Volume 294, number 1,2, 125–128 FEBS 10436 December 1991

Histone H1 inhibits eukaryotic DNA topoisomerase I

Arndt Richter and Marion Kapitza

Division of Biology, University of Konstanz, 7750 Konstanz, Germany

Received 23 September 1991; revised version received 10 October 1991

Histone H1 inhibits the catalytic activity of topoisomerase I in vitro. The relaxation activity of the enzyme is partially inhibited at a molar ratio of one histone H1 molecule per 40 base pairs (bp) of DNA and completely inhibited at a molar ratio of one histone H1 molecule per 10 base pairs of DNA. Increasing the amount of enzyme at a constant histone H1 to DNA ratio antagonizes the inhibition. This indicates that topoisomerase I and histone H1 compete for binding sites on the substrate DNA molecules. Consistent with this we show on the sequence level that histone H1 inhibits the cleavage reaction of topoisomerase I on linear DNA fragments.

Topoisomerase I; Relaxation; Cleavage; Histone H1

1. INTRODUCTION

The topological state of DNA in eukaryotic cells is controlled by topoisomerases [1]. Two types of enzymes, namely type I and type II DNA topoisomerases have been identified, purified, and extensively characterized. A key feature of both types of enzymes is their ability to remove superhelical tension from DNA molecules in a concerted nicking and releasing reaction (for review see [2,3]). It has been shown that at least three parameters affect the catalytic activity of the enzymes: (i) the topology of the DNA substrate, i.e. the torsional tension present in a DNA molecule; (ii) the nucleotide sequence of the DNA substrate; and (iii) its chromosomal organization [4–6]. In addition, modifications of the enzymes such as phosphorylation [7,8] or ADP-ribosylation [9,10] modulate their activities.

Recently we have shown that the interaction of topoisomerase I (EC 5.99.1.2) with DNA, organised in histone octamer complexes, is reduced, albeit not entirely blocked [11]. Here we analyse the accessibility of DNA for topoisomerase I in the presence of histone H1. We find that histone H1 inhibits the relaxation of supercoiled plasmid DNA by the enzyme as well as the cleavage reaction on linear DNA substrates.

2. MATERIALS AND METHODS

2.1. *Materials and general methods*

Topoisomerase I was prepared from calf thymus and HeLa cells as described [12]. The 154 base pairs (bp) long *Eco*RI/*Not*I DNA fragment was from the promoter region of the topoisomerase I gene [13]. Gel electrophoresis on agarose and polyacrylamide gels was performed as described [14]. The dried gels were exposed to Kodak X-ray films.

Correspondence address: A. Richter, Division of Biology, University of Konstanz, D-7750 Konstanz, Germany. Fax: (49) (7531) 88 3688.

2.2. *Relaxation of superhelical plasmid DNA*

DNA topoisomerase I activity was determined with 10 ng of superhelical pUC 18-390 DNA in a total volume of 100 μl reaction buffer (20 mM Tris-HCl, pH 7.5, 75 mM KCl, 5 mM $MgCl_2$, 1 mM dithiotreitol, 0.05 mg/ml bovine serum albumin) containing the indicated amounts of topoisomerase I and histone H1 (Boehringer, Mannheim). After preincubation of DNA and histone H1 for 15 min at 20°C, topoisomerase I was added and the incubation was continued for another 15 min. The reaction was terminated on ice by the addition of 200 μl of 0.5% sodium dodecyl sulfate in TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM ethylenediamine tetraacetic acid). After treatment with proteinase K for 2 h at 50°C the DNA was phenol-extracted and ethanol-precipitated. The DNA was separated on 1% agarose gels [14] transferred to nitrocellulose membranes and hybridized with nick-translated substrate DNA as a probe.

2.3. *Agarose bandshift of plasmid DNA*

Increasing amounts of histone H1 were incubated with pUC 18-390 DNA under conditions of the relaxation assay except that topoisomerase I was omitted from the reaction. After 15 min at 20°C the assay mixture was directly applied and separated on 1% agarose gels. The DNA was transferred to nitrocellulose membranes and processed as described above.

