Comparative Enzymatic Degradation of H1 Subfractions from Syrian Hamster Tissues

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An additional hydrolysis site recognized by thrombin on histone H1 molecules was found. Snakes venom proteases from *Agkistrodon rhodostoma, Bothrops marajoensis* and *Bothrops moojeni* were further used for the analysis of H1 histones. The presence of the main cleavage site on H1 histone molecules has been established. This site is localized on main N-terminal thrombin peptide. The main venom protease peptides obtained from different H1 subfractions preserve differences of electrophoretic mobility in acid-urea polyacrylamide gels typical for the initial H1 subfractions.

Introduction

H1 histone subfractions isolated from various sources differ in their electrophoretic mobility on acid-urea PAG [1–4]. Particularly great difference was observed in our laboratory for one of H1 histone subfractions isolated from Syrian hamster Kirkman-Robbins hepatoma. This subfraction is absent in homologous normal liver tissue [5, 6].

In this paper we have looked for a method giving possibility for localization of differences within H1 subfraction molecules causing different behaviour in acid-urea PAG. For this purpose digestion with thrombin and snakes venom proteases was applied. Except the Kirkman-Robbins Syrian hamster hepatoma and liver H1 subfractions, lysine-rich histone from calf thymus was used as a standard system.

Experimental Procedures

The neoplastic tissue was obtained from Kirkman-Robbins hamster hepatoma tumors transplanted on Syrian hamster in a procedure described earlier [5].

Hamster liver was taken from healthy hamsters as described [5].

Total H1 histone was extracted with 5% (v/v) perchloric acid from chromatin isolated from purified tumor or liver nuclei as described [5].

Histone H1 subfractions were separated on Amberlite CG-50 according to the method of Kinkade and Cole [7] as described by Bonner *et al.* [8] and Smerdon *et al.* [9]. The final purification was carried out on acid-urea preparative gels as described [5].

Electrophoresis in acid-urea gels was performed according to method of Panyim and Chalkley [10], modified by using 20% cylindrical gels of 3 mm diameter, 10 or 25 cm long. Electrophoresis was carried out at 12 V/cm. The gels were stained with 0.1% aminoblack in 7% acetic acid containing 20% ethanol and were destained by electrophoresis in 0.9 N acetic acid.

Thrombin degradation was carried out essentially according to the method described by Chapman *et al.* [11]. The digestion times were 2 or 5 h. Trascolan (Bayer) was used as the plasmin inhibitor at the concentration 100 U/ml of incubation mixture.

Snake venom proteases degradation were performed as follows: H1 histone or H1 subfractions were dissolved in 0.05 M Tris-HCl buffer, pH 8.0. The digestion was carried out at 36 °C for 5 h with Ancrod, Reptilase R or Defibrase. The enzyme protein ratio was 10 or 20 units of enzyme per 1 mg of protein. Ancrod, Reptilase R and Defibrase are generic names assigned to the coagulant fraction from *Agkistrodon rhodostoma, Bothrops marajoensis* and *Bothrops moojeni,* respectively, by the World Health Organization.

Abbreviation: PAG, polyacrylamide gel.

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Results and Discussion

Thrombin digestion

The typical electrophoretic pattern of a histone H1 subfraction after thrombin degradation is shown in Fig. 1. Generally, it resembles the results of Chapman *et al.* [11] for the calf thymus H1 histone. However, more detailed analysis based on electrophoresis in 25 cm long acid-urea PAG of the mixture of digested during 2 h and undegraded H1 subfraction (Fig. 2) reveals heterogenous peak consisting of undigested and partially hydrolyzed molecules. Similar electrophoretic pattern was noticed for different H1 subfractions from Syrian hamster hepatoma and liver as well as for the calf thymus H1 histone. Thus, the observed phenomenon of slightly increased electrophoretic mobility after short thrombin digestion is a common feature for H1 histone molecules. These experiments allow to postulate the existence of an additional thrombin digestion site. The hydrolysis at this site proceeds much faster than cleavage at the site which divides H1 molecules into two main N- and C-terminal peptides described by Chapman *et al.* [11]. An additional prove confirming the existence of fast thrombin hydrolysis site is the presence of very fast migrating band on acid-urea PAG which was observed after a short thrombin digestion time. This band migrates faster than highly basic C-terminal thrombin peptide (data not shown).

