THE OCCURRENCE OF *N*-ε-TRIMETHYL LYSINE IN TWO HISTONE-LIKE PROTEINS FROM WHEAT GERM

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1. Introduction

Chromatin contains many non-histone proteins among which the two major groups of proteins most studied are the high mobility group (HMG) proteins [1] and the histone-like proteins [2,3]. The HMG proteins can be extracted from chromatin with 0.35 M NaCl and have high and nearly equal amounts of acidic and basic amino acid residues. Localization in chromatin and/or function of the HMG proteins have been studied extensively [4,5]. The histone-like proteins are basic proteins and are not extracted with 0.35 M NaCl but co-extracted with histone. One of those proteins, known as protein A24 [2], has been reported to accompany both activation and repression of transcription [6,7].

In plants however, there have been only a few reports on the non-histone chromatin proteins. During germination of wheat seeds, the template activity of chromatin remarkably increases [8], and in reconstitution experiments, this increase can be strongly inhibited by the non-histone chromatin proteins isolated from wheat germ but not by those from the seedlings [9]. They have reported decrease of some specific non-histone proteins which accompany activation of transcription during germination [10]. Among those non-histone proteins, two proteins co-extracted with histone are most remarkable [10].

In an attempt to characterize these proteins in relation to their function in transcription, the unique occurrence of *N*-ε-trimethyl lysyl residues in the two histone-like proteins are observed.

2. Materials and methods

Wheat germ (spring and unknown variety) was a generous gift from Nisshin Seifun Company at Chiba and stored at 4°C. Chromatin was prepared from germ by the method in [11] in the presence of phenylmethylsulfonyl fluoride (PMSF) according to [10].

CM-Sephadex C25 column chromatography (0.5 × 4 cm) was performed at pH 9.0 [12] equilibrated with 7.5 mM sodium borate buffer. The protein solution of histone fraction plus an equal volume of 15 mM borate buffer was applied to the column. After washing with the buffer, the proteins were eluted stepwise with the borate buffer containing 0.3, 0.4, 0.5 and 0.6 M NaCl. The eluted proteins were analyzed by SDS–polyacrylamide gel electrophoresis [13]. The proteins were stained as in [14].

The two histone-like proteins were purified by preparative SDS–polyacrylamide gel electrophoreses. SDS and Coomassie blue were removed from the samples by passing slowly through a column of Dowex-X2 as in [15]. The proteins were extensively dialyzed against 2 mM sodium acetate buffer (pH 6.0) and the dialyzed samples were then lyophylized.

The lyophylized proteins were resuspended in 6 N HCl and hydrolyzed for 24 h at 110°C under vacuum. Amino acid analyses were performed on a Durrum D-500 automatic amino acid analyzer, and the basic amino acids fractionated by the method in [16] were also analyzed by paper chromatography on Whatman no. 1 paper developed with a mixture of *m*-cresol–phenol (1:1) saturated with 0.1 M borate buffer (pH 9.3).

For standards, *N*-ε-trimethyl-L-lysine was purchased from Calbiochem (code no. 644264), and *N*-ε-monomethyl-L-lysine from Sigma (M6004). *N*-ε-dimethyl-L-lysine was synthesized from *N*-α-acetyl-L-lysine (Sigma, A2010) as in [17].

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3. Results and discussion

The histone fraction from wheat germ chromatin contains two major basic non-histone proteins [10], and these are indicated by arrows in fig.1. These two proteins have been designated as HLP-1 and HLP-2 and M_r 24 000 and 21 000, respectively. They were scarcely extracted by 0.35 M NaCl which has been used for extraction of the HMG proteins [1] but were soluble in 0.4 N H_2SO_4. The patterns of eluted proteins of CM-Sephadex column chromatography shown in fig.1 indicate that neither HLP-1 nor HLP-2 can be eluted with as low a concentration of NaCl as can the

