PHOSPHORYLATED SITES OF CHICKEN ERYTHROCYTE HISTONE H5 BY A CYCLIC AMP-INDEPENDENT PROTEIN KINASE FROM MOUSE PLASMOCYTOMA CELLS

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1. Introduction

During erythropoiesis, the chicken erythrocyte-specific histone H5 progressively but incompletely substitutes histone H1. In early stages, newly synthesized histone H5 is phosphorylated to a high extent. Later on, dephosphorylation of histone H5 occurs in mature erythrocyte [1].

Structural analogy between histones H1 and H5 suggests a similar role for both proteins and phosphorylation of histone H1 has been correlated with the rate of cell division in different tumors [2] and with the condensation of chromatin [3] but phosphorylation of histone H5 is related to cell maturation and its degree of phosphorylation is proportional to the number of immature cells in anemic chickens and, at the terminal stage of development in mature red blood cells histone H5 is completely dephosphorylated [4]. This dephosphorylation has been detected when the chromatin reaches a highly condensed state [5]. The progressive displacement of histone H1 by histone H5 during cell maturation can be compared with that of histones by protamines during spermiogenesis where protamines are synthesized, then extensively modified by phosphorylation and dephosphorylated when spermatozoa leave the testis.

Phosphorylation also modifies histone–DNA interactions and chromatin conformation: for example, phosphorylation can modulate histone H5–nucleosome interactions [6]. Sites of phosphorylation of histone H5 have been investigated in vivo [4], and in vitro using the catalytic subunit of cyclic AMP-dependent protein kinase from pig brain [7] and from rat pancreas [8]. Even if the specificity is different from one kinase to another, the sites of phosphorylation in histone H5 are distributed in vitro as in vivo in two distinct regions of the protein: the amino-terminal region (residues 1–21) and the carboxy-terminal region (residues 101–189); in all cases, no site has been detected in the globular part of the molecule (residues 22–100) [9]. This fact has been already mentioned for histone H1 which, like histone H5, contains 3 different domains: a short apolar amino-terminal part in random coil (residues 1–34); a globular central part (residues 35–120); and a highly basic carboxy-terminal part in random coil (residues 121–213) [10]. Phosphorylation of histone H1 by a cyclic AMP-dependent protein kinase only takes place at serine residue 37 in the amino-terminal region of the molecule while phosphorylation by a cyclic AMP-independent protein kinase occurs at serine and threonine residues, mainly in the carboxy-terminal region.

This paper deals with the in vitro phosphorylation of chicken erythrocyte histone H5 by a cAMP-independent growth-associated protein kinase isolated from mouse plasmocytoma cells. This kinase has been shown to specifically phosphorylate histone H1; the core histones H2A, H2B, H3 and H4 are not phosphorylated.

Due to the close structural and functional relationship between histones H1 and H5, the kinase was assayed with histone H5. Five serine residues at positions 3, 7, 104, 117 and 148 were phosphorylated.

2. Materials and methods

2.1. *Preparation of histone H1-specific protein kinase from mouse plasmocytoma cells*

A cAMP-independent protein kinase with high specificity for histone H1 (PKH1) was purified from mouse plasmocytoma by 30–50% ammonium sul-

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fate precipitation followed by chromatography on DEAE-52 cellulose, hydroxylapatite, phosphocellulose and Sephadex G-200 columns as in [11]. It had a specific activity of 0.7- 1 unit/mg histone H1.

2.2. *Phosphorylation of histone H5*

The phosphorylation of chicken erythrocyte histone H5 (25 mg) by purified histone H1 kinase (PKH1) (enzyme/histone ratio 1/1000) was performed for 12 h at 34°C in 12 ml reaction mixture containing: 30 mM Tris–HCl (pH 7.6); 10 mM Mg-acetate; 1 mM dithiothreitol; 0.2 mM EGTA and 0.3 mM ([γ-^{32}P]-ATP). The reaction mixture was then dialysed 15 h against deionised water and lyophilised.

2.3. *Limited acid hydrolysis*

For identification of phosphoserine and phosphothreonine ^{32}P-labelled histone H5 was hydrolysed for 2 h at 110°C under vacuum with 6 N HCl [12]. The hydrolysate was submitted to electrophoresis at 2500 V for 2 h on Whatman 3 MM paper at pH 1.9 (acetic acid/formic acid/water, 35:10:400, by vol.). Unlabelled phosphoserine and phosphothreonine were used as markers.

2.4. *Localization of phosphorylation sites*

Tryptic hydrolysis, fractionation of tryptic hydrolysate and identification of phosphorylated sites were performed as in [8].

