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DNA binding of histone H1 is modulated by nucleotides

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Histone H1 acts as a general repressor of transcription in eukaryotes by organizing nucleosomes into inaccessible condensed forms of chromatin. The capability of H1 to bind to DNA with some sequence specificity is likely to be critical in the control of these processes. We show here that ATP and several other nucleotides, including non-hydrolyzable derivatives, can inhibit DNA binding of H1. The results also show that ATP differentially affects binding of H1 to DNA in a fashion enhancing nucleotide sequence specificity of the binding. The study suggests a novel mechanism of modulation of H1 activity that has important implications for the role of H1 as a transcriptional regulator.

Chromatin; Nucleotide; Histone H1; DNA binding

1. INTRODUCTION

Histone H1 plays a central role as general repressor of transcription in eukaryotes by organizing nucleosomes into condensed forms of chromatin, thereby rendering the DNA inaccessible [1–4]. Antirepression of H1-mediated inhibition of transcription is a distinct activity of transcription factors [5].

H1 displays nucleotide sequence specificity in its DNA binding [6–11]. The sequence recognized by H1 is closely related to that of other proteins that bind to NF1 sites [10,11]. The fold of the central globular domain of histone H5, an H1 variant, is very similar to the DNA binding domain of the cAMP receptor protein of *Escherichia coli* [12]. Both basic ends of H1 contain repeats of a motif, Ser-Pro-Lys/Arg-Lys/Arg, with DNA-binding capacity [13,14]. H1 also contains a conserved segment with homology to nucleotide binding domains in diverse nucleotide-binding proteins [15–17], and nucleotides can bind to H1 [16,18,19]. In this report we analyze the importance of the nucleotide interaction and show that nucleotides differentially affect the DNA binding of H1.

2. MATERIALS AND METHODS

H1 was purified from rat liver as previously described [11,16]. Electrophoretic mobility shift assays (EMSA) were as described [20] except that reactions contained 25 ng each of poly (dI-dC) and poly(dA-dT). 13 ng of H1 per reaction was used, and where indicated, nucleotides (Sigma) were included in preincubation of H1 for 10 min at room temperature prior to addition of DNA.

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Double-stranded DNA segments (numbers 2, 34, 36, 42 and 43 of [10], and the κ3 motif of the κ chain enhancer [21]) were prepared from oligonucleotides (Symbicom) as described [20].

3. RESULTS AND DISCUSSION

Increasing concentrations of ATP were added to H1 in an EMSA, using a double-stranded oligonucleotide with the H1 binding site of the SL3-3 virus enhancer [10]. To minimize non-specific H1-DNA interaction, each reaction contained a 2,000-fold excess of double-stranded poly(dI-dC) and poly(dA-dT) and 0.1 pmol of single-stranded DNA. The data show a clear inhibition of H1–DNA binding by ATP (Figs. 1 and 2). 50% inhibition was obtained at 2.3 mM ATP and 80% inhibition at 8 mM ATP (Fig. 1). These concentrations are in the range of the physiological ATP concentration of 3–5 mM [22,23].

The less abundant nucleotides, GTP, UTP, CTP, dATP and dGTP, all inhibited H1–DNA binding to about the same extent, and at about the same concentration, as did ATP (Fig. 2). Thus, inhibition of H1–DNA binding is not specific for the type of base or for the presence of ribose or deoxyribose. Non-hydrolyzable nucleotide analogues, 5′-adenylylimidodiphosphate (AMP-PNP) and 5′-guanylylimidodiphosphate (GMP-PNP) also inhibited H1–DNA binding. ADP gave variable results, but in all experiments ADP was less inhibitory than the nucleoside triphosphates. Neither of the nucleoside monophosphates, AMP or cAMP, resulted in any inhibition (Fig. 2). Thus, the observed inhibition depended on the presence of more than one phosphate in the compound. Finally, pyrophosphate or β-glycerophosphate had no effect on the binding of H1 (data not

