ε-N-DIMETHYLLYSINE IN HISTONES

Woon Ki Paik and Sangduk Kim

Fels Research Institute, Temple University School of Medicine, Philadelphia, Pa. 19140

Received April 13, 1967

When isolated calf thymus nuclei were incubated with S-adenosyl-L-methionine-methyl-^{14}C and the acid -hydrolyzate of the protein was analyzed by paper chromatography, two radioactive ninhydrin spots were detected, whose R_f values corresponded to those of ε-N-methyl-L-lysine and ε-N-dimethyl-L-lysine (1). Although ε-N-methyllysine has been shown to be present in the flagella proteins of Salmonella typhimurium (2) and in histone of higher animals (3), the presence of ε-N-dimethyllysine in these sources has not yet been reported. These facts prompted us to re-examine the resolution of the analytical methods employed by other, particularly column chromatography with a Beckman automatic amino acid analyzer (3,4).

We report in this paper that hydrolyzates of calf thymus arginine-rich histone, a commercial product, type IV of Sigma Chemical Company, were found to contain ε-N-dimethyllysine as well as ε-N-monomethyllysine. These were identified by using a Beckman automatic amino acid analyzer with 0.35 M citrate buffer, pH 5.84 at 28°. Further identification of ε-N-dimethyllysine was carried out by paper chromatography.

We thank Dr. M. P. Brigham for allowing us to use the Beckman automatic amino acid analyzer, and Mr. E. Pearson for skillful technical assistance with the analysis.

Supported by NIH (AM09602-01A1 PC), NSF (B7-0280R) and NCI (CA 07174).

When authentic ε-N-methyl-L-lysine and ε-N-dimethyl-L-lysine (5) were chromatographed following the procedure of others (3,4) on a 50-cm column of a Beckman automatic amino acid analyzer using 0.35 M citrate buffer, pH 5.28 at 50° and with a flow rate of 30 ml per hour, both compounds appeared as a single peak between lysine and histidine, as shown in Figure 1. Therefore, the above elution conditions failed to separate these two methylated lysines.

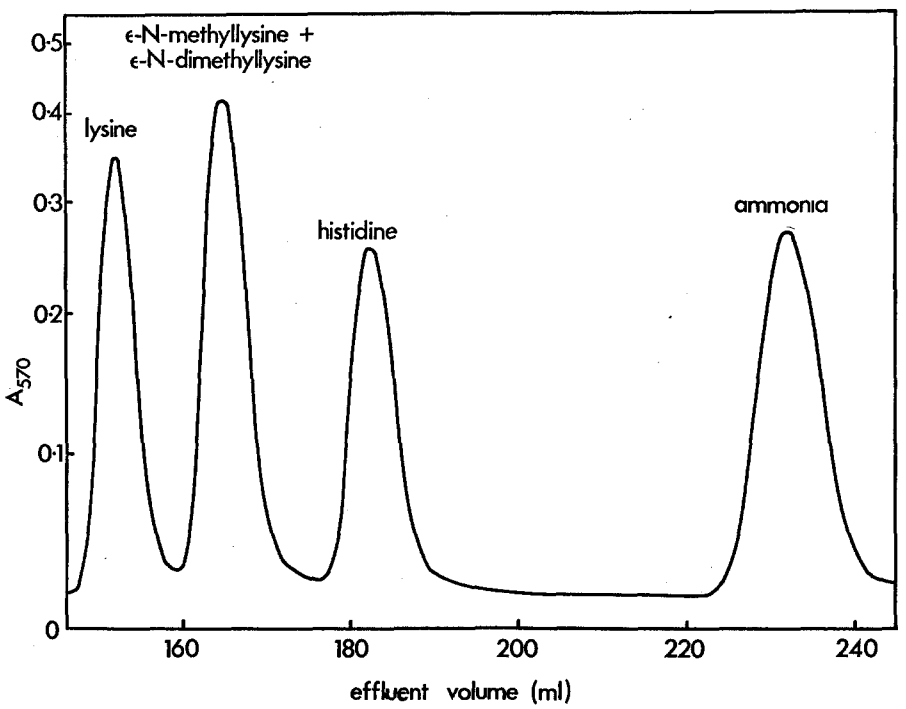

Figure 1. Elution pattern of standard compounds with the Beckman automatic amino acid analyzer
The 50-cm column was used and the elution was carried out with 0.35 M citrate buffer of pH 5.28 at 50°. The flow rate: 30 ml/hour. 0.26 μmole of ε-N-methyl-L-lysine and 0.62 μmole of ε-N-dimethyl-L-lysine were used.