Table 1
Sites of in vitro phosphorylation of histone H5 determined from ^{32}P-labelled tryptic peptides

	Amounts of phosphorylation[a]
A: By a cAMP-independent protein kinase from mouse plasmocytoma cells	
(H) Thr–Glu–Ser(P)$_3$–Leu–Val–Leu–Ser(P)$_7$–Pro–Ala–Pro–Ala–Lys–Pro–Lys	5
(Lys–Arg)[b]–Ser(P)$_{104}$–Pro–Gly–Lys–Lys	10
Ser–Thr–Ser(P)$_{117}$–Pro–Lys	43
Ser–Thr–Ser(P)$_{117}$–Pro–Lys–Lys	6
Ser–Arg–Ala–Ser(P)$_{148}$–Pro–Lys	36
B: By a cAMP-dependent protein kinase from rat pancreas [8]	
(Arg–Arg)–Ser(P)$_{22}$–Ala–Ser–His–Pro–Thr–Tyr–Ser(P)$_{29}$–Glu–Met–Ile–Ala–Ala–Ala–Ile–Arg	20
Lys–Lys–Ser(P)$_{145}$–Arg	50
Lys–Ala–Ser(P)$_{166}$–Lys–Ala–Lys–Lys	30

[a] The amounts of phosphorylation are expressed in % of the total amount of [^{32}P]phosphate incorporated into the protein

[b] Residues in parentheses are not included in the tryptic peptides. They are written to show that most of the phosphorylation sites are located in a sequence B–X–Ser(P) where B is a basic amino acid and X any amino acid

3. Results and discussion

Phosphoserine only was shown to be present in the 2 h-hydrolysate of histone H5 phosphorylated by the cAMP-independent protein kinase isolated from mouse plasmocytoma cells. By comparison calf thymus histone H1 was preferentially phosphorylated with this kinase on threonine residues (P-Ser/P-Thr ratio, 0.48).

Radioautography of the peptide map of the tryptic hydrolysate of the histone H5 labelled with [^{32}P]-phosphate shows 5 labelled peptides. Structural studies of the phosphorylated tryptic peptides isolated by ion-exchange chromatography on Chromobeads P (Technicon Corp.) allowed us to locate the phosphoserine residues in the sequence of the protein [13].

The sites of phosphorylation of histone H5 by the H1-specific cAMP-independent protein kinase from mouse plasmocytoma cells are presented in table 1. The phosphorylation sites of histone H5 phosphorylated with the cAMP-dependent protein kinase from rat pancreas [8] are given by comparison.

Five serine residues in histone H5 were phosphorylated to different extents by the H1-specific kinase. Two minor sites (serine-3, serine-7) were identified in the short hydrophobic sequence (residues 1–11) of the amino-terminal region of histone H5. The 3 other phosphorylation sites were found in the highly basic carboxy-terminal half of the protein, at serine residues 104, 117 and 148. Serine 117 is indeed the major site of phosphorylation of histone H5 for the H1-specific kinase since ~50% of the total ^{32}P-labelling was incorporated in that residue. The incomplete cleavage by trypsin of the Lys_{119}–Lys_{120} bond generated two peptides and accounted for the presence of two radioactive spots corresponding to [^{32}P]Ser_{117} on the peptide map of histone H5.

As shown in table 1, the phosphorylation sites of histone H5 by the H1-specific cAMP-independent kinase from mouse plasmocytoma cell are different from those phosphorylated by the cAMP-dependent kinase from rat pancreas.

However both enzymes phosphorylate essentially serine residues located in the highly basic domain of the protein which is thought to interact strongly in the chromatin with the phosphate groups of DNA but, whereas serine residues phosphorylated by the cAMP-independent kinase are adjacent to a proline residue, those phosphorylated by the cAMP-dependent kinase are located just before a basic residue.

The specificity of these two enzymes contrasts sharply with that of cAMP-dependent protein kinase from pig brain which preferentially phosphorylates serine residues located in the globular domain (residues 22–100) of histone H5 [7,9].

Four of the 5 serine residues phosphorylated in vitro in H5 by the H1-specific cAMP-independent kinase are identical to those found phosphorylated in vivo [5]. The extent of labelling is however different. Nearly 50% of the ^{32}P-label are incorporated in vivo in the amino-terminal region of histone H5 (residues 1–21), equally distributed between serines at positions 3, 7. The remaining 50% are located in the carboxy-terminal half of the molecule at Ser_{104} and Ser_{148}. Ser_{117}, major site of the in vitro phosphorylation by the cAMP-independent kinase does not appear to be phosphorylated in vivo.

As observed in histone H5 phosphorylated with a cAMP-dependent kinase from rat pancreas [8], the serine residues phosphorylated with the H1-specific cAMP-independent kinase are all located within regions in predicted β-turn conformation except Ser_3 [14,15]. In fact, the specificity of a given protein kinase is basically related to the recognition of specific amino acid sequences. Thus, the cAMP-independent kinase from mouse plasmocytoma cell phosphorylates with a marked specificity the serine residues in histone H5 just before a proline residue.

Obviously this specificity is similar to that of the growth-associated histone kinase which is bound to chromatin and catalyzes cAMP-independent phosphorylation of a number of serine and threonine residues in histone H1 [16]. The four sites of growth-associated phosphorylation which are located at positions 16, 136, 153 and 180 of the H1 subfraction RTL-3 [17] are all included in a repeat structure where a proline residue occurs next to a serine or threonine residue such as in Ser_{16}–Pro–Ala–Lys, Thr–Pro–Lys–Lys (positions 136 and 153) and Ser_{180}–Pro–Lys–Lys.

The recognition of such sequences by the cAMP-independent kinase from mouse plasmocytoma cells explains the narrow specificity of this kinase for the phosphorylation of histone H1 and of histone H5 which is closely related to histone H1 [13]. These sequences are only encountered in histones H1 and H5 but not in core histones H2A, H2B, H3 and H4.

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