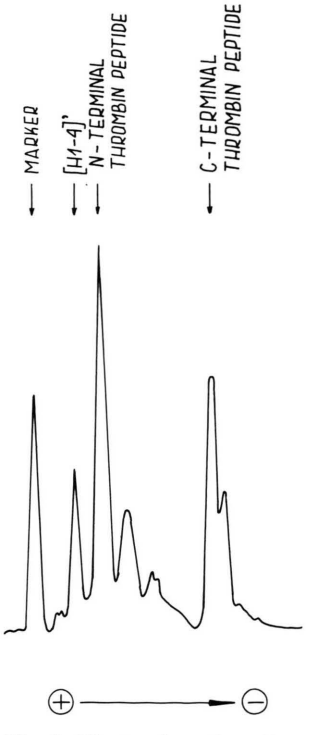

Fig. 1. Electrophoretic pattern of the H1-4 Syrian hamster Kirkman-Robbins hepatoma subfraction after digestion with thrombin obtained on 10 cm long acid-urea PAG. Enzyme protein ratio was equal to 2 units of thrombin per 1 mg of H1-4 histone subfraction. Digestion time 5 h. Marker – bovine serum albumin.

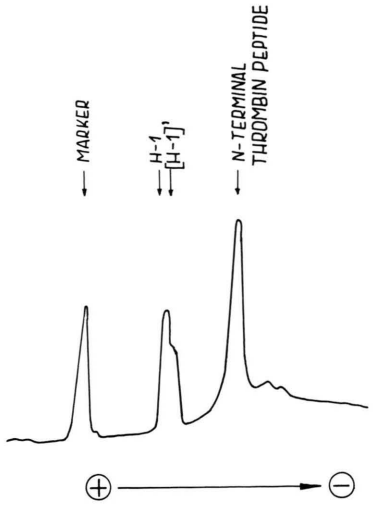

Fig. 2. Electrophoretic pattern of H1-1 Syrian hamster Kirkman-Robbins hepatoma subfraction digested for 5 h with thrombin and mixed with initial preparation of this subfraction obtained on 25 cm long acid-urea PAG. H1 corresponds to initial undegraded H1-1 subfraction and [H-1]′ to the peptide arising after cleavage of H1-1 molecule at the additional thrombin hydrolysis site.

Data concerning specificity of thrombin digestion, including also the proteins which are not physiological substrates, may suggest (i) degradation proceeds entirely after arginine residue, (ii) 6, 7 or 8 positions to the right of susceptible bond is occupied by glutamic or aspartic acid or respective amine [12]. Among known aminoacid sequences of mammalian H1 histone the regions compatible with these data are not present [13, 14].

The thrombin cleavage site dividing calf thymus H1 molecules into two main N- and C-terminal peptides is localized after lysine residue which corresponds to the 122 lysine on RTL-3 H1 histone subfraction from rabbit thymus [13]. The aminoacid sequence in the region of 122 lysine is –pro–lys–lys–ala–gly–ala–ala–lys–pro–lys–. The closest var-

iant to this sequence localized on the same RTL-3 subfraction is −pro−lys−lys−ala−lys−ala−val−lys− pro−lys−. There are two differences between these sequences. The 125 glycine is replaced by 185 lysine and the 127 alanine corresponds to the 187 valine [13]. Moreover, there are shorter sequences −pro−lys−ala− localized at the proximity of C-terminal aminoacid of RTL-3 H1 subfraction which can also candidate for the additional thrombin cleavage site [13].

Unresolved question in thrombin digestion of H1 histone molecules is the heterogeneity of C-terminal peptides revealed by acid-urea PAG electrophoresis. This phenomenon is observed even if single H1 subfraction is cleaved (Fig. 1), similary to that described by Chapman *et al.* [11] for the total calf thymus H1 histone. It seems not to be possible that the reason of the observed heterogeneity comes from the absence of the described additional thrombin cleavage site in a part of H1 subfraction molecules, for example, as result of modification of aminoacid residues, since even after short thrombin digestion all molecules of the cleaved H1 subfraction increase their electrophoretic mobility.

Electrophoresis in acid-urea PAG has not shown any difference between main N- and C-terminal thrombin peptides obtained from H1-1 and H1-4 Syrian hamster hepatoma subfractions (data not shown).