2.4. *Mapping of DNA topoisomerase I cleavage sites*

The 154 bp long DNA substrate was exised from pUC18-390 DNA [11] with *Eco*RI/*Sma*I, end-labeled with Klenow polymerase in the presence of [^{32}P]dATP at the *Eco*RI site, and purified by gel filtration and ethanol precipitation. In the cleavage reaction, 10 ng of the labeled DNA fragment was preincubated with the indicated amounts of histone H1 in a total of 100 μl of cleavage buffer (20 mM Tris-HCl, pH 7.5, 75 mM KCl, 5 mM $MgCl_2$, 1 mM dithiotreitol, 0.05 mg/ml bovine serum albumin). Camptothecin (CPT; lactone form; Sigma Chemie, Deisenhofen; 50 μM final concentration) in dimethyl sulfoxide (DMSO; 0.5% final concentration), DMSO or H_2O alone were included in the reaction as indicated in the figure legends. After 15 min at 20°C, 1 unit of purified topoisomerase I was added, and the incubation was continued for 15 min. The reaction was terminated by the addition of 200 μl of 0.5% sodium dodecyl sulfate in TE. After treatment with proteinase K for 2 h at 50°C the DNA fragments were phenol-extracted, ethanol-precipitated, and analysed on 4% polyacry-

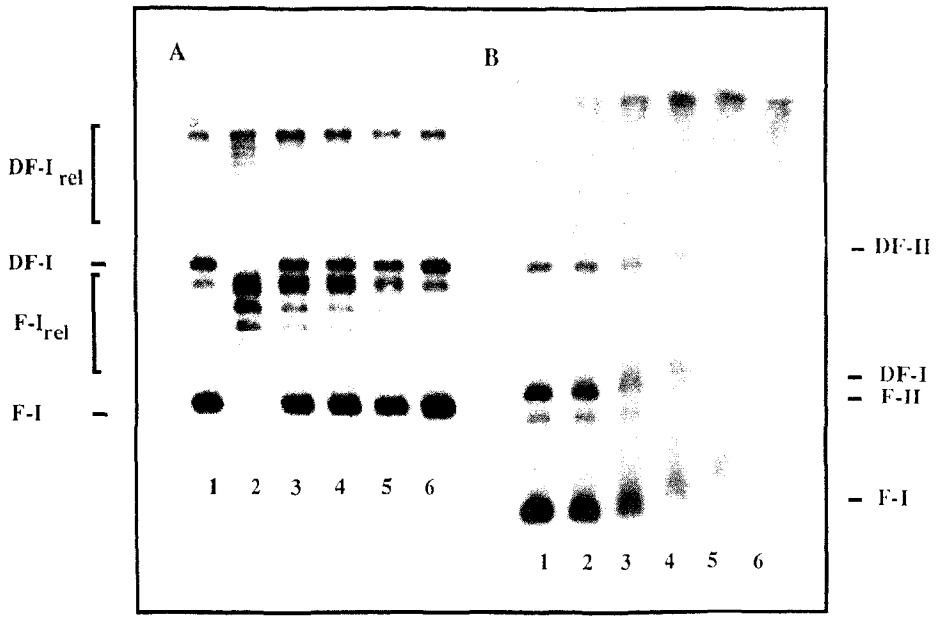

Fig. 1. Histone H1 inhibits the relaxation activity of topoisomerase I. (A) 10 ng supercoiled pUC-390 DNA (lane 1) were incubated for 15 min at 20°C with one unit of topoisomerase I without histone H1 (lane 2) or at molar ratios of histone H1 to DNA (bp) of 0.025, 0.05, 0.1 and 0.. (lanes 3–6), respectively. The DNA was then purified, separated on agarose gels, transferred to nitrocellulose filters and hybridized with nick translated substrate DNA. (B) shows the corresponding agarose bandshift of histone H1/DNA complexes formed under assay conditions. 10 n; of supercoiled pUC-390 FI-DNA (lane 1) were incubated in the absence of topoisomerase I without H1 (lane 2) and with increasing amounts o histone H1 (lanes 3–6) as in (A), separated on agarose gels and analysed as described above. DF-I, dimer of pUC-390; rel, relaxed DNA.

lamide sequencing gels with the same labeled DNA fragment treated in a Maxam-Gilbert 'G+A' reaction separated in parallel as marker.

3. RESULTS

Histone H1 inhibits the relaxation activity of topoisomerase I in a concentration-dependent manner. In the experiment shown in Fig. 1A, supercoiled plasmid DNA was incubated with one unit of purified topoisomerase I in the presence of increasing amounts of histone H1. Under these conditions an almost complete inhibition was observed at a ratio of one histone H1 molecule per 40 bp of DNA (compare lanes 2 and 3, Fig. 1A). Agarose bandshift experiments (Fig. 1B) performed in parallel showed that the DNA/histone H1 complexes enter the gel at a molar ratio of 0.025 (one histone H1 per 40 bp of DNA; lane 3) when the enzymatic activity was strongly inhibited. At higher histone H1/DNA ratios parts of the DNA substrate (lanes 4,5) or the total DNA (lane 6) remained on top of the agarose gels. But even at the highest histone H1 to DNA ratio no complexes are formed which can be pelleted by low-speed centrifugation (2 min, 10 000 × *g*, 20°C) indicating that the inhibition of topoisomerase I observed does not result from histone H1-mediated precipitation of the supercoiled DNA substrate.

