HOMOCITRULLINE FORMATION FOLLOWING CARBAMYLATION OF HISTONES WITH CARBAMYL PHOSPHATE*

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1. Introduction

The modification of histone structure by group substitution reactions appears to be one of the major new aspects of nuclear protein metabolism. Most of the reactions thus far described for the acetylation, methylation and phosphorylation of the amino acid residues are enzymatically catalyzed reactions. Formation of such derivatives may have biological significance due to the capacity of histones to inhibit ribonucleic acid synthesis.

The incorporation of radioactively labeled acetate occurs preferentially in the arginine-rich histone fractions, and most of the radioactive acetyl groups become attached to the ϵ amino-nitrogen of lysine residues in the polypeptide chain [1]. Methylation also occurs mostly in the arginine-rich histones and again the modified amino acid is lysine, which becomes ϵ-*N*-methyl lysine [2]. Phosphorylation, on the other hand, occurs more extensively in histones very rich in lysine and the amino acid which is modified is serine [3].

Recently Ramponi and Grisolia [4] have demonstrated that carbamyl phosphate (CP) and 1,3, diphosphoglyceric acid (1,3 PGA) can react with histones to give carbamyl and 3-phospho-glyceryl histones. It was suggested that such non-enzymic modification of proteins might be of much biological significance. Since then a report by Paik et al. [5] has appeared demonstrating non-enzymatic acylation of histone with acetyl CoA.

It thus appeared of interest to determine the site of the carbamyl group attachment for the arginine-rich and the lysine-rich histones.

2. Methods and materials

Histone was prepared from calf thymus nuclei as described by Cruft et al. [6], and the lysine-rich (F_1) and arginine-rich (F_3) fractions were then isolated by chromatography on CM-cellulose [7]. ^{14}C-CP was prepared from ^{14}C cyanate [8]. Carbamylation of lysine-rich and arginine-rich histones was carried out in a system containing ^{14}C-CP, phosphate buffer and histone as described by Ramponi and Grisolia [4].

The carbamylated histones were passed through a Sephadex G-25 column in order to remove ^{14}C-CP excess and any other small molecular weight contaminants.

Amino acid analyses were performed according to the procedure of Spackman et al. [9] in a Beckman Unichrom amino acid analyzer, equipped with a microcuvette. Protein samples were dissolved in 6 N HCl, sealed under vacuum, and hydrolyzed at 110° for 24 hr.

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After hydrolysis the samples were dried and the residues dissolved in 0.2 M citrate buffer pH 2.2. Portions of either 0.5 ml or 1 ml were applied to each ion exchange column. The elution conditions of the acid and neutral amino acids were slightly modified. The pH of the first buffer was increased by 0.04 pH units, in order to allow the displacement of the cystine peak towards alanine. The gives a distict separation of homocitrulline, which is now eluted between cystine and valine. The relative elution positions and the quantitative determination of these amino acids were checked using homocitrulline synthesized according to Greenstein and Winitz [10]. All the other conditions for the routine analysis of protein hydrolyzates were unchanged (temperature, buffer changes, etc.).

The radioactivities of the amino acid peaks were determined as described previously [11].

3. Results and discussion

Fig. 1 illustrates the amino acid elution pattern, as well as the distribution of radioactivity of the hydrolyzate of lysine-rich ^{14}C carbamylated histone.

It can be noted that under the conditions used in these experiments there is a new amino acid peak which is not found with non-carbamylated histones. This peak, located between valine and alanine, corresponds to homocitrulline since the lysine-rich histone lacks cystine. Furthermore all the radioactivity detected coincides with the new amino acid.

Fig. 2 shows the profile for the amino acid analysis of carbamylated arginine-rich histone. Again, homocitrulline is present and is located this time between valine and cystine. The radioactivity is again found entirely in the peak corresponding to homocitrulline.

Table 1 illustrates the amino acid analyses of the lysine-rich and arginine-rich histones compared with those of the same proteins after carbamylation. The amino acid residues expressed as moles/100 moles of histone before and after carbamylation are very close, except that the lysine content is reduced in the carbamylated histones. This loss of lysine on carbamylation can be accounted for by the appearance of homocitrulline. Furthermore, after correction (assuming that 36% of the homocitrulline was lost during hydrolysis [12]), it was calculated that all the radioactivity occurring in the ^{14}C carbamylated histones can be accounted for by the radioactivity found associated with the homocitrulline peaks. Moreover, the