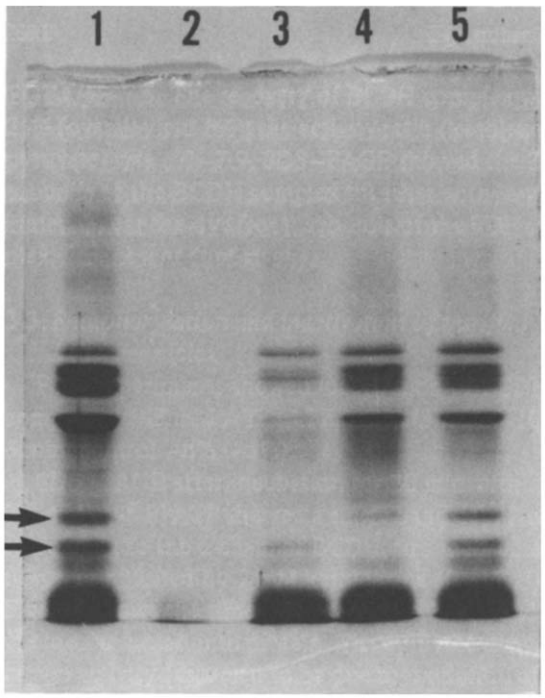

Fig.1. SDS–polyacrylamide gel (10%) electrophoresis of the eluted proteins from the CM-Sephadex C25 chromatography: (1) total wheat germ histone fraction; (2) the eluates with 0.3 M NaCl, (3) 0.4 M NaCl; (4) 0.5 M NaCl; (5) 0.6 M NaCl. The arrows indicate two histone-like proteins, HLP-1 (above) and HLP-2 (below).

