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PHENYLMETHYLSULFONYL FLUORIDE STIMULATES PROTEOLYSIS OF NUCLEAR PROTEINS FROM CHICK LIVER

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Summary: The degradation of H1 histone and high mobility group (HMG) non-histone proteins was stimulated when the homogenate from chick liver was incubated in the presence of phenylmethylsulfonyl fluoride (PMSF). Two proteinase inhibitors, elastatinal and chymostatin, significantly inhibited the PMSF-stimulated degradation of H1 histone and HMG proteins. On the contrary, other proteinase inhibitors like leupeptin, pepstatin, trypsin inhibitor, antipain, o-phenanthroline and EDTA had no effect on the degradation of the nuclear proteins. These results warn the researcher to be cautious while using PMSF for preparation of nuclear proteins such as H1 histone and HMG proteins.

Isolation of nuclear proteins such as histones and non-histone proteins is often hampered by proteolytic degradation of the desired protein. In order to minimize this nuisance, PMSF has been used most extensively as an inhibitor of proteolysis of nuclear proteins (1,2). However, in the course of our studies on nutritional (3) and developmental (4) changes in chromatin-associated high mobility group (HMG) proteins in chick liver, we came to notice that the addition of PMSF to the chick liver homogenate stimulated, rather than inhibited, proteolytic degradation of not only HMG proteins but also H1 histone. The present report describes this unexpected finding.

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MATERIALS AND METHODS

Animals and materials: Newly hatched male White Leghorn chicks were obtained from a local hatchery (Ishii Hatchery, Tokushima). PMSF was purchased from Sigma Chemical Co.(U.S.A.) and Calbiochem(U.S.A.). Trypsin inhibitor, leupeptin, antipain, pepstatin, elastatinal and chymotrypsin were from Boehringer Mannheim(Germany). o-Phenanthroline was supplied from Dojin Laboratories (Japan).

Assay of H1 histone and HMG protein degradation: The livers from newly hatched chicks were homogenized in 2.5 volumes of cold 0.25 M sucrose in buffer A [50 mM Tris-HCl (pH 6.5), 25 mM KCl and 10 mM $MgCl_2$] with a Potter-Elvehjem type homogenizer. The homogenate (200 µl) was then incubated with or without 5 mM PMSF at 37°C. After incubation, H1 histone and HMG proteins were extracted by 0.2 M H_2SO_4, precipitated by 18 % trichloroacetic acid and the extracted proteins were electrophoresed on acetic acid-urea gel of Panyim and Chalkley (5). Following staining of the gel with Coomassie brilliant blue, the degradation of H1 histone and HMG proteins was estimated by observing the intensity of the stained band.

Preparation of antisera against H1 histone and HMG2 protein: H1 histone was isolated from 70 day-old chick liver by 5 % perchloric acid extraction and acetone precipitation. H1 histone was then purified on a Bio-Gel P60 column according to the procedure of Böhm et al.(6). Male New Zealand white rabbits were immunized with H1 histone and antiserum was prepared as described previously (4). The preparation of antiserum against HMG2 protein was also previously described (4).

Immunoblotting: Immunoblotting of H1 histone and HMG proteins was performed as described previously (4).

Protein measurement: The amount of protein was determined according to Lowry et al.(7) using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

Following the incubation of chick liver homogenate with or without 5 mM PMSF at 37°C for different time intervals, the fraction containing H1 histone and HMG proteins was extracted from the homogenate and electrophoresed on acetic acid-urea gels (Fig.1). HMG1 and HMG2 clearly decreased during incubation of the homogenate containing PMSF. After 4 h of incubations, HMG proteins disappeared completely. The content of H1 histone also decreased during incubation in the presence of PMSF. However, neither H1 histone nor HMG proteins showed any noticeable degradation even after 4 h incubation of the homogenate without PMSF. PMSF from Sigma and Calbiochem showed similar effect on the degradation of H1 histone and HMG proteins (data not shown).

Immunostaining by anti-HMG2 and anti-H1 histone sera of the total homogenate proteins after sodium dodecylsulfate-polyacrylamide gel electrophoresis also showed time-dependent decrease in the presence of PMSF (Fig. 2). These results indicate that the decrease of HMG proteins and H1 histone after incubation with PMSF, depicted in Fig. 1, does not occur due to changes in extractability of these proteins.

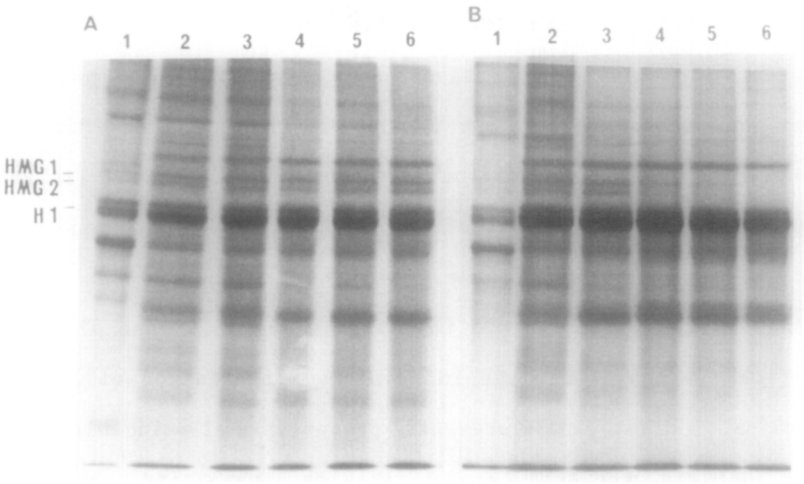

Fig.1. Effect of PMSF on the disappearance of H1 histone and HMG proteins from the chick-liver homogenate. The homogenate (200µl) was incubated at 37℃ without (A) or with (B) 5 mM of PMSF, and then the fraction containing H1 histone and HMG proteins was extracted and subjected to acid-urea gel electrophoresis as described in "Materials and Methods". Lane 1, HMG fraction from chick erythrocytes as the marker; lanes 2, 3, 4, 5, and 6 indicate incubation times 0, 1, 2, 3 and 4 h, respectively.