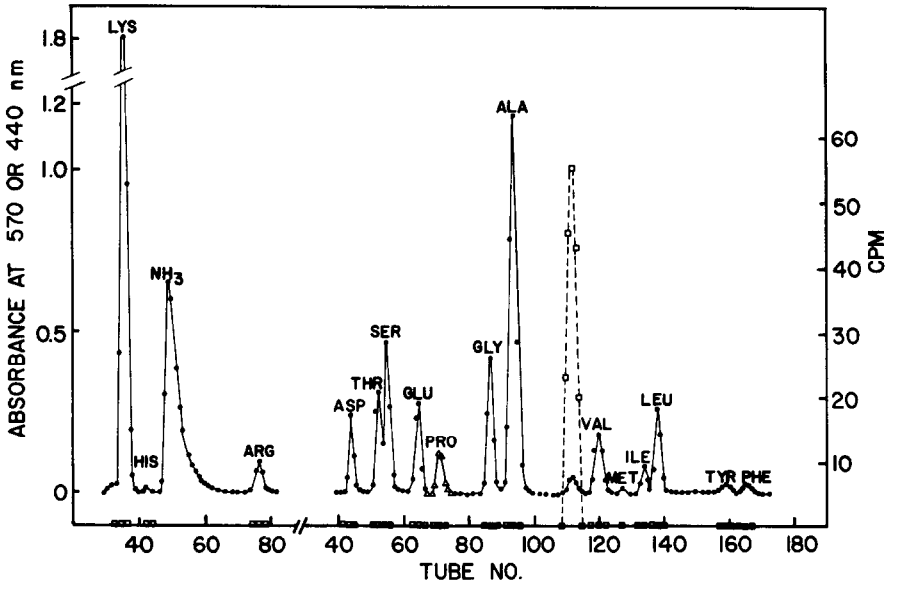

Fig. 1. The amino acid elution pattern of carbamylated F_1 lysine-rich histone. ●—● Absorbance at 570 nm. △—△ Absorbance at 440 nm. □—□ Counts per minute.

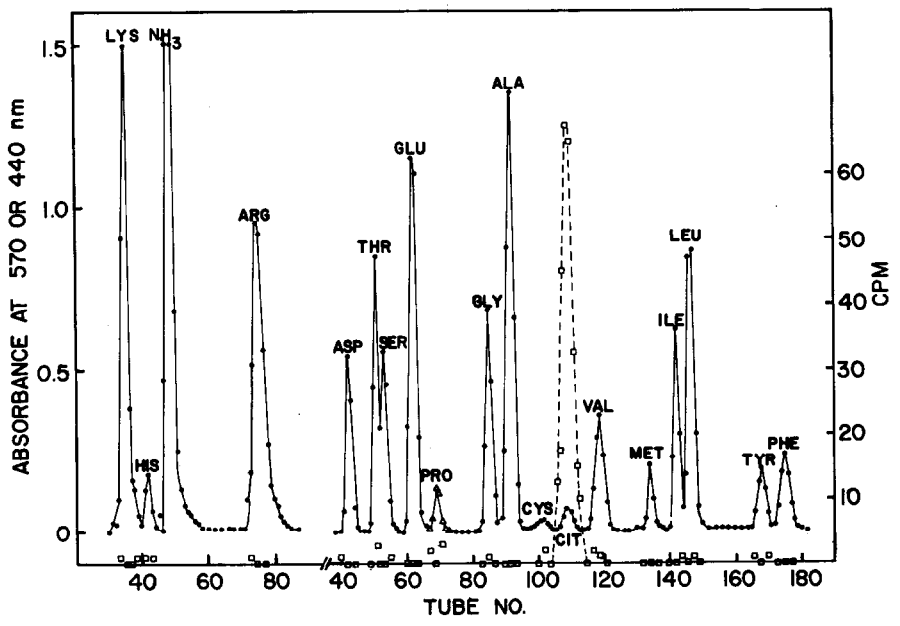

Fig. 2. The amino acid elution pattern of carbamylated F_3 arginine-rich histone. ●—● Absorbance at 570 nm. △—△ Absorbance at 440 nm. □—□ Counts per minute.

Table 1
Amino acid composition of histone fractions before and after carbamylation.

	F_1 Lysine-rich histone		F_3 Arginine-rich histone	
	Control	Carbamylated	Control	Carbamylated
	moles/100 moles total amino acids			
Lysine	23.96	22.53	10.25	9.38
Histidine	0.47	0.42	1.52	1.54
Arginine	2.82	2.90	11.94	12.05
Aspartic acid	4.02	3.80	4.79	4.63
Threonine	5.45	5.42	6.78	6.73
Serine	6.34	6.39	4.48	4.41
Glutamic acid	6.42	5.72	11.83	11.79
Proline	8.82	9.17	4.87	5.01
Glycine	7.59	7.66	6.07	6.07
Alanine	21.43	21.66	12.61	12.58
Cystine/2	–	–	1.10	0.89
Homocitrulline	–	1.36	–	0.76
Valine	4.97	5.05	4.47	4.57
Methionine	0.23	0.21	1.55	1.54
Isoleucine	1.48	1.61	4.38	4.49
Leucine	4.73	4.64	8.37	8.61
Tyrosine	0.42	0.55	2.21	2.09
Phenylalanine	0.81	0.70	2.75	2.83

quantity of homocitrulline found in the carbamylated F_1 fraction is twice that found with the F_3 fraction in good agreement with the previous study of Ramponi and Grisolia [4], which determined that lysine-rich histones more readily carbamylated than the arginine-rich histones. It seems logical therefore to conclude that during the non-enzymatic carbamylation of histones, the carbamyl groups are attached solely to the ϵ-amino-nitrogen of lysine residues in the polypeptide chain to yield homocitrulline residues.

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