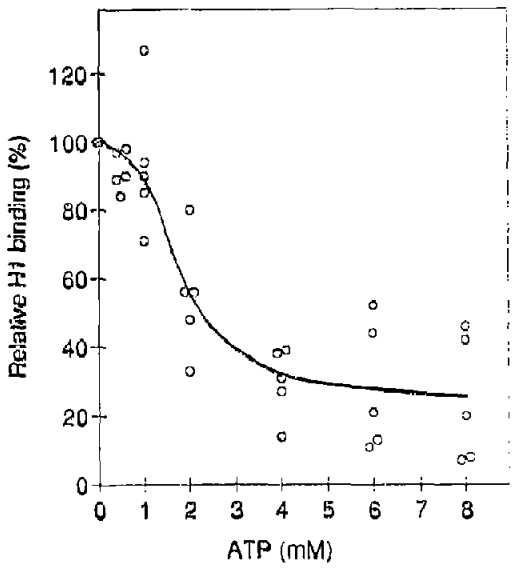

Fig. 1. Inhibition by ATP of complex formation between histone H1 and the H1 binding site of the SL3-3 virus enhancer in EMSA.

shown). Thus, there are clear structural requirements on the compounds capable of inhibition.

It was of interest to compare the effect of ATP on H1 binding to the SL3-3 site and to other DNA sequences. The sequences were as follows; the H1 site in the SL3-3 enhancer, two others of roughly the same intermediate binding strength [10]; and two much stronger H1 binding sites, the site of adenovirus 2/5 (Ad2/5) and our proposed optimized H1 binding sequence [10]. Non-specific control DNA did not compete with H1 binding to the SL3-3 site (Fig. 3). The site of SL3-3, the half-site of the albumin gene and the optimized half-site could all compete without any differential effect of 3 mM ATP (Fig. 3). Thus, half-sites are not more sensitive to inhibition with nucleotides than palindromic binding sites of similar strength. In contrast, the strong H1 binding site of Ad2/5 and our proposed optimized binding site were both clearly better competitors in the presence of 3 mM ATP (Fig. 3). Thus, ATP increases the stringency of sequence-specific H1–DNA binding. This interpretation is supported by analyses of the amounts of complexes with the DNA segments, both in the presence and absence of poly(dI-dC) and poly(dA-dT) competitors (data not shown).

Our data argue against the possibility that nucleotides inhibit H1–DNA binding by functioning as a DNA analogue, or that hydrolysis of the nucleotide

