LIMITED PROTEOLYSIS OF THE VERY-LYSINE-RICH HISTONE H1 BY A MEMBRANE-BOUND PROTEINASE FROM RAT LIVER

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1. Introduction

Histone degrading enzymes in various tissues have been described by several authors [1–6]. From our studies on the subcellular distribution of histone degrading enzymes an enzyme with high specificity for chromosomal proteins has been found [6]. In the present communication we report on the limited proteolysis of the very-lysine-rich histone H1 by this proteinase.

2. Materials and methods

The insoluble proteinase was prepared from rat liver mitochondria which had been treated with digitonin to remove lysosomal contamination [7]. The digitonin-treated mitochondria were frozen and thawed twice, centrifuged through 1.7 M sucrose–10 mM Tris, pH 8 for 2 h at 160 000 *g*. The sediment, which contained approx. 22% of the proteinase activity, was suspended in distilled water and used for the experiments described. Solubilization and purification to homogeneity of the enzyme could be achieved after treatment with 0.5 M potassium phosphate buffer, pH 8.5 for 2 days, acetone fractionation (60% v/v), adsorption to hydroxylapatite and Sephadex G 75 gel filtration [8].

3. Results

Histone H1, which was isolated from total rat liver histones by extraction with 0.5 N $HClO_4$ [9], was incubated with proteinase isolated from the mitochondrial fraction. The degradation pattern of histone H1 by the action of the neutral rat liver proteinase is depicted in fig.1a–e. The formation of one major degradation product is observed, even if the time of incubation is extended or the amount of enzyme is increased indicating a limited proteolysis of H1. From the electrophoretic mobility in SDS-acrylamide gels a mol. wt. of 13 000 ± 10% has been estimated for the fragment.

When a mixture of total rat liver histones, including H1, was incubated with the proteinase under conditions that led to complete degradation of pure H1 alone, only 20% of H1 was degraded. Under these conditions all the other histones were degraded at about the same rate (not shown). When histone H1 was complexed to calf thymus DNA, only 30% of H1 was found to be degraded; a similar observation has already been made by Bartley and Chalkley using proteinases associated with calf thymus chromatin [1]. To study the effect of phosphorylation on the susceptibility of H1 to the mitochondrial proteinase, histone H1 was isolated from chromatin of regenerating rat liver 24 h after partial hepatectomy. No difference in the degree and pattern of degradation was observed between this preparation and the usual, unphosphorylated preparation from normal rat liver.

The histone degrading enzyme purified from the rat liver mitochondrial fraction [8] shows a high degree of substrate specificity. It is shown in table 1 that, among a variety of proteins tested, only histones, non-histone chromosomal proteins and glucagon were found to be degraded. Although it was possible to solubilize the mitochondrial proteinase in 0.5 M

potassium phosphate buffer, pH 8.5, we have used the insoluble form of the proteinase for the incubation with the various substrates, since the reaction could easily be stopped by removal of the enzyme from the incubation mixture by centrifugation. The proteinase action on the various protein substrates was examined by SDS-acrylamide gel electrophoresis as detailed in the legend to table 1. Among several synthetic substrates only *N*-acetyl-L-tyrosine ethyl ester and benzoyl-L-arginine ethyl ester were suitable substrates.

