# INVESTIGATION OF SITES PHOSPHORYLATED IN HISTONE H5 BY PROTEIN KINASE FROM PIG BRAIN

S. N. KUROCHKIN, N. B. ANDREEVA, K. G. GASARYAN and E. S. SEVERIN
*Institute of Molecular Biology of the Academy of Sciences of the USSR, Moscow 117312*

and

I. V. KURCHATOV
*Institute of Atomic Energy, Moscow, USSR*

Received 16 February 1977

## 1. Introduction

Serine-rich histone H5 (Y, F2c) is a tissue-specific histone found only in the erythroid tissue of birds and some cold-blooded vertebrates [1,2]. It has been suggested that this histone is responsible for the total inactivation of nuclear transcription in matured erythrocytes [3,4]. In immature erythroid cells histone H5 is phosphorylated to a considerably higher degree than in mature erythrocytes [5], and this difference influences the capacity of this histone to restrict transcription [5,6].

The present communication is concerned with sites of phosphorylation of histone H5 by the catalytic subunit of cyclic-AMP-dependent histone kinase from pig brain [7], which was shown earlier to phosphorylate lysine-rich histones [8].

## 2. Materials and methods

[$\gamma$-$^{32}$P]ATP (0.1 Ci/mmol) was obtained from Amersham, DNS-Cl from Merck, the latter was twice recrystallized from benzene. The catalytic subunit of the histone kinase from pig brain was isolated according to the method described elsewhere [7]. Histone H5 was obtained from chromatin of pigeon erythrocytes by extraction with 5% perchloric acid. The mixture of histones H1 and H5 was chromatographed on a CM-cellulose column as described [9].

The phosphorylation of histone H5 (6 mg) by homogeneous catalytic subunit of the histone kinase (enzyme/histone ratio is about 1:500) was run for 12 h at 37°C in 6 ml reaction mixture containing $5 \times 10^{-2}$ M Tris–HCl, pH 7.4, $1 \times 10^{-2}$ M $MgCl_2$, $1 \times 10^{-3}$ M dithiothreitol, $3 \times 10^{-4}$ M EGTA and $3 \times 10^{-4}$ M [$\gamma^{32}$P]ATP.

The tryptic digest of [$^{32}$P]phosphohistone (37°C, 20 h, histone: trypsin 100 : 1) was fractionated on a Bio-gel P-4 column ($1 \times 180$ cm) equilibrated with $1 \times 10^{-3}$ M HCl followed by finger-printing on Whatman No. 3MM paper, in the first direction electrophoresis (80 V/cm) at pH 3.5, for 45 min and in the second chromatography in pyridine/butanol/acetic acid/water (10:15:3:12). The position of radioactive peptides in the fingerprint was determined by radioautography using RT-1 X-ray film (4 h exposure).

The amino acid composition of eluated [$^{32}$P]-phosphopeptides was studied using a quantitative method of amino acid analysis [10] based upon their dansylation followed by two-dimensional thin-layer chromatography on polyamide plates. DNS-Amino acids were scanned using PMQ-II (Opton) spectrophotometer with a $6 \times 0.1$ mm slit. All the peptides obtained were partially sequenced using Edman procedure with the identification of N-terminal amino acid residues in the form of their DNS-derivatives.

Carboxy-terminal amino acid residues were deter-


*North-Holland Publishing Company – Amsterdam*

mined after hydrolysis of peptides with carboxypeptidase B and DNS-Cl treatment.

## 3. Results and discussion

The amount of radioactive phosphate incorporated into the histone during 12 h incubation is more than 2.5 mol $H^{32}PO_3^-$/mol histone H5.

The elution profile of a tryptic digest of [$^{32}$P]-phosphohistone H5 fractionated by gel-filtration on Bio-gel P-4 is shown in fig.1.

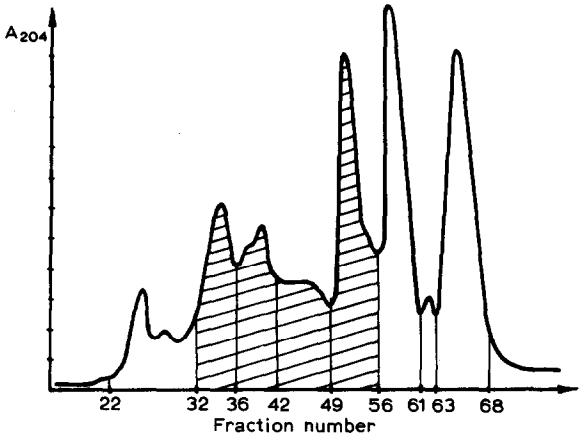


Fig.1. Chromatographic separation of a tryptic digest of [$^{32}$P]phosphohistone H5 on a Bio-gel P-4 column (1 × 180 cm). Fractions containing radioactive phosphate are marked by hatching. Fraction vol. 2.5 ml.

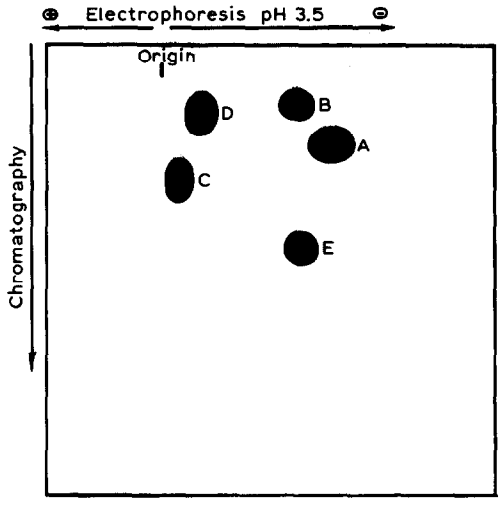


Fig.2. The autoradiograph of a fingerprint of peptide material from fractions 31–55.