Defibrase digestion

Three snake venom proteases: Ancrod from *Agkistrodon rhodostoma,* defibrase preparation from *Bothrops moojeni,* and reptilase R derived from *Bothrops marajoensis* were tested as a tool to localize differences between H1 subfractions causing various electrophoretic behaviour in acid-urea PAG system. Initial experiments have shown that all three tested proteases when applied for digestion of H1 histones give the same digestion products. Therefore, for further experiments only defibrase was used.

The general feature of these thrombinlike enzymes is the ability to cleave peptide bonds between Arg-X. The more often aminoacid X is glycine. However, the presence of Arg-X bond is not sufficient for substrate recognition. They split fibrinopeptide A from fibrinogen (A) chains by hydrolysis of the Arg-Gly bond split by thrombin, but unlike thrombin do not cleave the corresponding Arg-Gly bond in the (B) chains [15−17]. These proteases do not split many other proteins, like native casein and denatured hemoglobin despite of the presence of Arg-X bonds [18, 19]. The described proteases act with a species-dependent preference on fibrinogen of different mammals, hence, coagulation of rabbit fibrinogen proceeds about 10 times longer then the coagulation of human fibrinogen after addition of the same amounts of these enzymes. This phenomenon might be explained by structural differences of rabbit and human fibrinogen as reflected the sequence analysis of fibrinopeptide A from different mammals [20]. To sumarize, these enzymes recognize aminoacid differences in much longer regions of protein molecules than hydrolyzed bond.

Defibrase was applied for digestion of: total calf thymus H1 histone, H1-1, H1-4, H1 histone subfractions from Syrian hamster hepatoma, and H1-1 and H1-2, H1 histone subfraction from Syrian hamster liver. For all histones tested main degradation peptide and small amounts of several other peptides were released (Fig. 3). Despite of the same conditions of hydrolysis H1-1 subfractions derived from Syrian hamster hepatoma appeared to be more resistant to the action of defibrase than remaining histones under analysis, probably, reflecting structural differences of the H1 histones under investigation (Fig. 3).

Digestion of main thrombin N- and C-terminal peptides with defibrase localizes the cleavage site on N-terminal thrombin peptide (Fig. 4). Thus, main defibrase peptide contains all C-terminal domain − "tail" and at least part or whole central globular domain. It may be suggested that main defibrase degradation site should be localized after one of four arginine residues. Their positions in aminoacid sequence of CTL-1 H1 subfraction are: 31, 32, 53, 78 [14]. The fact that arginines at the positions 32, 53 and 78 of aminoacid sequence of CTL-1 H1 subfraction are also conserved in RTL-3 and trout testis H1 subfractions [13] and that defibrase digestion patterns for H1 histones from different sources are similar, suggests that the main defibrase cleavage site is localized at analogous position in different H1 subfractions. Presumable localization of thrombin and defibrase cleavage sites are summarized on Fig. 5.

Main defibrase peptides obtained from the H1-1 and H1-4 Syrian hamster hepatoma H1 subfractions when analyzed on 25 cm long, acid-urea PAG show

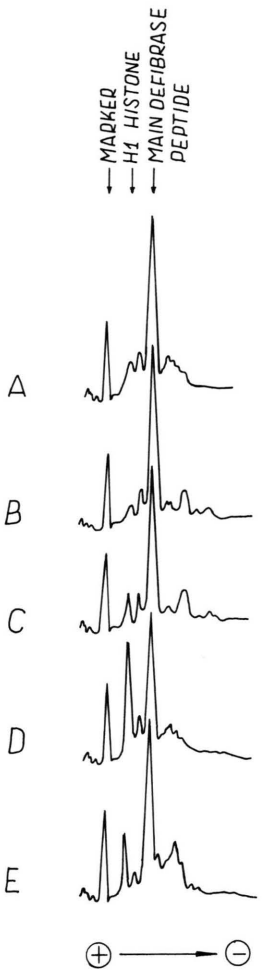

Fig. 3. Electrophoretic patterns of different H1 histone preparations after digestion with defibrase. Digestion time 5 h. Enzyme/substrate ratio was equal to 20 units of enzyme per 1 mg of histone. 10 cm long acid-urea gels were applied. A – total H1 histone from calf thymus; B and C – H1-1 and H1-2 subfractions from Syrian hamster liver, respectively; D and E – H1-1 and H1-4 subfractions from Syrian hamster Kirkman-Robbins hepatoma, respectively. Marker – bovine serum albumin.