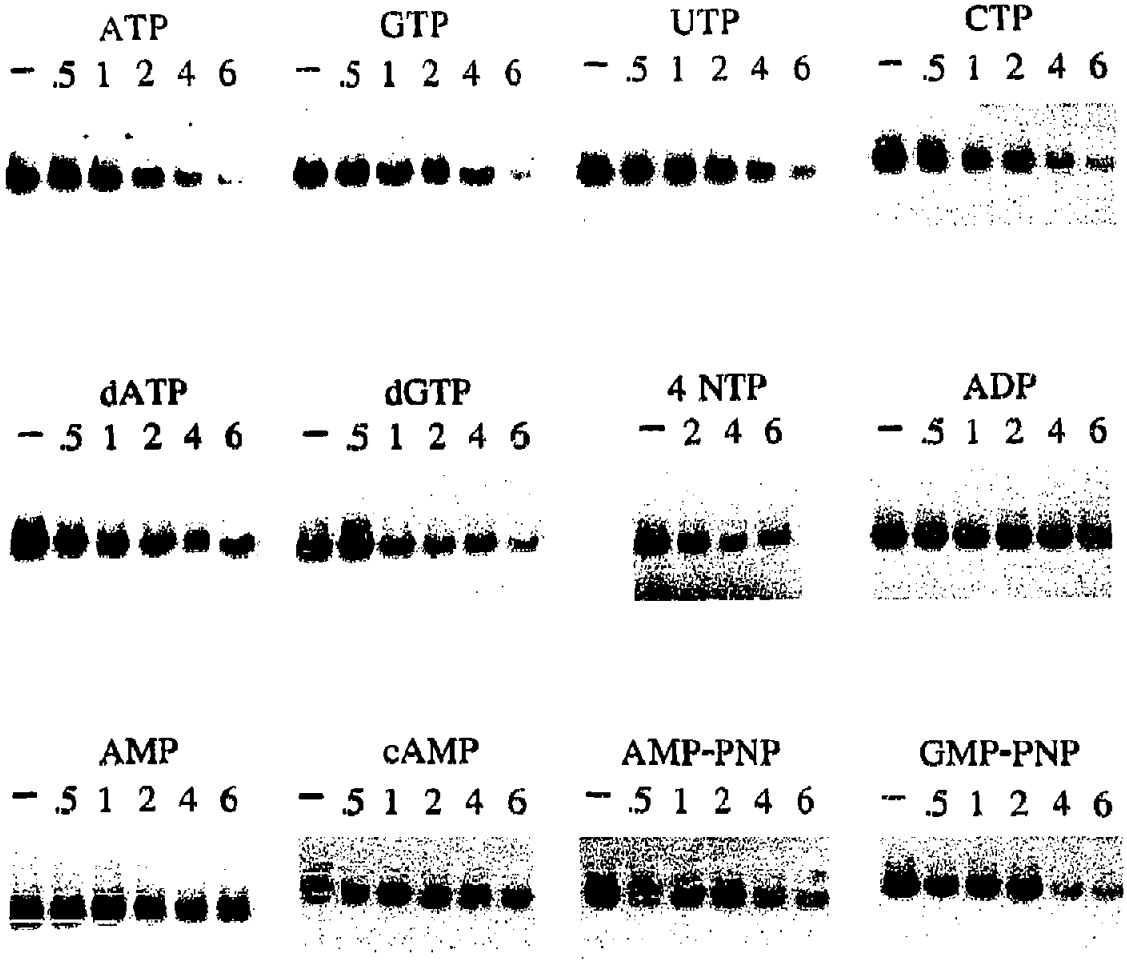

Fig. 2. Effects of different nucleotides and related analogues on histone H1–DNA binding. The final concentrations of reagents (mM) are indicated above each lane.

