measurement of ω-N-methyl-arginine and present results which show a relatively large amount of the compound in rat liver histone, but not in calf thymus histone.

MATERIALS AND METHODS

Purification of histones - Prior to the purification of histones from rat liver,

rat liver nuclei were isolated by the Method of Widnell and Tata (10), using a high density sucrose solution. The isolated "pure" nuclei were extracted with 0.20 M H_2SO_4, and the extracted histones were further purified by CM-cellulose column chromatography (6,11). Calf thymus histones were purified from histone type II-A (mixture of various histones) of Sigma Chemical Co. as above.

Subcellular fractions of rat liver were prepared according to the method of Schneider (12), and rat liver chromatin, NaCl-soluble protein from isolated nuclei were obtained by the method of Wang (13). Protein concentration was determined by the method of Lowry et al. (14), using histone type II-A or bovine serum albumin as standard for the measurement of histone and other proteins, respectively.

Amino acid analysis - Thirty to 50 mg of carefully measured protein suspensions were hydrolyzed in approximately 10 ml of 6 N HCl at 110° under reflux for 20

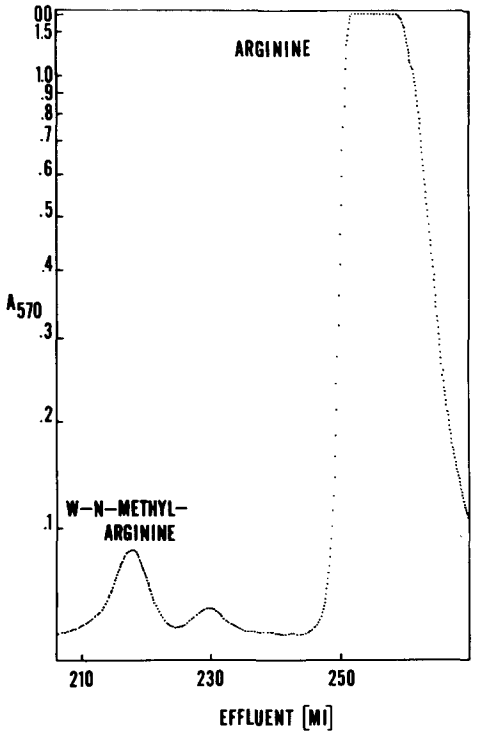

Fig. 1. Detection of ω-N-methylarginine with an overloading amount of protein hydrolyzate. One ml of the hydrolyzate of slightly lysine-rich histone from rat liver nuclei was analyzed according to the method described under Methods. The amount of ω-N-methylarginine was found to be 0.077 μmoles.

hours, removed the HCl under reduced pressure, further washed three times with water, and the residues were dissolved in water to give the concentration of 10 mg of the original protein per ml. Since the amount of ω-N-methylarginine is very small as shown later, the following assay method was devised; first, 1.0 ml of the above hydrolyzate was charged on Aminex A-5 cation exchanger resin (0.9 x 25 cm; product of Bio-Rad Laboratories) in the Beckman automatic amino acid analyzer, and the column was eluted with 0.20 M citrate buffer at pH 5.28 at a flow rate of 60 ml per hour at 50° (2). Under this condition, all the amino acids except ω-N-methylarginine were overloaded (Fig. 1). However, if ω-N-methylarginine is present, its ninhydrin peak is clearly visible and can be precisely measured. The limit of detection is a function of the amount of hydrolyzate used and in some cases further overloading of the column might be necessary for detection of this amino acid. Next, 0.2 ml of the above same hydrolyzate was again analyzed under exactly identical conditions. Now, the ninhydrin peak of ω-N-methylarginine

