PRESENCE OF A HISTONE H2B PROTEASE IN SNAKE VENOM PHOSPHODIESTERASE

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1. Introduction

We wish to report the accidential discovery of protease activity towards histone H2B in a commercial sample of snake venom phosphodiesterase EC 3.1.4.1 supplied by Worthington Biochemicals, obtained from *Crotolus adamanteus* venom.

The lysine-rich histone H2B (mol. wt ~13 700 – 125 amino acids) forms part of the histone octomer of the nucleosome particle which is the basic structural unit of chromatin [1]. In contrast to H4, H3 and H2A, however, its sequences do not display complete evolutionary conservation of structure. There are definite species and cell variations around the N-terminal part of the molecule [2] whereas the C-terminal (hydrophobic regions) are more conservative. It has a single N-terminal proline and the molecule can be phosphorylated both in vivo (cell cycle-dependent) and in vitro [3].

In the course of our recent structural investigations in which we were looking for possible polyADPR links in nuclear proteins we digested various fractions with many phosphate splitting enzymes including the phosphodiesterase described above. Whereas the non-histone protein profile on SDS–gel electrophoresis was relatively unchanged after incubating with the enzyme we found that H2B both singly and in histone mixtures was extensively degraded to at least two lower molecular weight species.

2. Materials and methods

Several batches of snake venom phosphodiesterase I were purchased from Worthington Biochemicals (catalog no. LS00 03926; preparation of Williams et al., J. Biol. Chem. 236 (1961) 1130). All possessed the protease activity towards H2B. The incubation buffer originally used was that recommended by the suppliers, 0.2 M Tris–HCl (or glycine buffer) (pH 8.0), 50 mM Mg^{2+} but later the Mg^{2+} was found to be unnecessary for the activity. Initially the enzyme concentration used was 2% (w/w) of that of the substrate.

Electrophoresis was carried out mainly in 15% SDS–polyacrylamide gels [4] but results were checked in the Panyim and Chalkley system (15% acrylamide, pH 2.6). Samples (5–10 μg) were loaded onto 12 × 0.5 cm gels. Loss of stained bands was checked and monitored in a Joyce Loebl chromoscan.

Rat liver non-histones were extracted from isolated nuclei by 8 M urea 50 mM phosphate (pH 7.6) [5] and the histones removed with dil. acid (0.2 M H_2SO_4 or 0.2 N HCl).

Calf thymus H1, H4 and H2B histones were obtained from Boehringer Mannheim GmbH.

3. Results and discussion

The limit digest under the original conditions used was found to be obtained at 37°C for 2 h. The results of these digestions on rat liver nuclear proteins and calf thymus histones is shown in fig.1. Clearly no breakdown of the non-histone proteins or H1 and H4 histones occurred. Dansylation [6] of the 2 h hydrolysate at 37°C revealed the formation of at least 14 new end groups (not from the enzyme itself) indicating extensive exopeptidase activity.

The crude whole histone standard we used (fig.1) was deficient in H2A but other samples containing this histone showed no breakdown. This is interesting in view of the discovery of a unique

