Modulation of the relative trypsin sensitivities of the core histone 'tails'

Nerina Harborne and James Allan

Department of Biophysics, Kings College, London WC2B 5RL, England

Received 9 February 1983

The order in which the core histone tails in chicken erythrocyte chromatin are attacked by trypsin has been reinvestigated. Results are presented to demonstrate that in the absence of linker histones H1 and H5 the relative order of core histone degradation by trypsin can be altered by changing the salt environment. In native chromatin, the presence of linker histones H1 and H5 inhibits this salt-dependent transition.

Chromatin *Core histone* *H1* *Trypsin* *Proteolysis* *Electrophoresis*

1. INTRODUCTION

Each of the chromosomal histone proteins contains both folded and unstructured domains. Histones H3, H4, H2a and H2b all have, at their NH-termini, a stretch of unfolded residues [1–3]. Histones H3 and H2a also have short COOH-terminal 'tails' [1,3]. The linker histones, H1 and H5, have unstructured 'tails' at both NH- and COOH-termini of the molecule [4,5]. The core histone tails are not essential for maintaining the core particle integrity [6] and their primary function may involve the determination and stabilisation of the chromatin higher order fibre [6–8]. Similarly, although located on the nucleosome by its globular domain [5], the capacity of H1, or H5, to condense the chromatin fibre is a characteristic property of the tails of this molecule [5].

Each of the histone proteins is sensitive to proteolytic degradation by trypsin, which cleaves primarily at the arginine and lysine residues within the tails of these molecules, giving rise to a relatively trypsin-resistant folded region for each histone [1–5]. Trypsin attacks each of the core histones at different rates, a property which is apparent after gel analysis of the histones derived from a time course of trypsin digestion of chromatin [9–11]. For example, authors in [9] showed that for chicken erythrocyte chromatin the degradation order was H3 = H4 > H2a = H2b. The order obtained in [10] was similar, H3 > H4 > H2a = H2b, although H4 was clearly relatively more resistant. In contrast, Lilley and Tatchell [11], using chicken erythrocyte core particles, observed an order of H3 > H2a > H4 > H2b. Although the manner in which chromatin is prepared can influence the sequence of trypsin sensitivity of the core histones [10], and may be responsible for other published degradation orders [12,13], the basis for these conflicting results has not been explained. Here, we show that the sequence in which the core histones are degraded by trypsin is dependent on both the salt environment and on the presence or absence of linker histone in the chromatin.

2. MATERIALS AND METHODS

2.1. *Chromatin*

Chicken erythrocyte polynucleosomes were prepared by mild micrococcal nuclease digestion of erythrocyte nuclei [8] and were fractionated into size classes on sucrose gradients [8]. The linker histones, H1 and H5, were depleted from the chromatin by passage over DNA cellulose [8]. Core particles were prepared from depleted polynucleosomes by digestion with micrococcal nuclease.

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

2.2. *Trypsin digestion*

Chromatin (A_{260}/ml) was treated at 4°C with trypsin (0.5 μg/ml) (Sigma, type II) in 5 mM Tris (pH 7.5), 0.1 mM EDTA, at the relevant salt concentration. Aliquots of 150 μl, removed from the reaction mix at various times, were added to a large excess (2 μg) of soya bean trypsin inhibitor (SBTI, Sigma, type III) and stored on ice. Proteins were precipitated from the samples by addition of 6 vol. acetone.

2.3. *SDS–acrylamide gels*

Trypsin-digested chromatin samples were analysed by electrophoresis in 18% SDS–acrylamide gels essentially as in [14]. The gels were stained with Coomassie brilliant blue and evaluated by densitometry. The relative areas under the histone peaks obtained by densitometry were determined by weighing and planimetry, and were normalised by reference to the SBTI band on the gels.

