STUDY OF THE SPECIFICITY OF HISTONE KINASE USING SYNTHETIC SUBSTRATES

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Received 26 January 1978

1. Introduction

The important role of the protein kinases, phosphorylating various protein substrates, in the regulation of a number of metabolic processes has been elucidated recently. Protein kinases have been reported to phosphorylate different histone fractions. Thus, histone kinase from pig brain phosphorylates certain serine residues in lysine-rich histones: Ser-37 in histone H1; Ser-18 in histone H2a; Ser-14 and Ser-36 in histone H2b; Ser-45, Ser-49 and Ser-91 in histone H5 [1–4].

It has been suggested that the specificity of histone kinase action is determined by the local primary structure of protein substrates. For a more detailed study of the specificity of histone kinase a number of peptides, which are analogues of phosphorylation sites in histones H1 and H2b, have been synthesized.

2. Materials and methods

[γ-^{32}P]ATP (0.1 Ci/mmol) from Amersham was used. The catalytic subunit of histone kinase was obtained as in [5]. The peptides were synthesized as in [6–8]. The peptides obtained were purified by chromatography on a silica gel column in the system butan-1-ol/acetic acid/pyridine/water (15:3:10:12, v/v/v/v). Trifluoroacetyl derivatives were obtained as in [9].

Phosphorylation of peptides by the homogeneous catalytic subunit of histone kinase was carried out for 20 min at 37°C in 50 μl incubation mixture containing 50 mM Tris·HCl buffer, pH 7.4, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.3 mM EGTA (ethylene glycol tetraacetic acid) and 2.5 mM [γ-^{32}P]ATP at an enzyme/substrate ratio of 1:50. Upon the completion of the reaction, pH of the mixture was adjusted to 4.5 and the mixture was separated by electrophoresis (80 V/cm), at pH 3.5 for 40 min on Whatman 3 MM paper. The positions of the radioactive spots on the electrophoregram were determined by autoradiography using an RT-2 X-Ray film (12 h exposure). The positions of the spots containing peptide material were determined by staining the electrophoregram with ninhydrin. The spots were cut out and the amount of radioactive phosphate incorporated into peptides was determined using an SL-30 spectrometer (Intertechnique).

3. Results and discussion

From a study of the phosphorylation of histone fractions by the catalytic subunit of histone kinase we found the following peptide fragments containing phosphorylated serine residues:

	37
Histone H1	Lys–Ala–Ser(HPO_3^-)–Gly–Pro–Pro–Val–Ser–Glu–Leu–Ile–Thr–Lys

14
Histone H2b Lys–Gly–Ser(HPO_3^-)–Lys
36
Lys–Glu–Ser(HPO_3^-)–Tyr–Ser–Val–Tyr–Lys

In histone H1, besides Ser-37, the serine residue 105 is also phosphorylated in the following sequence:

105
Gly–Thr–Gly–Ala–Ser–Gly–Ser(HPO_3^-)–Phe–Lys

We have synthesized a number of peptides with similar primary structures (table 1). The aim of the present work was to study the effect of various amino acid substitutions and changes in the length of the polypeptide chain of substrates upon the rate of their phosphorylation by the catalytic subunit of histone kinase.

As can be seen from table 1, peptides 1–5, which have a greater similarity to the phosphorylated site of histone H1 containing Ser-37 are effective substrates of histone kinase. The decrease of the number of lysine residues in the N-terminal part of the peptide molecule leads to a slight decrease of the phosphorylation rate (peptides 1–3). At the same time the elimination of three C-terminal amino acids (Gly–Pro–Pro) (peptides 7–8) decreases the phosphorylation rate drastically. The substitution of glycine (peptide 4) or D-alanine (peptide 6) for the alanine residue preceding serine as well as substitution

Table 1
Structure of synthetic peptides and their phosphorylation rates

Histone	Peptide no.	Amino acid sequence	Phosphorylation rate (%)
H1	1.	H–Lys–Lys–Lys–Ala–(Ser)–Gly–Pro–Pro–OMe	100
H1	2.	H–Lys–Lys–Ala–(Ser)–Gly–Pro–Pro–OMe	78
H1	3.	H–Lys–Ala–(Ser)–Gly–Pro–Pro–OMe	32
H1	4.	H–Lys–Lys–Lys–Gly–(Ser)–Gly–Pro–Pro–OMe	91
H1	5.	H–Lys–Lys–Lys–Ala–(Ser)–Gly–Ala–Ala–OMe	70[a]
H1	6.	H–Lys–Lys–Lys–(D)Ala–(Ser)–Gly–Pro–Pro–OMe	60[a]
H1	7.	H–Lys–Lys–Lys–Ala–(Ser)–OMe	< 1
H1	8.	H–Lys–Ala–(Ser)–OMe	< 1
H1	9.	H–Lys–Lys–Ala–Ala–(Ser)–Gly–Pro–Pro–OMe	29
H1	10.	H–Ala–(Ser)–Gly–Ser–Phe–Lys–OMe	29
H1	11.	H–Ala–(Ser)–Gly–Ser–Phe–OMe	23
H1	12.	TFA–Ala–(Ser)–Gly–Ser–Phe–OMe	< 1
H2b	13.	H–Lys–Lys–Gly–(Ser)–Lys–Lys–OMe	92
H2b	14.	H–Lys–Lys–Ser–Lys–Lys–Glu–(Ser)–Tyr–Ser–Val–OMe	88
		Histone H1	240

[a] The values of phosphorylation rate of peptides 5 and 6 are given with accuracy of ± 10%

of –Gly–Ala–Ala– for fragment –Gly–Pro–Pro– (peptide 5) causes only an insignificant decrease of the phosphorylation rate.

On the other hand, increase of the distance between the N-terminal lysines and the serine residue to two amino acids (peptide 9) results in a sharp decrease of the phosphorylation rate. This accords with the earlier finding that the phosphorylation reaction requires the presence of a lysine or arginine residue near the serine residue [1].

Peptide 10 which is the analogue of the site (103–108) of histone H1 is also phosphorylated by this enzyme. Omitting the lysine residue at the carboxyl terminus of the peptide (peptide 11) affects the phosphorylation rate rather weakly. Whereas upon blockage of α-amino group of peptide 11 by a trifluoroacetyl group (peptide 12), phosphorylation of the peptide was practically zero. This, apparently, indicates that in peptide 11 the phosphorylated residue is serine in the second position, and not in the fourth. The absence of a basic amino acid residue in peptide 11 is, most probably, compensated by the positive charge on the free α-amino group.

Peptides 13 and 14 which are analogues of the phosphorylated sites of histone H2b have also been found to be good substrates of histone kinase.

It should be noted that at concentrations greater than 80 μM for all peptides, except peptide 14, a strong substrate inhibition was observed which made estimation of K_m values rather difficult. Nevertheless, K_m values for the following peptides were found:

Peptide 2, $K_m = 3.3 \times 10^{-4}$ M
Peptide 10, $K_m = 8.3 \times 10^{-5}$ M
Peptide 14, $K_m = 1.1 \times 10^{-4}$ M
Histone H1, $K_m = 5.0 \times 10^{-5}$ M

Thus, the conclusion can be drawn that the peptides with a greater length of amino acid chain have a higher affinity for the active site of the enzyme.

The substrate properties of synthetic peptides are affected most by:

1. The position of the basic amino acid in the polypeptide chain in relation to the serine residue.

2. The presence of one or more amino acid residues following the serine residue in the C-terminal part of the peptide molecule.

Acknowledgements

We are thankful to Dr L. I. Maryash for compound 14 (table 1) which was used in our tests.

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