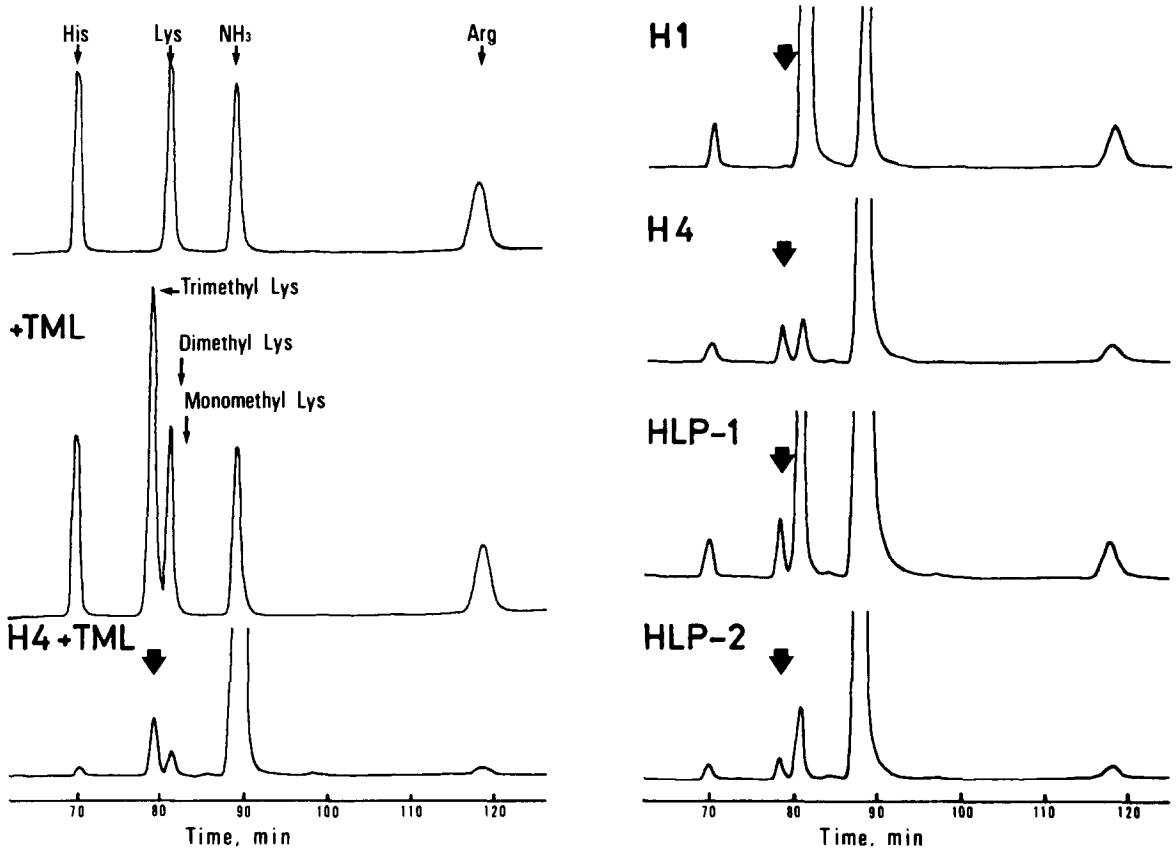

Fig.2. Amino acid analyses of acid hydrolyzed histones and histone-like proteins. The thick solid arrows designate the position expected for *N*-ε-trimethyl lysine (TML) if it had been present.

HMG proteins [12]. The patterns of elution of the two proteins and the characteristics of solubility show that both HLP-1 and HLP-2 are histone-like proteins rather than so-called HMG proteins [10] and that HLP-1 is more basic than HLP-2. High basicity of HLP-1 has also been shown by its high content of the lysyl residues (see below).

Amino acid analyses of some acid-hydrolyzed chromosomal proteins were performed by an amino acid analyzer, and the representative chromatograms are compared in fig.2. A peak immediately before free lysine, which did not correspond to any of the normal free amino acids, was detected in the acid hydrolysates of histone H4, HLP-1, HLP-2 but not of histone H1, H2A/2B, H3, and HMG-a (in preparation). The most likely candidate for this unknown residue was a methylated lysyl residue because methylated residues were found in histones from various species and in cytochrome *c* from wheat [18]. Among methylated lysines, *N*-ε-mono- or di-methyl lysine did not correspond to the unknown peak, whereas *N*-ε-trimethyl lysine corresponded to the peak and co-eluted with the amino acid in question. Identification of the residue as *N*-ε-trimethyl lysyl residue was further confirmed by paper chromatography developed with a mixture of *m*-cresol–phenol (1:1) (not shown). R_f-values of lysine, *N*-ε-monomethyl lysine, *N*-ε-dimethyl lysine, *N*-ε-trimethyl lysine, and arginine were 0.45, 0.60, 0.77 (bluish), 0.85 (reddish) and 0.52, respectively.

In table 1, the extent of trimethylation of the lysyl residues of the histones and the histone-like proteins are summarized. The occurrence of *N*-ε-trimethyl lysine in some but not all chromatin proteins may exclude the possibility of an artifact caused by the isolation procedure employed. High content of *N*-ε-trimethyl lysine in some chromosomal proteins may be unique to wheat germ chromatin, and the work reported here represents the first observation of *N*-ε-trimethyl lysine in the histone-like proteins.

Table 1
The extent of trimethylation of lysyl residues in some chromosomal proteins as measured by an amino acid analyzer

Protein	mol% total lysyl residues/mol protein	% *N*-ε-trimethyl lysine/total lysine
HLP-1	14.3	12
HLP-2	10.0	20
HMG-a	12.9	0
histone H1	21.6	0
histone H2A/2B[a]	18.7	0
histone H3	12.4	0
histone H4	7.3	44

[a] Histone H2A and H2B could not be separated by the method in section 2

The function of these histone-like proteins is unknown though the decrease of these proteins in the histone fraction accompanies the activation of transcription [9,10]. The meaning of methylation of these histone-like proteins and its relationship to the function of these proteins are of interest.

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