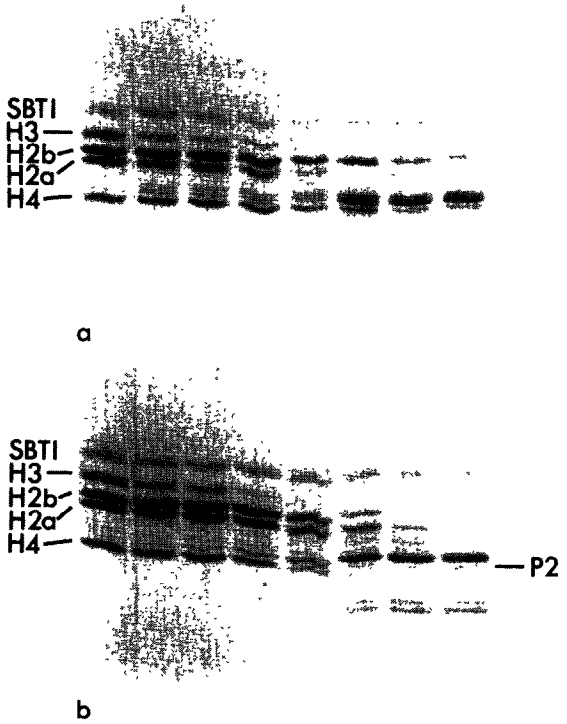

Fig.1. SDS–acrylamide gel analysis of protein products obtained after trypsin digestion of stripped chromatin at (a) 5 or (b) 80 mM NaCl. Times of digestion, from left to right, were: 0, 0.25, 0.5, 1, 2, 5, 10 and 30 min.

3. RESULTS

Stripped (H1- and H5-depleted) chicken erythrocyte chromatin was digested with trypsin at either 5 mM or 80 mM NaCl and the products of these digests were analysed in 18% SDS–acrylamide gels. A comparison of the digestion products obtained under these two conditions reveals that the order in which the various core histones are degraded is dependent on the salt environment (fig.1). At 80 mM NaCl, the order was H3 > H4> H2a = H2b while at 5 mM NaCl the order was H3 > H2a > H4 > H2b. The change in the sequence of histone digestion was mainly due to alteration in the sensitivities of histone H2a and to a lesser extent, histone H4. This conclusion is revealed more dramatically in fig.2. Here the extent of histone loss (degradation) after a standard time of trypsin treatment, but at various salt concentrations, was determined. The curves reveal a marked increase in the relative sensitivity of H2a, particularly when the salt is reduced below 20 mM. Fig.2 also suggests a corresponding increase in the

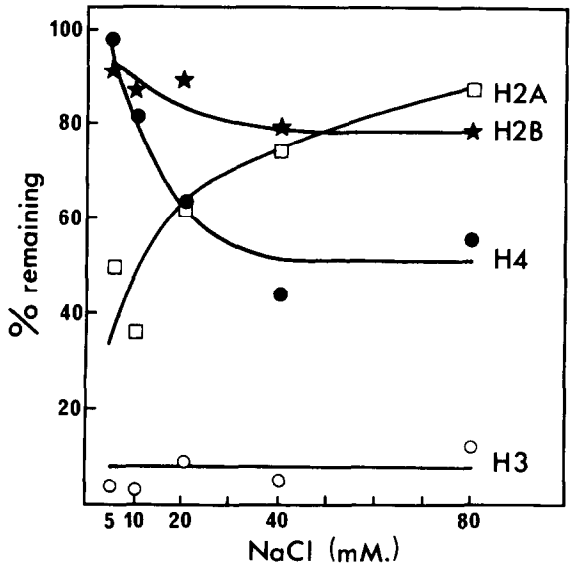

Fig.2. Relative trypsin sensitivities of each core histone type in stripped chromatin as a function of salt. Stripped chromatin was digested under standard conditions, at the [salt] indicated, for 1 min. The percentage of intact histone remaining was determined after SDS–acrylamide gel analysis by densitometry of the stained gel.

resistance of histone H4. However, some caution should be exercised with this latter result, for during the course of digestion a trypsin-resistant fragment, P2 [3,9], is formed from H2a which during electrophoresis migrates in the same position as H4. These two components cannot be distinguished (fig.1). Furthermore, the result with H4 was not very reproducible between experiments. In fig.1, for example, a change in H4 sensitivity is not very convincing, whereas in fig.3, H4 clearly becomes more resistant at low salt. For these reasons, a change in sensitivity of H4 to trypsin should be regarded tentatively. The salt-dependent sensitivity of H2a was, however, firmly established and was consistently observed in many experiments.