Since methylation of an amino group enhances the basicity of the molecule, ε-N-dimethyllysine should be more basic than ε-N-methyllysine. Therefore, it is highly likely that a change of pH during elution might

separate these two compounds on a column. When authentic ε-N-methyl-L-lysine and ε-N-dimethyl-L-lysine were eluted with 0.35 M citrate buffer of pH 5.84 at 28° instead of pH 5.28 at 50°, the two compounds were clearly separated, ε-N-methyl-L-lysine follwed by ε-N-dimethyl-L-lysine. Fig. 2 is the chromatographic pattern of the basic amino acids of the acid-hydrolyzate of arginine-rich histone, prepared by hydrolyzing the protein in 6 N HCl at 110° for 18 hours under reflux. In the figure there are two

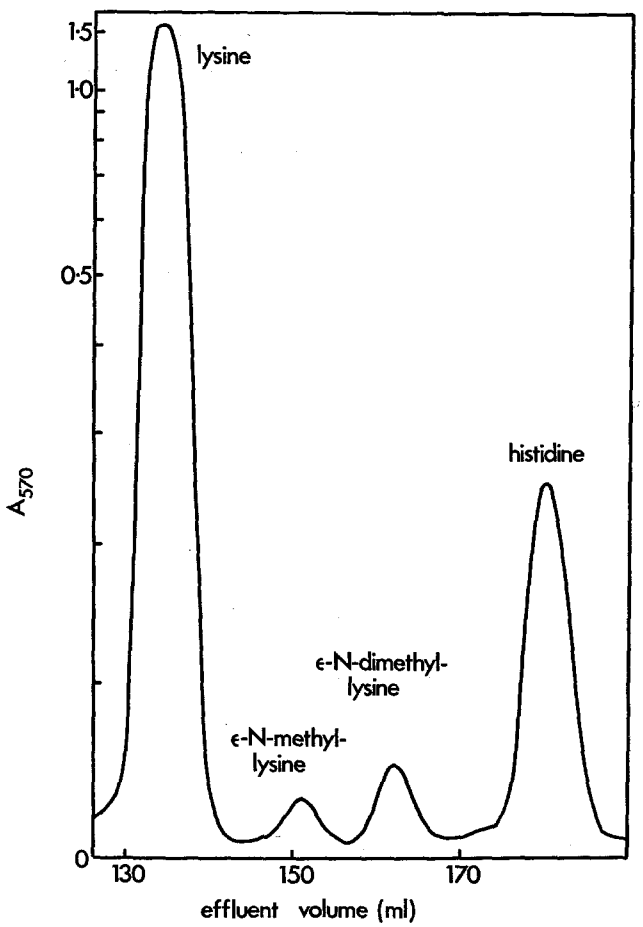

Figure 2. Chromatographic separation of some of the basic amino acids in histone hydrolyzate
The 50-cm column was used and the elution was carried out with 0.35 M citrate buffer, pH 5.84 at 28°. The flow rate: 30 ml/hour. 10.4 mg of commercial calf thymus arginine-rich histone (Type IV of Sigma) was hydrolyzed.

peaks between lysine and histidine. The first and second peaks, respectively, coincided with those of synthetic ε-N-methyl-L-lysine and ε-N-dimethyl-L-lysine, synthesized in our laboratory (5).

The fractions corresponding to these two peaks on the column were collected from the analyzer in order to identify the compounds as ε-N-methyl-lysine and ε-N-dimethyllysine, and the accompanying salts were removed by the method of Stein and Moore (6). Table I lists the results of analysis of the sample by paper chromatography using Whatman No. 1 paper in m-cresol-phenol (1:1) solvent system (7). The standard compounds were run together with the sample, since some remaining salt in the sample affected the R_f values of the standard compounds.

Table I

R_f Values of Lysine, ε-N-Methyl-L-lysine, ε-N-Dimethyl-L-lysine and the Unknown Compounds on Paper Chromatogram

	R_f Values of		
	Lysine	ε-N-Methyl-L-lysine	ε-N-Dimethyl-L-lysine
Standard Compounds + the Unknown	0.11	0.33	0.70
The Unknown	0.12	0.36	0.73

Chromatogram was developed on Whatman No. 1 paper in 1:1 m-cresol-phenol, pH 9.3 borate buffer (7). Prior to the application of the samples, the paper was sprayed with 0.06 M borate buffer, pH 7.3.

Because of recent suggestions that histone may be a gene-repressant (8), investigation of histones in relation to DNA action must take into account the significance of ε-N-methyllysine and ε-N-dimethyllysine as histone components. Therefore, it is desirable to know the content of these two methylated amino acids in the protein. It is obvious from the above results that calculations based solely on the amount of ε-N-methyllysine (3,4) are erroneous. It was found in the present study that arginine-rich histone

contains approximately 1.7 moles of ε-N-methyl-lysine and 3.5 moles of ε-N-dimethyllysine per 100 moles of lysine. Further investigation on the distribution of these two methylated amino acids in protein other than arginine-rich histone is presently under way.

REFERENCES

1. S. Kim and W. K. Paik, J. Biol. Chem., 240, 4629 (1965).
2. R. P. Ambler and M. W. Rees, Nature, 184, 56 (1959).
3. K. Murray, Biochemistry, 3, 10 (1964).
4. D. G. Comb, N. Sarkar, C. J. Pinzino, J. Biol. Chem., 241, 1857 (1966).
5. L. Benoiton, Can. J. Chem., 42, 2043 (1964).
6. W. H. Stein and S. Moore, Cold Spring Harbor Symp., 14, 179 (1949).
7. A. L. Levy and D. Chung, Anal. Chem., 25, 396 (1953).
8. de Reuck, A. V. S. and Knight, J. (Editors), Histones, Little, Brown and Company, Boston, 1966.