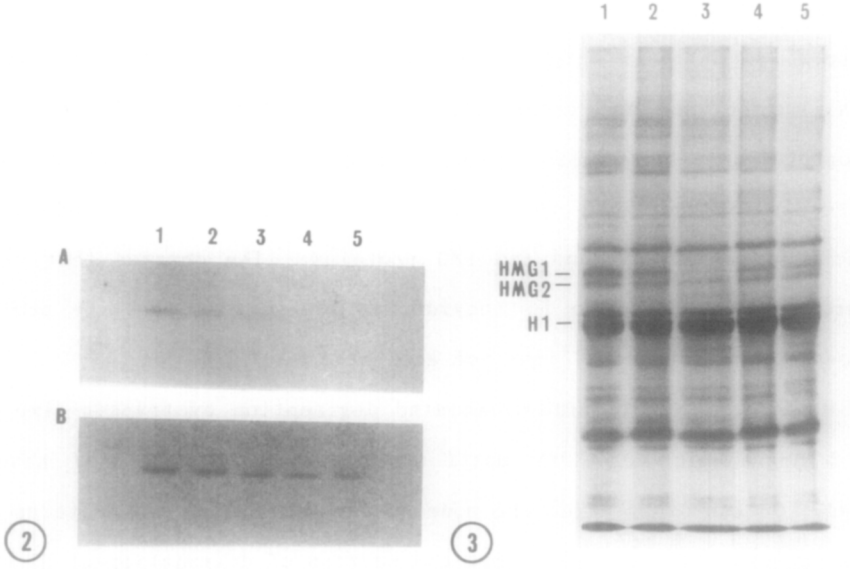

Fig.2. Immunoblotting of chick-liver homogenate proteins with anti-HMG2 or anti-H1 histone antibodies. After incubation of the homogenate at 37℃ in the presence of 5 mM PMSF, 30 µg of the total homogenate protein was subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis and immunoblotted with anti-HMG2 antibody (A) or anti-H1 histone antibody (B). Lanes 1, 2, 3, 4 and 5 indicate incubation times 0, 1, 2, 3 and 4 h, respectively.

Fig.3. Effect of proteinase inhibitors on the degradation of H1 histone and HMG proteins. Chick-liver homogenate (200 µl) was incubated at 37℃ for 2 h in the presence of various proteinase inhibitors, and then the fraction containing H1 histone and HMG proteins was extracted and subjected to acid urea gel electrophoresis as in Fig. 1. Lane 1, non-incubated control; lane 2, incubated without PMSF; lane 3, incubated with 5 mM PMSF alone; lane 4, incubated with 5mM PMSF and 5 mM elastatinal; lane 5, incubated with 5 mM PMSF and 5 mM chymostatin.

The most probable cause for the disappearance of H1 histone and HMG proteins is proteolytic digestion. To evaluate this possibility, we incubated the homogenate for 2 h in the presence of 5 mM PMSF and various proteinase inhibitors. As previously observed, incubation with PMSF caused a drastic decrease in HMG proteins (Fig. 3, lane 3). Incubation in the presence of various proteinase inhibitors showed that two agents, elastatinal (Fig. 3, lane 4) and chymostatin (Fig. 3, lane 5), were capable of significantly inhibiting the PMSF-induced degradation of H1 histone and HMG proteins. Elastatinal prevents proteolysis by inhibiting elastase, while chymostatin prevents proteolysis by inhibiting chymotrypsin-like enzyme. Interestingly, both inhibitors contain an unusual urea linkage and a peculiar amino acid, 2-iminohexahydro-(4S)-pyrimidyl-(S)-glycine (8). Furthermore, neither leupeptin which inhibits plasmin, trypsin and related enzymes nor pepstatin which inhibits acid protease had any effect on the degradation of H1 histone and HMG proteins (data not shown). Trypsin inhibitors and antipain which prevent proteolysis by inhibiting trypsin or trypsin-like enzymes and o-phenanthroline and EDTA which inhibit various metalloproteinases were also found to have no effect (data not shown). These results suggest that the stimulation of proteolysis by PMSF accounts for decreases in H1 histone and HMG proteins. The enzymes that catalyze this proteolysis appear to be chymotrypsin- and elastase-like proteinase that does not require metal ions for activity.

For H1 histone, nucleotide-stimulated degradation by trypsin-like enzyme (9) and degradation of SPKK motif by thiol proteinase (10) have been reported. On the other hand, the mode of degradation of HMG proteins in the cell is unknown. It is likely that the pattern of degradation of H1 histone and HMG proteins is similar. However, it cannot be concluded from these experiments whether one or more enzymes are involved in the degradation of H1 histone and HMG proteins. To our knowledge, this is the first report on the degradation of H1 histone and HMG proteins by chymotrypsin- and elastase-like enzymes. Further studies are required to elucidate the physiological roles of these proteinases. Also we warn the workers to be cautious while using PMSF for preparation of H1 histone and HMG proteins.

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