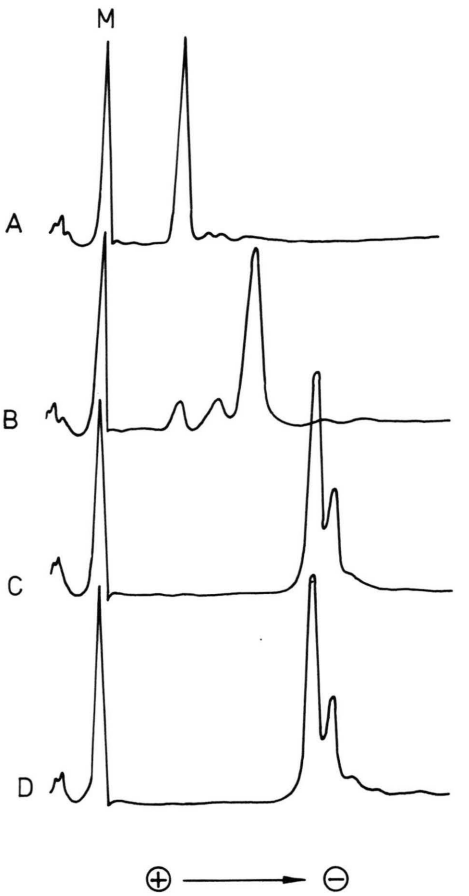

Fig. 4. Defibrase digestion of the main thrombin N- and C-terminal peptide obtained from Syrian hamster liver H1-1 histone subfraction. Digestion time 5 h. Enzyme/histone ratio was equal to 20 units per mg of protein. A – N-terminal thrombin peptide; B – N-terminal thrombin peptide digested with defibrase; C – C-terminal thrombin peptide; D – C-terminal thrombin peptide digested with defibrase.

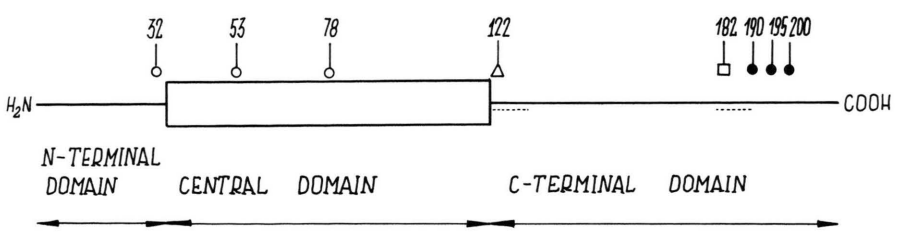

Fig. 5. Schematic diagram of possible localization of thrombin and defibrase cleavage sites within histone H1 molecules. ♁ The most probable positions of defibrase cleavage site; △ thrombin cleavage at lysine 122 giving two main N- and C-terminal peptides; □ lysine 182; ● positions of lysine residues near C-end of H1 molecules appearing in aminoacid sequence pro–lys–ala. 9 aminoacids region starting at proline 121 and the region showing maximal homology to that starting at proline 181 are underlined.

differences in electrophoretic mobility similar to that observed for initial subfractions: the peptide originated from H1-1 subfraction migrates slower than that from H1-4 (Fig. 6). Thus, the putative differences in aminoacid sequences between subfractions under analysis are localized within main defibrase peptide and probably they are outside of the N-terminal domain – "nose" which contains first 35 N-terminal aminoacids [21, 22].

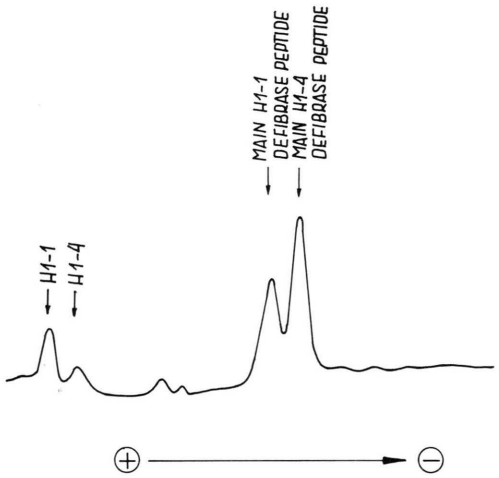

Fig. 6. Electrophoretic pattern of H1-1 and H1-4 histone subfractions from Syrian hamster Kirkman-Robbins hepatoma digested with defibrase. Digestion time 5 h; enzyme protein ratio was equal to 20 units per mg protein. 25 cm long acid-urea gel was used.