The experiments described above for chicken erythrocyte polynucleosomes were repeated using

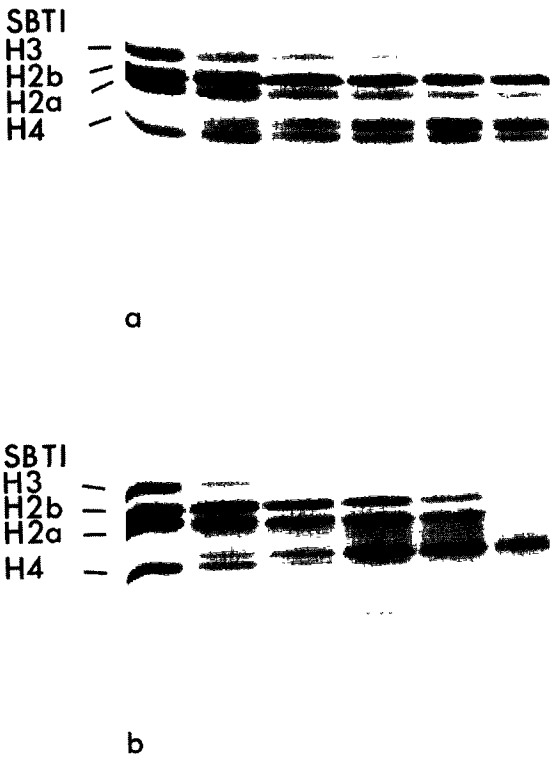

Fig.3. SDS–acrylamide gel analysis of the protein products obtained after trypsin treatment of core particles at (a) 5 or (b) 80 mM NaCl. Times of digestion, from left to right, were: 0, 0.25, 0.5, 1, 2 and 5 min.

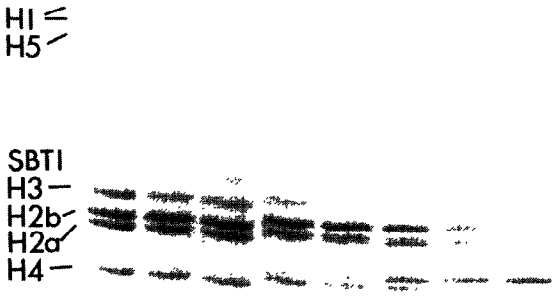

Fig.4. SDS–acrylamide gel analysis of the protein products obtained after trypsin treatment of native chromatin at 5 mM NaCl. Times of digestion, from left to right, were: 0, 0.25, 0.5, 1, 2, 5, 10 and 30 min.

core particles from the same source. The results were essentially the same (fig.3).

When digested with trypsin in 80 mM NaCl, native chicken erythrocyte chromatin (containing H1 and H5) displayed an order of core histone degradation which could not be distinguished from that observed with stripped chromatin (not shown; see fig.1b). At 5 mM NaCl, however, native chromatin, in contrast to stripped chromatin, did not display a significantly altered order of core histone sensitivity. The order at low salt was seen to be H3 > H4 > H2 = H2b (fig.4) which is the same order as that obtained with either chromatin at high salt. H1 and H5, therefore, appear to exert an influence on the salt-dependent accessibility of the core histone tails to trypsin. In this context it should be noted that at both low and high salt the linker histones, H1 and H5, are by far the most trypsin sensitive of all the histone types ([3,9]; see fig.4).

4. DISCUSSION

The results presented here demonstrate that the availability, for trypsin cleavage, of the tails of histones H2a and, possibly, H4 is dependent on both the salt environment and the presence or absence of linker histone.

Our observations help to explain the apparent conflicting results made in previous studies. In [9],

native chicken erythrocyte chromatin was digested with trypsin at low salt (1 mM) and therefore the suppressive effect of the linker histone would have maintained the high salt order of core histone cleavage (H3 = H4 > H2a = H2b). However, in [11] chicken erythrocyte core particles were digested in low salt (1 mM) and, therefore, due to the absence of H1 or H5 in their substrate, the low salt pattern that we observe (fig.1a) was expressed. Their order was H3 > H2a > H4 > H2b. Some disagreement between these studies with respect to the relative sensitivity of histone H4 [9,10] remains unexplained by our results. We observe that H3 is always more sensitive to trypsin than H4. This is in agreement with [10] but contrary to [9]. Perhaps the problems we have mentioned concerning accurate estimation of H4 and the non-reproducibility in its digestion properties may contribute to the uncertainty surrounding this histone.