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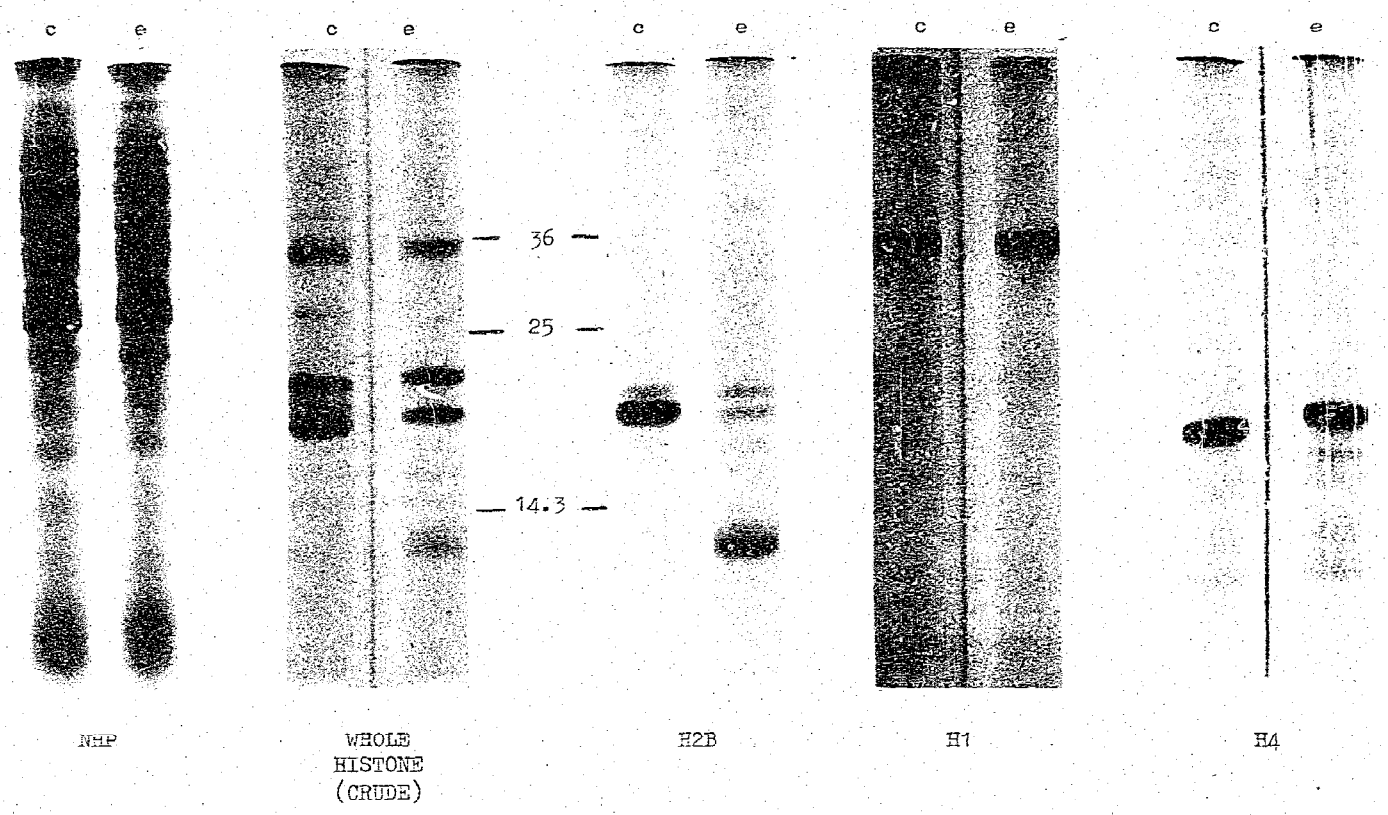

Fig.1. SDS–polyacrylamide gels of control (c) and enzyme treated (e) nuclear proteins. Left to right: 8 M urea soluble rat liver non-histone protein (75 μg); a crude histone fraction (5 μg); Boehringer calf thymus histones H2B, H1 and H4 (2.5 μg each). Gels stained with Coomassie brilliant blue. Figures show the positions of molecular weight marker polypeptides for histone gels only. Non-histone proteins were run in 10% (w/v) gels.

protease that specifically degrades H2A and has been shown to be tightly bound to calf thymus chromatin [7]. A single fragment, cH2A, similar in electrophoretic mobility to H4, and a pentapeptide were produced.

Table 1
Effect of various chemicals and inhibitors on the breakdown of histone H2B by snake venom protease

Sodium phosphate (0.25 M)	++
Sodium phosphate (0.5–1.5 M)	+++
NaCl (0.5–3 M)	+++
Urea (1–5 M)	–
Sodium bisulphite (10–25 mM)	+
Sodium bisulphite (50 mM)	++
Phenylmethylsulphonylfluoride (1–2 mM)	+++
Trypsin inhibitor (soya bean) (50–100 μg)	+++

+++ = complete inhibition. Assay based on disappearance of H2B band on gel electrophoresis after digestion for 1.5 h at 37°C with 2% (w/w) of crude enzyme

In contrast to these findings (table 1) our enzyme was inhibited by increasing NaCl concentrations and not affected by up to 5 M urea. We also found (as they did) that proteolytic activity was observed over a wide pH range, the optimum observed being in the pH range 7–9. In addition the enzyme was completely inhibited by the usual proteolytic inhibitors (see table 1).

Attempts to isolate the digest fragment (mol. wt ~10 000) by column chromatography on G-100 resulted in the isolation of two peaks one of which seemed to be an aggregate mixture. The other was hydrolysed to its constituent amino acids and the composition of the hydrolysate was indicative of the removal of ~29–30 amino acids from the N-terminal (marked loss of proline and lysine residues). Dansylation of the new end groups released gave an equivocal answer indicating a number of products to be present.

Further work on these fragments is required before the composition can be stated with confidence.

This protease activity was not found to be present in a sample of bovine spleen phosphodiesterase (Sigma, phosphodiesterase type II used EC 3.14.1.18).

We also found the enzyme in solution to be stable for 2–3 weeks at −15°C.

Many authors have reported the presence of histone degrading enzymes in isolated chromatin and nuclei [8–11]; they are usually inhibited by sodium bisulphite. However, these seem to lack any specificity towards a particular histone class except that H1 is more sensitive; probably because it does not form part of the nucleosome complex. It remains to be determined whether the enzyme we have described is able to degrade H2B in the nucleosome. If it could be separated from the venom phosphodiesterase activity this enzyme might prove useful for physico-chemical studies on the role of H2B in nucleosome structure.

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