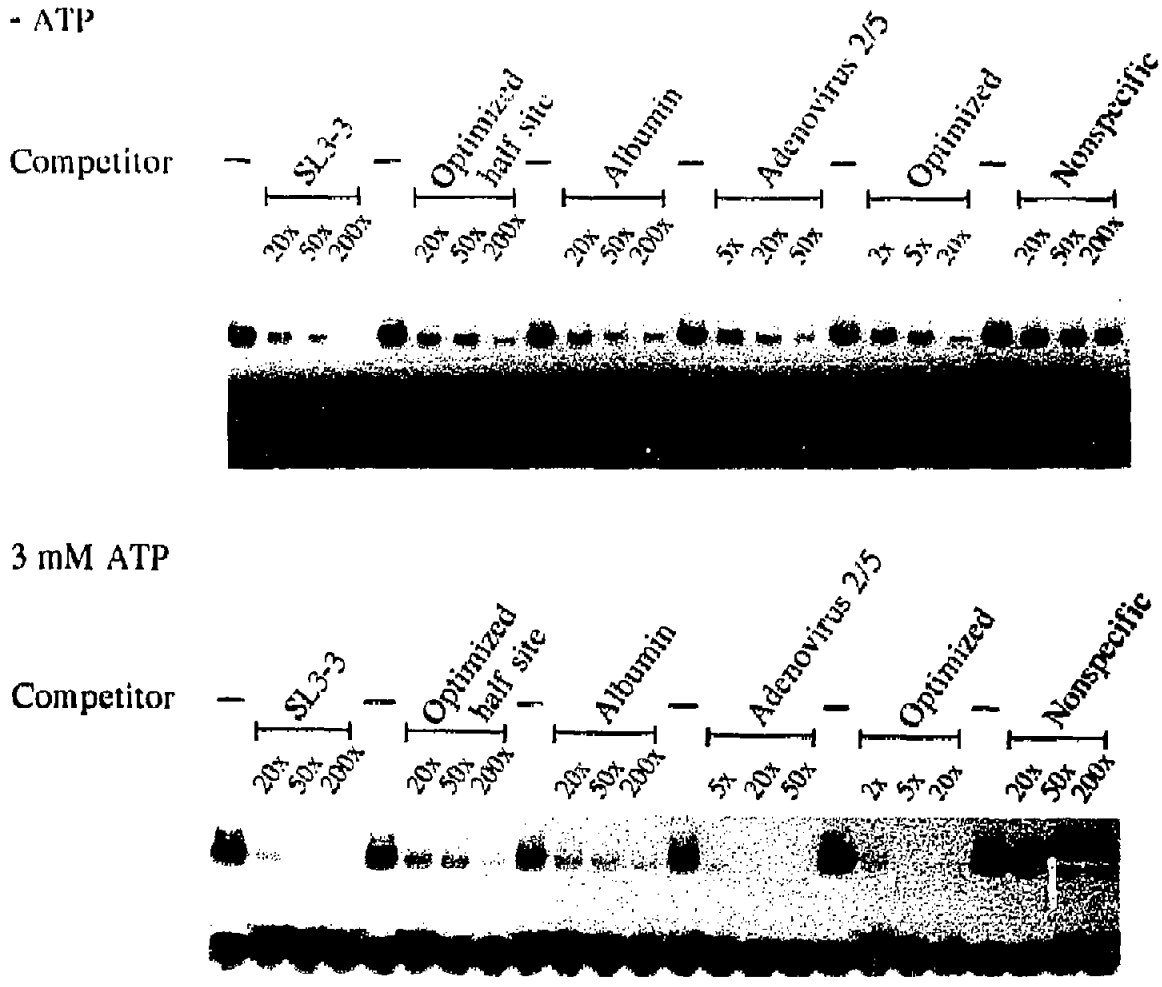

Fig. 3. Effect of ATP on competition of histone H1 binding to the SL3-3 site by various histone H1 binding sites. The molar excess of the competitor is indicated above each lane.

could lead to interference with the ability of H1 to interact with DNA. The data rather favour the possibility that the inhibition is an effect of binding of nucleotide to H1, either due to a physical overlap between the nucleotide and DNA binding sites, or to an allosteric effect on the DNA binding. The preferential inhibition of moderate strength H1 binding sites over strong sites could indicate competition between nucleotide and specific DNA for binding of H1. On the other hand, the slope of the inhibition curve could indicate transition between two conformations.

The finding that physiological levels of ATP affect H1–DNA binding in vitro indicates a direct connection between the availability of energy as ATP in the cell nucleus and the ability of H1 to bind to DNA in vivo. Since H1 acts as a general repressor, our results favour a direct coupling between the availability of energy as ATP and the accessibility of various genes to transcriptional activators.

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REFERENCES

[1] Weintraub, H. (1985) Cell 42, 705–711.
[2] Garrard, N.T. (1991) BioEssays 13, 87–88.
[3] Wolffe, A.P. (1989) EMBO J. 8, 527–538.
[4] Zlatanova, J. (1990) Trends Biochem. Sci. 15, 273–276.
[5] Croston, G.E., Kerrigan, L.A., Lira, L.M., Marshak, D.R. and Kadonaga, J.T. (1991) Science 251, 643–649.
[6] Berent, S.L. and Sevall, J.S. (1984) Biochemistry 23, 2977–2983.
[7] Mannermaa, R.-M. and Oikarinen, J. (1991) FEBS Lett. 278, 115–119.
[8] Zlatanova, J. and Yaneva, J. (1991) DNA Cell Biol. 10, 239–248.
[9] Sevall, J.S. (1988) Biochem. 27, 5038–5044.
[10] Nilsson, P., Hallberg, B., Thornell, A. and Grundström, T. (1989) Nucleic Acids Res. 17, 4061–4075.
[11] Ristiniemi, J. and Oikarinen, J. (1989) J. Biol. Chem. 264, 2164–2174.
[12] Clore, G.M., Gronenborn, A.M., Nilges, M., Sukumaran, D.K. and Zarbock, J. (1987) EMBO J. 6, 1833–1842.
[13] Churchill, M.E.A. and Suzuki, M. (1989) EMBO J. 8, 4189–4195.
[14] Suzuki, M. (1989) EMBO J. 8, 797–804.
[15] Ristiniemi, J. and Oikarinen, J. (1988) Biochem. Biophys. Res. Commun. 153, 783–791.

[16] Mannermaa, R.-M. and Oikarinen, J. (1992) Biochem. Biophys. Res. Commun. 182, 309-317.
[17] Mannermaa, R.-M. and Oikarinen, J. (1990) Biochem. Biophys. Res. Commun. 168, 254–260.
[18] de Petrocellis, L., Quagliarotti, G., Tomei, L. and Geraci, G. (1986) Eur. J. Biochem. 156, 143–148.
[19] Oikarinen, J., Mannermaa, R.-M., Tarkka, T., Yli-Mäyry, N. and Majamaa, K. (1991) Neurosci. Lett. 132, 171–174.
[20] Thornell, A., Hallberg, B. and Grundström, T. (1988) Mol. Cell. Biol. 8, 1625–1637.
[21] Sen, R. and Baltimore, D. (1986) Cell 46, 705–716.
[22] Johnson, G.S. and Schwartz, J.P. (1976) Exp. Cell. Res. 97, 281–290.
[23] Rapaport, E., Garcia-Blanco, M.A. and Zamecnik, P.C. (1979) Proc. Natl. Acad. Sci. USA 76, 1643–1647.