Increasing amounts of topoisomerase I antagonize the inhibition by histone H1. The extent of histone H1-

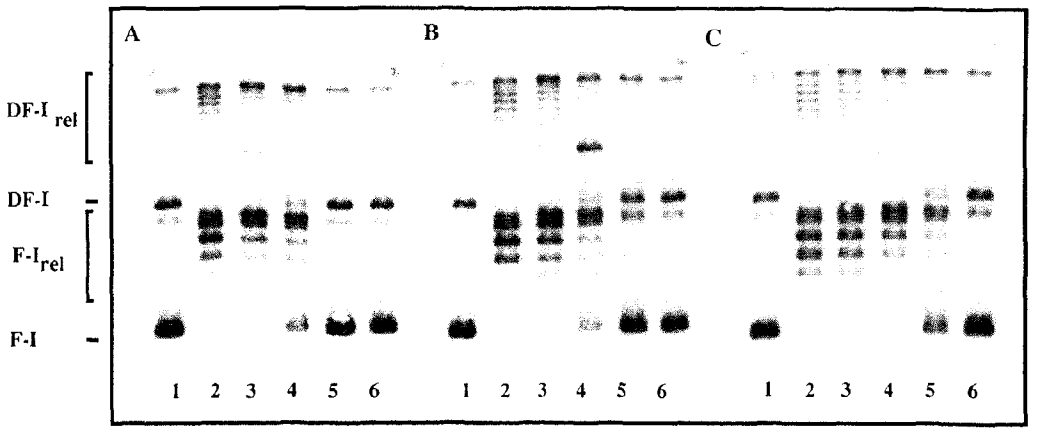

Fig. 2. Effect of increasing amounts of topoisomerase I on the inhibition by histone H1. Experiments, as shown in Fig. 1A were repeated with 2 (A), 4 (B) and 20 units (C) of topoisomerase I. After the reaction the DNA was purified, separated on agarose gels, transferred to nitrocellulose filters and hybridized with nick-translated substrate DNA. DF-I, dimer of pUC-390; rel, relaxed DNA.