All radioactive fractions (31–55) were pooled and then fractionated by fingerprinting. The developed X-ray film of a fingerprint of this peptide material is shown in fig.2.

Table 1 shows the amino acid composition of the radioactive peptides, their N- and C-terminal amino acid residues, and partial N-terminal sequences. It also shows the relative yields of the peptide established from the C-terminal studies and the relative amount of the radioactive phosphate determined by scanning the corresponding spots on developed autoradiographic X-ray film.

Table 1

| Peptide | Yield (%) | Amount of $^{32}$P | N-Terminal amino acid | C-Terminal amino acid | Amino acid composition and sequence |
|---|---|---|---|---|---|
| A | 40 | 100 | Gly– | –Lys | $\overrightarrow{Gly}-\overrightarrow{Thr}-(Arg,Ser_{2-3},Glu_2,Ile)-\overleftarrow{Lys}$ |
| B | 50 | 50 | Gly– | –Arg | $\overrightarrow{Gly}-\overrightarrow{Thr}-(Ser_2)-\overleftarrow{Arg}$ |
| C | 50 | 60 | Glx– | –Lys | $\overrightarrow{Glx}-\overrightarrow{Ser}-(Ile,Glx)-\overleftarrow{Lys}$ |
| D[a] | 20 | 30 | Thr– | –Arg | $\overrightarrow{Thr}-(Ser,Pro)-\overleftarrow{Arg}$ |
| E | 100 | 40 | Gly– | –Arg | $\overrightarrow{Gly}-\overrightarrow{Val}-\overrightarrow{Gly}-(Ala,Gly,Ser_2)-\overleftarrow{Phe}-\overleftarrow{Arg}$ |

[a] Only a limited amount of the peptide was available; the data are qualitative

(—→) Amino acid sequences established using the Edman procedure
(←—) Carboxypeptidase B-cleaved amino acids

Comparison of the data obtained with the known primary structure of histone H5 from chicken erythrocytes [11] demonstrates that phosphorylated peptides of tryptic hydrolysate have the following amino acid sequences:

A – unknown

B – Gly–Thr–Ser–Ser–Arg

Peptide 43–47 – Gly–Gly–$\overset{45}{\text{Ser}}$–$\overset{46}{\text{Ser}}$–Arg

C – Glx–Ser–Ile–Glx–Lys

Peptide 48–52 – Gln–$\overset{49}{\text{Ser}}$–Ile–Gln–Lys

D – unknown

E – Gly–Val–Gly–(Ala–Gly–Ser–Ser)–Phe–Arg

Peptide 86–94 – Gly–Val–Gly–Ala–Gly–$\overset{91}{\text{Ser}}$–$\overset{92}{\text{Ser}}$–Phe–Arg

Peptide A seems to be the sum of peptides B and C and to arise from incomplete selectivity of tryptic hydrolysis. Peptide D appears to belong to the C-terminal part of a histone molecule. The fact that peptide E was present in large amounts and was only rather weakly labelled with radioactive phosphate, suggests a high degree of Ser 91 (or 92) phosphorylation in vivo.

Thus, there are four definite sites which are phosphorylated by the catalytic subunit of protein kinase from pig brain: Ser 45 (or 46), Ser 49, Ser 91, (or 92) and one Ser or Thr residue in the amino acid sequence 111–200 of a histone H5 molecule.

## References

[1] Hnilica, L. S. (1964) Experientia 20, 13–14.
[2] Edwards, J. L. and Hnilica, L. S. (1968) Experientia 24, 228–230.
[3] Dick, C. and Johns, E. W. (1969) Biochim. Biophys. Acta 175, 414–419.
[4] Billett, M. A. and Hindley, J. (1972) Eur. J. Biochem. 28, 451–462.
[5] Adams, G. H. M., Vidali, G. and Neelin, J. M. (1970) Can. J. Biochem. 48, 33–37.
[6] Gasaryan, K. G., Andreeva, N. B. and Vishnevskaya, T. Yu. (1977) Differentiation, in press.
[7] Nesterova, M. V., Sashchenko, L. P., Vasiliev, V. Yu. and Severin, E. S. (1975) Biochim. Biophys. Acta 377, 271–281.
[8] Shlyapnikov, S. V., Arutyunyan, A. A., Kurochkin, S. N., Memelova, L. V., Nesterova, M. V., Sashchenko, L. P. and Severin, E. S. (1975) FEBS Lett. 53, 316–319.
[9] Gasaryan, K. G., Andreeva, N. B. and Penkina, V. I. (1976) Differentiation 5, 21–28.
[10] Spivac, V. A., Fedoseev, V. A., Orlov, V. M. and Varshavsky, Ya. M. (1971) Anal. Biochem. 44, 12–31.
[11] Santiere, P., Briand, G., Kmiecik, D., Loy, O., Biserte, G. and Champagne, M. (1976) FEBS Lett. 63, 164–166.