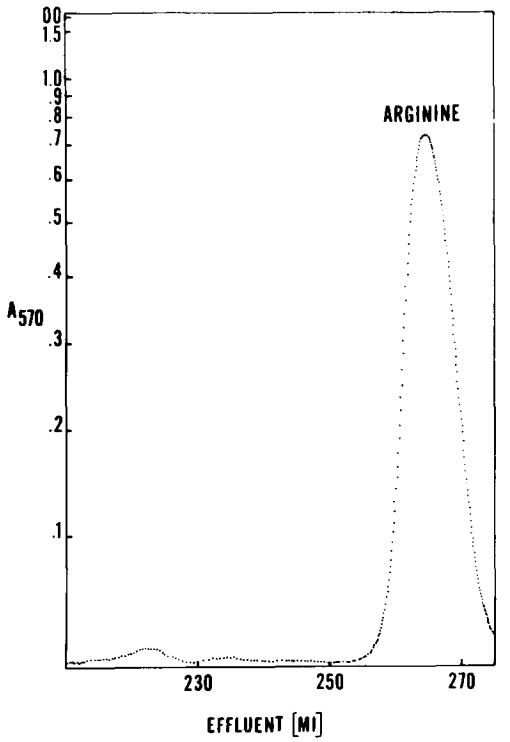

Fig. 2. Measurement of arginine in the same sample used in Fig. 1. Two tenth ml of the identical sample used in Fig. 1 was analyzed under the identical conditions. The amount of arginine was found to be 1.117 μmoles.

Table I

Amount of ω-N-methylarginine in various proteins

Proteins	Source	μmole of ω-N-methyl-arginine in 1.0 ml of hydrolyzate*	μmole of arginine in 0.2 ml of hydrolyzate*	ω-N-methylarginine / Arginine x 100
Histones:				
Slightly lysine-rich	Calf thymus nuclei	trace	0.746	-
Lysine-rich	Calf thymus nuclei	N. D. **	1.445	-
Arginine-rich	Calf thymus nuclei	N. D.	1.223	-
Slightly lysine-rich	Rat liver nuclei	0.069	1.042	1.32
Lysine-rich	Rat liver nuclei	0.021	1.613	0.26
Arginine-rich	Rat liver nuclei	0.059	1.778	0.66
Mitochondria + microsome	Rat liver	0.016	0.858	0.36
Cytosol	Rat liver	N. D.	0.900	-
Chromatin	Rat liver	0.020	1.770	0.23
NaCl-soluble + ribosome	Rat liver nuclei	0.025	1.916	0.26

* One ml of the hydrolyzate contains 10.0 mg of protein before hydrolysis.
** Not Detectable.
Subcellular fractions such as mitochondria, microsome, cytosol or chromatin were treated three times with 15% trichloracetic acid, once with hot alcohol, and were hydrolyzed according to the method described under Methods.

is not detectable. However, the amount of arginine is within the range for precise determination (Fig. 2). After multiplying the amount of arginine by the factor of 5, the ratio of the amounts of these two amino acids were calculated. Quantitative measurement of ninhydrin color was carried out with Infotronics Amino Acid Analyzer CRS-100A Digital Readout System, and the color value of ω-N-methylarginine was assumed to be equal to that of arginine (22.5).

RESULTS AND DISCUSSION

Table I lists the amount of ω-N-methylarginine in various proteins as well as subcellular fractions of rat liver. It is evident that rat liver histones contain relatively large amount of the amino acid. On the other hand, the amino acid is hardly detectable in various histones from calf thymus. Among the rat liver histones, slightly lysine-rich histone contains the most. Furthermore, it is noted that the cytosol protein does not seem to contain any detectable amount of the amino acid. The NaCl-soluble protein in the table is mainly composed of acidic proteins. The fact that an enzyme which methylates the guanidino group of arginine in histones is largely situated in the cytosol of rat liver (4) and that rat liver histones contain a relatively large amount of ω-N-methylarginine suggest that histone methylation at the guanidino group of arginine residues should occur before transport into the nucleus of the cell. It should be noted that, although not detected with the ninhydrin reaction, histones from calf thymus serve as a good methyl acceptors (1,4,6). This is quite similar to the situation with t-RNA methylation (15): methyl-deficient t-RNA serves well for methylation with S-adenosyl-L-methionine.

Significance of ω-N-methylarginine as well as the function of protein methylase I remains to be clarified. However, it was reported that treatment of immature rats with estradiol-17 resulted in the decrease of the protein methylase activity by almost one-half (4). Similar decrease in the enzyme activity was also observed during thyroxine-induced amphibian metamorphosis (to be published).

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