The phosphorylated site of calf thymus F2b histone by the cyclic AMP-dependent protein kinase

THE phosphorylation of histones is a complex process which occurs at different stages of the cell cycle[1-3]. Different types of histone kinases are responsible for the phosphorylation of the seryl (or threonyl) residues of the histone fractions. One of them is the ubiquitous cyclic AMP-dependent protein kinase[4,5], whereas the histone kinase predominant in mitotic cells is not activated by the cyclic nucleotide[6]. The cyclic AMP-dependent enzyme of rat liver, phosphorylates the seryl residue in the position 37 of calf thymus F1 histone, and the growth associated histone kinase catalyses the phosphorylation of other seryl and threonyl residues of the F1 fraction[4-6].

The cytosol and nucleus of human tonsillar lymphocytes also contain cyclic AMP-dependent and independent histone phosphorylating enzymes[7,8]. *In vivo*, F2b histone is phosphorylated to a small extent only[1-3] but *in vitro* it is about as good a substrate as F1 for both of the lymphocyte histone kinases, and a better substrate than the other histone fractions. The lymphocyte enzymes, however, differ from each other in their kinetic parameters and in the sites of phosphorylation of the F2b fraction[7,8]. When phosphorylation was carried out using the cyclic AMP-independent enzyme four different, approximately equally, phosphorylated peptide fragments were detected on the two dimensional paper electrophoretic pattern from the tryptic digest of F2b histone fraction; whereas one of them was phosphorylated preferentially when the reaction was catalysed using the cyclic AMP-dependent enzyme[7,8]. The same single peptide fragment of calf thymus F2b was phosphorylated also by the cyclic AMP-dependent enzyme of rat Harderian gland[9]; hence, this specificity seems to be the common feature of cyclic AMP-dependent protein kinases of different species and of different tissues.

The amino acid composition of the tryptic peptide fragment phosphorylated by the cyclic AMP-dependent enzyme has been determined (Table 1). The complete amino acid sequence of the F2b histone fraction is known[10], and the amino acid composition of the phosphorylated peptide is consistent with that of only one tryptic fragment of F2b. There is a single tryptic peptide containing glutamic acid, serine, trypsine, valine and lysine together, that is, the residues from Glu-35 to Lys-43 (Fig. 1). In addition, the C-terminal sequence of the isolated peptide, (Val-Tyr-Lys-COOH, see legend to Table 1) agrees well with that of the F2b fragment in question.

The phosphorylated segment of F2b contains two seryl groups in positions 36 and 38. Our data provide no information as to which one is modified but the electrophoretic pattern suggests that only one of them is phosphorylated. Assuming phosphorylation of Ser-36, thus Arg-34-Lys-35 of F1 and Arg-33-Lys-34 of F2b are identical residues in the vicinity of the sites phosphorylated by the cyclic AMP-dependent kinase, although the surrounding sequences differ significantly in calf thymus F1 (ref. 11) and F2b histone fractions. Considering the total molecules, however, the similarity between the positions of the phosphorylated groups is obvious and the modification occurs adjacent to the N-terminal clusters of basic amino acids in both cases.

H_2N-Pro-Gln-Pro-Ala-Lys-Ser-Ala-Pro-Ala-Pro-Lys-Lys-Gly-Ser-Lys-Lys-Ala-Val-Thr-Lys-Ala-Gln-Lys-Lys-Asp-Gly-Lys-Lys-
Arg-Lys-Arg-Ser-Arg-Lys-Glu-Ser-Tyr-Ser-Val-Tyr-Val-Tyr-Lys-Val-Leu-Lys-Gln-Val-His-Pro-Asp-Thr-Gly-Ile-Ser-Ser-Lys-Ala-
Met-Gly-Ile-Met-Asn-Ser-Phe-Val-Asn-Asp-Ile-Phe-Glu-Arg-Ile-Ala-Gly-Glu-Ala-Ser-Arg-Leu-Ala-His-Tyr-Asn-Lys-Arg-Ser-
Thr-Ile-Thr-Ser-Arg-Glu-Ile-Gln-Thr-Ala-Val-Arg-Leu-Leu-Leu-Pro-Gly-Glu-Leu-Ala-Lys-His-Ala-Val-Ser-Glu-Gly-Thr-Lys-
Ala-Val-Thr-Lys-Tyr-Thr-Ser-Ser-Lys-COOH

Fig. 1 Amino acid sequence of calf thymus F2B histone[10].

Table 1 Amino acid composition of the phosphorylated peptide fragment of calf thymus F2b histone

Amino acid	Concentration (nmol)	Molar ratio to lysine
Serine	280	2
Glutamic acid	160	1
Valine	280	2
Tyrosine	220	>1, 5*
Lysine	150	1

F2B was purified according to the method of Oliver *et al.*[12] and phosphorylated using the cyclic AMP-dependent protein kinase of tonsillar lymphocytes. The incubation mixture contained 10^{-6} M cyclic AMP and 1.25×10^{-4} M γ ^{32}P-ATP (16 mCi mmol^{-1}). Generally one out of every five molecules of histone was phosphorylated in these conditions. The incubation procedure, the precipitation and tryptic digestion of the phosphorylated samples were performed as described previously[4,5]. The ^{32}P-labelled phosphopeptide was isolated as before[4,5] applying two-dimensional paper electrophoresis at *p*H 6.5 and at *p*H 1.9. The labelled peptide fraction was further purified by ascending paper chromatography (pyridine:*iso*amyl alcohol:water—35:35:30 by volume). A sample of the purified phosphopeptide was hydrolysed in boiling HCl (5.7 N) for 24 h at 105°C, and the amino acid analysis was performed on a JEOL JAH 6 analyser. The C-terminal sequence of the isolated peptide was determined according to the method of Sajgó and Dévényl[13]. A sample of the peptide (40 nmol) dissolved in 0.1% NH_4HCO_3 was digested by carboxipeptidase A and B (SERVA, free of trypsine and chymotrypsine) at 37°C. The molar ratio of enzyme to substrate was 1:100. Aliquots were taken out after 0, 5, 15, 30 and 60 min digestion into 0.01 M HCl. The aliquots were freeze-dried then resolved in 0.01 M HCl and were put on a resin-coated chromatoplate (FIXION 50-X8, Chinoin, Hungary). Control mixture of all the amino acids in 0.01 M HCl was also put on the chromatoplate. After ascending chromatography in sodium citrate buffer (Na^+ 0.4 M, citric acid 0.4 M, *p*H 3.28) at 45°C., the amino acid spots were developed using ninhydrin–cadmium–acetate–collidine reagent. After 5 min digestion the only detectable amino acid was lysine. After 15 minutes digestion a small amount of tyrosine appeared and the amount of lysine increased. In the last two samples a spot in the position of valine could be observed, beside the strong tyrosine spot.

*This method destroys a part of the tyrosine residues.

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