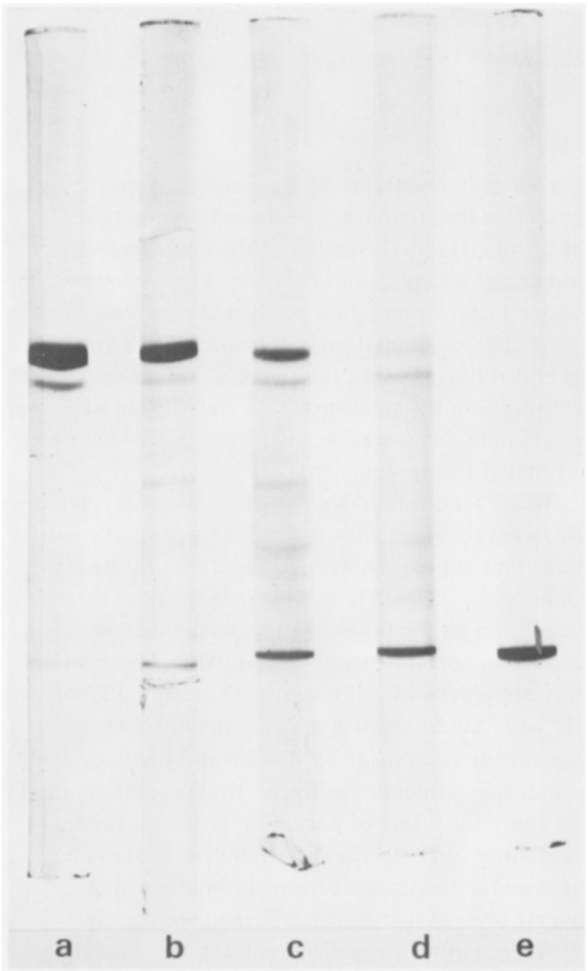

Fig.1. Degradation pattern of rat liver histone H1. (a) Histone H1, (b) histone H1 plus proteinase, incubated for 3 min, (c) 6 min (d) 10 min (e) 30 min. The incubation mixture contained, in a total volume of 0.1 ml: 5 μmol of Tris/HCl, pH 8, 18 μmol of histone H1 and 8 μg of proteinase (specific activity 2.0 μmol *N*-acetyl-L-tyrosine ethyl ester split/min × mg protein) (b–e). After incubation at 37°C the reaction was stopped by the addition of 20 μl of 4.5 N acetic acid. The acrylamide gel electrophoresis was carried out according to [10].

Table 1
Substrate specificity of the proteinase

Substrate	Degradation % of control
Histone H1	100
Total histones	46
Non-histone chromosomal proteins	48
Ovalbumin	3
Myoglobin	6
Cytochrome *c*	3
Bovine serum albumin	5
Lysozyme	7
Insulin	3
Glucagon	95
Azocasein	0
Azocoll	0

The proteinase assay contained in a final volume of 0.1 ml, Tris/HCl, pH 8.0 5 μmol; protein substrate, 15–50 μg; and insoluble proteinase, 0.02 mg (specific activity 0.2 μmol *N*-acetyl-L-tyrosine ethyl ester split per min × mg). After vigorous shaking for 60 min at 37°C the incubation mixture was centrifuged for 5 min at 15 000 *g*. Twenty μl of supernatant was mixed with 20 μl of 2% SDS, 2% 2-mercaptoethanol and 0.05 M Tris, pH 8.1 and kept at 95°C for 5 min. After the addition of 5 μl of bromophenolblue (0.01%) and 10 μl of glycerol (87%), the mixture was applied to SDS gels [11]. The protein of the band obtained from incubations without added proteinase was used as control (= 100%).

In experiments with azocasein and azocoll the incubation mixture contained, in a total volume of 0.5 ml; Tris/HCl, pH 8, 0.05 mmol; azocoll or azocasein, respectively, 12 mg; and proteinase, 0.12 mg (specific activity 0.2 μmol *N*-acetyl-L-tyrosine ethyl ester split per min × mg). After 60 min at 37°C, 0.5 ml of 10% TCA was added. After centrifugation the clear supernatants were measured at 520 nm or 422 nm, respectively, against a blank without addition of enzyme.

4. Discussion

As can be seen from fig.1, a weaker second protein band in addition to H1 is found. Microheterogeneity of histone H1 has been observed by others [12].

From the primary structure of histone H1 [12] it is known that the histone molecule consists of a hydrophobic, α-helical part and a hydrophilic part

containing most of the lysine and proline residues. Probably, only this hydrophilic, basic part of the molecule is bound to DNA [12]. Although we have not carried out an amino acid analysis on the histone H1 fragment obtained after the limited proteolysis, it is likely that it is formed from the basic part of the histone H1 molecule, because of its high mobility in low pH acrylamide gel electrophoresis [10].

Further work is required to establish whether the proteinase described here has any physiological role in the degradation of chromosomal proteins.

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