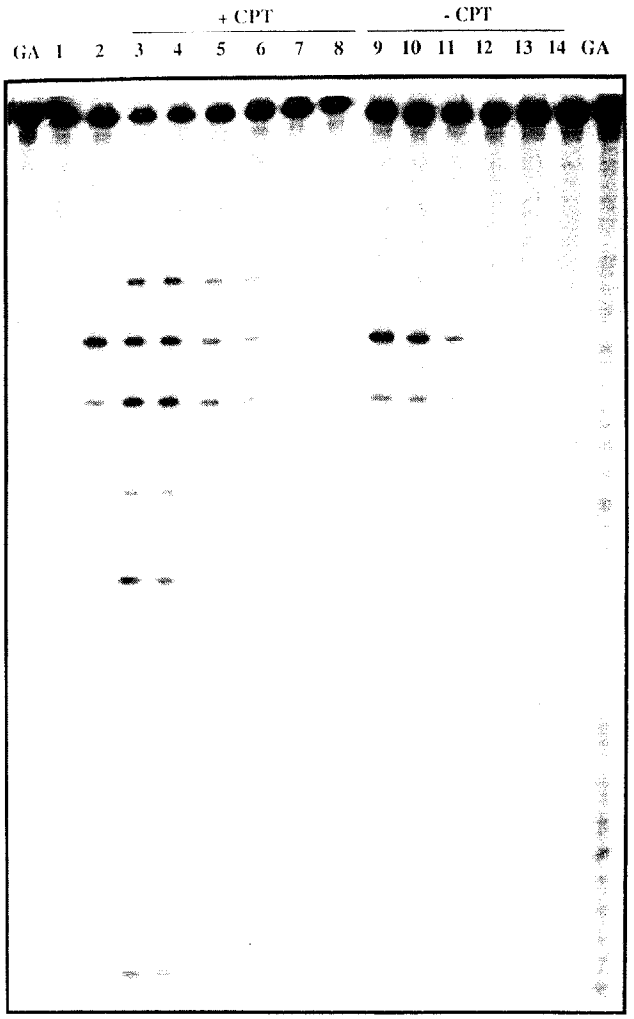

Fig. 3. Histone H1 inhibits the cleavage activity of topoisomerase I. 10 ng of an end-labeled 154 bp long DNA fragment of known sequence (lane 1) were incubated for 15 min at 20°C with one unit of topoisomerase I (lanes 2–14) without histone H1 (lanes 2,3,9) or at molar ratios of histone H1 to DNA (bp) of 0.025 (lanes 4,10), 0.05 (lanes 5,11), 0.1 (lanes 6,12), 0.2 (lanes 7,13), and 0.4 (lanes 8,14), respectively. The reactions were performed in the presence of CPT in DMSO (lanes 3–8), DMSO alone (lanes 9–14) or without any addition (lane 2). GA: Maxam-Gilbert 'G+A' reaction of the topoisomerase I substrate.

mediated inhibition of the topoisomerase I activity depended on the amount of enzyme added. At low enzyme concentrations (one unit of topoisomerase I; see Fig. 1A) a molar ratio of one histone H1 molecule per 40 bp of supercoiled plasmid DNA was sufficient to almost completely inhibit the relaxation reaction. With 2 and 4 units of topoisomerase I, a comparable inhibition was observed at a molar ratio of one histone H1 molecule per 10 bp (Fig. 2A,B: lane 5) and with 20 units of enzyme at a molar ratio of one histone H1 molecule per 5 bp of DNA (Fig. 2C: lane 6). A further 10-fold increase in the amount of topoisomerase I completely abolished the inhibition by histone H1 (data not shown). These results may indicate that topoisomerase I and histone H1 compete for binding sites on the DNA substrate.

We further determined the influence of histone H1 on the cleavage activity of DNA topoisomerase I directly on the sequence level. Two types of cleavage sites can be distinguished, namely sites where cleavages introduced by the enzyme are found in the absence of camptothecin and additional sites where cleavage is only detectable in the presence of the drug [15]. Fig. 3 shows the influence of histone H1 on the cleavages introduced by topoisomerase I into a 154 bp long linear DNA fragment in the absence (lanes 2,9–14) or in the presence of camptothecin (lanes 3–8). Cleavage inhibition was observed when the DNA fragment was incubated with increasing amounts of histone H1 for 15 min at 20°C, followed by the addition of enzyme. As in the case of the relaxation of supercoiled plasmid DNA, inhibition of cleavage was observed at a histone H1 to DNA (bp) ratio of 0.025 (lanes 4,10) and cleavage was completely abolished at a molar ratio of 0.2 and above (lanes 7,8,13,14). It is also evident from the experiment shown in Fig. 3 that histone H1 affects both types of cleavage sites, namely those observed in the absence and in the presence of CPT, and that the cleavages were inhibited at all sites to a comparable extent. Analysis of cleavages introduced into the complementary strand in the 154-bp fragment as well as cleavages introduced into several other unrelated DNA fragments confirmed these observations (data not shown). Sucrose gradient centrifugation (5–25% sucrose in cleavage buffer) of the histone H1/DNA complexes showed that the sedimentation rate of the DNA fragment increased slightly upon addition of topoisomerase I and histone H1, but no large aggregates or precipitation occurred even at the highest amount of histone H1 added (data not shown).

4. DISCUSSION

Chromatin is the substrate upon which all nuclear enzymes in eukaryotes must operate. In chromatin the DNA is wrapped around nucleosomes. In addition to the tight interaction of DNA and core histones, the DNA may interact with histone H1 and a variety of other non-histone chromatin proteins with structural and regulatory functions (for a review see [16]).

DNA topoisomerase I is one of the most abundant non-histone chromosomal proteins. In vivo topoisomerase I interacts with replicating chromatin [17,18] and actively transcribed regions of the genome [19–24]. The interaction of topoisomerase I with these regions may be influenced and regulated by the superhelical tension created during replication and transcription. In addition, topoisomerase I activity can be modulated by the general accessibility of the DNA substrate, and the interaction of topoisomerase I with histone chromatin proteins as well as non-histone chromatin proteins. This modulation of the enzymatic activity may result from direct protein-protein interactions or from competition

with other proteins for binding sites on the same substrate DNA.

We have shown here that histone H1 interferes with the enzymatic activity of topoisomerase I in vitro in a concentration-dependent way. We observe an inhibition of the relaxation of supercoiled plasmid DNA, and in agreement with this a strong inhibition of the cleavage activity on linear DNA molecules. Javaherian and Liu [25] have shown that HMG17 stimulates the relaxation activity of topoisomerase I and the authors stated that histone H1 had a similar effect on the catalytic activity of the enzyme. However, under conditions where only half of the substrate DNA was relaxed by topoisomerase I alone we could not detect a stimulation of the relaxation activity of the enzyme in the presence of increasing amounts of histone H1 (data not shown).