Conclusions

Analysis of thrombin degradation products of H1 histone shows the additional thrombin cleavage site on the H1 histone molecules. The thrombin degradation of H1 histone at this site proceedes faster than for the site dividing H1 molecule into two main N- and C-terminal peptides. The putative localization of this site is near C-end of H1 protein chain. The main thrombin peptides do not conserve differences in electrophoretic mobility in acid-urea PAG observed for initial H1 subfractions.

Degradation patterns of H1 histones from different sources with defibrase reveals the presence of one main site of hydrolysis. The defibrase main peptide contains the whole C-terminal and a part N-terminal thrombin peptide. The main defibrase peptides derived from two H1 subfractions conserve differences in electrophoretic mobility in acid-urea PAG characteristic for initial H1 subfractions. Susceptibility to defibrase degradation was shown to vary depending on H1 subfraction tested, what may suggest the existence of aminoacid replacements near the cleavage sites in different H1 subfractions.

This results indicate that snake venom proteases may be useful tool for investigation of homology between related proteins.

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[1] T. Dupressoir and P. Sautiere, Biochem. Biophys. Res. Comm. **122,** 1136–1145 (1984).
[2] O. H. J. Destree, H. J. Hoenders, A. F. M. Moorman, and R. Charles, Biochim. Biophys. Acta **577,** 61–70 (1979).
[3] S. M. Seyedin and R. D. Cole, J. Biol. Chem. **256,** 442–444 (1981).
[4] A. M. Chrismar, M. Brito, and L. O. Burzio, Biochem. Int. **5,** 263–269 (1982).
[5] G. M. Graczyk, J. K. Bartkowiak, A. Plucienniczak, E. L. Hrabec, and H. T. Panusz, Cancer Res. **41,** 2457–2464 (1981).
[6] J. K. Bartkowiak, G. M. Graczyk, A. Plucienniczak, A. Jasinska, and H. T. Panusz, Cancer Res. **41,** 2465–2467 (1981).
[7] J. M. Kinkade jr. and R. D. Cole, J. Biol. Chem. **241,** 5790–5797 (1966).
[8] J. Bonner, G. R. Chalkley, M. Dahmus, D. Fambrough, S. Fujimura, R. C. Huang, J. Huberman, R. Jensen, K. Marushiga, H. Ohlenbusch, B. Oliviera, and J. Widholm, in: Nucleic Acids **12** (L. Grosman and K. Moldave, eds.), Part B, 3–84 (1968).
[9] M. J. Smerdon and J. Isenberg, Biochemistry **15,** 4233–4247 (1976).
[10] S. Panyim and R. Chalkley, Arch. Biochem. Biophys. **130,** 337–346 (1969).
[11] G. E. Chapman, P. G. Hartman, and E. M. Bradbury, Eur. J. Biochem. **61,** 69–75 (1976).
[12] W. H. Seegers, D. Houria, I. Hassouna, D. Hewett-Emmett, D. A. Walz, and T. J. Andary, Seminars in Thrombosis and Haemostasis, J. Jan., 3–62 (1975).
[13] R. D. Cole, cited by J. M. Walker *et al.*, Comprehensive Biochemistry, 19 B, Part II. Protein Metabolism, 507–573 (1982).
[14] L. W. Liao and R. D. Cole, J. Biol. Chem. **256,** 3024–3029 (1981).
[15] W. H. Holleman and L. J. Coen, Biochim., Biophys. Acta **200,** 587–589 (1970).
[16] M. R. Ewart, M. W. C. Hatton, J. M. Basford, and K. S. Dodge, Biochem. J. **118,** 603–609 (1970).
[17] K. Stocker and P. W. Straub, Thromb. Diath. Haemorrh. **24,** 248–255 (1970).
[18] M. W. C. Hatton, Biochem. J. **131,** 799–808 (1973).
[19] K. S. Soh and K. E. Chan, Toxicon **12,** 151–158 (1974).
[20] K. Wik, O. Tangen, and F. McKenzie, Br. J. Haematol. **23,** 37–41 (1972).
[21] Y. Ishimi, H. Yasuda, Y. Ohba, and M. Yamada, J. Biol. Chem. **256,** 8249–8251 (1981).
[22] L. G. Nikolaev, B. O. Glotov, A. V. Itkes, and S. Severin, FEBS Lett. **125,** 20–24 (1981).