The variability in the relative trypsin sensitivities of the core histone tails may have its basis in conformational changes which (poly)-nucleosomes undergo in response to alterations in their salt environment. When subjected to a gradual decrease in the level of monovalent cations, say from 100–1 mM, both polynucleosomes and nucleosomes display a gradual unfolding which is reflected in their sedimentation [15,18] or electron microscopic [16] properties. A process of gradual unfolding is also suggested by the overall reduction in the capacity of certain of their core histones (H4 and H2b) to be crosslinked in vitro [17]. At low ionic strength (7 mM Na^+) nucleosomes depleted of H1 display a dramatic reduction in their sedimentation coefficient, suggesting that a major unfolding event occurs under these conditions [18]. It seems possible, therefore, that the conformational changes in the nucleosome which accompany alterations to the salt environment could be linked to the variation in core histone tail accessibility which we have observed, occurring either as a result of, or giving rise to, these latter changes. In support of this suggestion it should be noted that there is an analogy between the capacity of the linker histones, H1 and H5, to block the low salt unfolding of the nucleosome [18] and to prevent the low salt-induced alteration in relative accessibility of the core histone tails.

With respect to this latter property one other comment should be made. As H1 or H5 are the most trypsin-sensitive of all the histones [3,9] their capacity to prevent a change in the relative accessibility of the core histone tails is unlikely to be a feature of the intact molecule. A more probable explanation is that the folded, or globular H1 domain [4,5], which is relatively trypsin-resistant, confers this property and does so by virtue of its capacity to seal off two turns of DNA in the nucleosome [5] and thus, potentially, restrict conformational changes within this structure.

As mentioned earlier, a function for the core histone tails has not been specifically identified. Although they contribute basic charge necessary to stabilise the higher order chromatin fibre [8], their highly conserved sequence [6] suggests that the core histone tails perform a more specific function. Their involvement in nucleosome unfolding, a process which could be biologically relevant to transcription or replication, may constitute such a function.

ACKNOWLEDGEMENTS

We thank Dr Hannah Gould for valuable comments on the manuscript. This work was supported by a Wellcome Research Fellowship.

REFERENCES

[1] Bohm, L., Crane-Robinson, C. and Sautiere, P. (1980) Eur. J. Biochem. 106, 525–530.
[2] Bohm, L., Briand, G., Sautiere, P. and Crane-Robinson, C. (1982) Eur. J. Biochem. 123, 299–303.
[3] Bohm, L., Briand, G., Sautiere, P. and Crane-Robinson, C. (1981) Eur. J. Biochem. 119, 67–74.
[4] Hartman, P.G., Chapman, G.E., Moss, T. and Bradbury, E.M. (1977) Eur. J. Biochem. 77, 45–51.
[5] Allan, J., Hartman, P.G., Crane-Robinson, C. and Avilles, F.X. (1980) Nature 288, 675–679.
[6] McGhee, J.D. and Felsenfeld, G. (1979) Annu. Rev. Biochem. 49, 1115–1156.
[7] Whitlock, J.P. and Simpson, R.T. (1977) J. Biol. Chem. 252, 6516–6520.
[8] Allan, J., Harborne, N., Rau, D.C. and Gould, H. (1982) J. Cell Biol. 93, 285–297.
[9] Weintraub, H. and Van Lente, F. (1974) Proc. Natl. Acad. Sci. USA 71, 4249–4253.
[10] Noll, M., Thomas, J. and Kornberg, R. (1975) Science 187, 1203–1206.

[11] Lilley, D.M.J. and Tatchell, K. (1977) Nucleic Acids Res. 4, 2039–2055.
[12] Marks, D.B. and Keller, B.J. (1976) Arch. Biochem. Biophys. 175, 598–605.
[13] Chatterjee, S. and Walker, I.O. (1973) Eur. J. Biochem. 34, 519–526.
[14] Laemmli, U.K. (1970) Nature 227, 680–685.
[15] Allan, J., Cowling, G.J., Harborne, N., Cattini, P., Craigie, R. and Gould, H. (1981) J. Cell. Biol. 90, 279–288.
[16] Thoma, F., Koller, Th. and Klug, A. (1979) J. Cell. Biol. 83, 403–427.
[17] Martinson, H.G., True, R.J. and Burch, J.B.E. (1979) Biochemistry 19, 3260–3269.
[18] Burch, J.B.E. and Martinson, H.G. (1980) Nucleic Acids Res. 8, 4969–4987.