Recently we have shown that topoisomerase I is partially but not entirely excluded from access to DNA wrapped around histone octamer complexes [11]. On the other hand, histone H1 completely inhibited topoisomerase I cleavage on linear DNA molecules at a molar ratio of one histone H1 molecule per 5 bp of DNA. Thus, the inhibition of the topoisomerase I cleavage reaction by histone H1 is much more efficient than the inhibition of cleavage on DNA wrapped around nucleosomes.

Increasing the amount of topoisomerase I antagonizes the inhibition of the relaxation activity of the enzyme by histone H1. One histone H1 molecule per 10 bp of DNA was sufficient to completely inhibit the relaxation catalyzed by 2 units topoisomerase I (Fig. 2A, lane 5). In the presence of a 10-fold amount of enzyme, however, two histone H1 molecules per 10 bp of DNA were necessary for the same inhibition of relaxation (Fig. 2C, lane 6). We conclude from this data that histone H1 competes with topoisomerase I for binding sites on the substrate DNA. The exclusion of topoisomerase I from binding sites by the competition with histone H1 (and perhaps other DNA binding proteins) may therefore represent another level of regulation of the enzymatic activity of topoisomerase I in chromatin.

Acknowledgements: The authors wish to thank Dr. R. Knippers for critical reading of the manuscript. This work was supported by Deutsche Forschungsgemeinschaft through SFB 156.

REFERENCES

[1] Liu, L.F. (1989) Annu. Rev. Biochem. 58, 351–375.
[2] Wang, J.C. (1985) Ann. Rev. Biochem. 54, 665–697.
[3] Vosberg, H.P. (1985) Curr. Top. Microbiol. Immunol. 114, 19–102.
[4] Camilloni, G., DiMartino, E., DiMauro, E. and Caserta, M. (1989) Proc. Natl. Acad. Sci. USA 86, 3080–3084.
[5] Shen, C.C. and Shen, C.K. (1990) J. Mol. Biol. 212, 67–78.
[6] Capranico, G., Jaxel, C., Roberge, M., Kohn, K.W. and Pommier, Y. (1990) Nucleic Acids Res. 18, 4553–4559.
[7] Kaiserman, H., Ingebritsen, T.S. and Benbow, R.M. (1988) Biochemistry 27, 3216–3222.
[8] Ackerman, P., Glover, C. and Osheroff, N. (1985) Proc. Natl. Acad. Sci. USA 82, 3164–3168.
[9] Ferro, A.M. and Baldomero, M.O. (1984) J. Biol. Chem. 259, 547–554.
[10] Darby, M.K., Schmitt, B., Jongstra-Bilen, J. and Vosberg, H.-P. (1985) EMBO J. 4, 2129–2134.
[11] Richter, A. and Ruff, J. (1991) Biochemistry (in press).
[12] Strausfeld, U. and Richter, A. (1989) Prep. Biochem. 19, 37–48.
[13] Kunze, N., Klein, M., Richter, A. and Knippers, R. (1990) Eur. J. Biochem. 194, 323–330.
[14] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, New York.
[15] Kjeldsen, E., Mollerup, S., Thomsen, B., Bonven, B., Bolund, L. and Westergaard, O. (1988) J. Mol. Biol. 202, 333–342.
[16] van Holde, K.E. (1988) Chromatin, Springer-Verlag, New York.
[17] Avemann, K., Knippers, R., Koller, T. and Sogo, J.M. (1988) Mol. Cell. Biol. 8, 3026–3034.
[18] Zhang, H., D'Arpa, P. and Liu, L.F. (1990) Cancer Cells 2, 23–27.
[19] Gilmour, D.S. and Elgin, S.C. (1987) Mol. Cell. Biol. 7, 141–148.
[20] Rowe, T.C., Couto, E. and Kroll, D.J. (1987) NCI Monogr. 4, 49–54.
[21] Rose, K.M., Szopa, J., Han, F.-S., Cheng, Y.-C., Richter, A. and Scheer, U. (1988) Chromosoma 96, 411–416.
[22] Culotta, V. and Sollner-Webb, B. (1988) Cell 52, 585–597.
[23] Ness, P.J., Koller, T. and Thoma, F. (1988) J. Mol. Biol. 200, 127–139.
[24] Stewart, A.F., Herrera, R.E. and Nordheim, A. (1990) Cell 60, 141–149.
[25] Javaherian, K. and Liu, L.F. (1983) Nucleic Acids Res. 11, 461–472.