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The role of charge neutralization and cooperative binding of linker histone in the higher-order structure of chromatin

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The binding mode and stoichiometry of interaction between soluble rat liver chromatin and histone H1 (H1) were studied. H1 binding to chromatin is cooperative. Chromatin accepts 3.6 molecules of H1/nucleosome at 0 M salt, close to the required ratio for neutralization of 90% of the charges on the phosphate groups of chromatin (4.0 H1 molecules/nucleosome). The proposal is put forward that critical charge neutralization (90%) has a significant influence on the irregular appearance of chromatin.

Chromatin; Histone H1; Cooperativity; DNA-protein interaction; Fluorescence

1. INTRODUCTION

Linker histone is essential in the formation of condensed chromatin [1,2]. However, little is known about the mechanism of chromatin condensation by binding to linker histone.

Further compaction of native chromatins in the condensed state via exogenous addition of linker histones has been reported [3]. This has raised the questions as to the number of linker histones that can be accepted by the chromatin and the physical properties that determine the stoichiometry. Examination of the mode of interaction between chromatin and linker histone would reduce the possibility of higher order structures of chromatin. However, the reported results would still require clarification; some workers have suggested a non-cooperative mode [4,5] while others have proposed a cooperative type [6,7].

The present results show that 90% charge neutralization plays a significant role in determining the stoichiometry and that H1 binds to chromatin cooperatively.

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2. EXPERIMENTAL

Rat liver chromatin (H1- and non-histone-protein-depleted) of length 80 ± 20 nucleosomes was prepared and characterized as in [8,9]. The concentration of chromatin was determined by assuming an $A_{260\,nm}$ value of 20 for 1 mg DNA/ml. Rat liver histone H1 was prepared exactly as in [3,9]. H1 was labelled with fluorescein isothiocyanate (FITC, Sigma) (approx. 1 mol FITC/mol H1) and its concentration evaluated as in [10].

On binding to chromatin, the fluorescence of FITC-labelled H1 prepared from FITC samples of various origins was 50% quenched (average) at 80 mM NaCl, provided samples were fresh. Since extensive quenching (≥75%) was observed previously [10,11], such samples were submitted to ion-exchange column chromatography. Commercial samples of FITC were found to comprise at least 5 main components. Hence, no attempt was made to resolve this further. FITC labelling does not affect the results of the present study, since: (i) the difference in binding isotherms determined for various molar ratios of labelled to unlabelled H1 [10] was within experimental error; (ii) the 168 bp nucleosomal DNA of chromatin reconstituted with FITC-labelled H1 was protected against micrococcal digestion [12].

Fluorescence was recorded on a Perkin-Elmer MPF-44A spectrofluorometer. Excitation was at 488 nm and the fluorescence intensity at 520 nm was noted. The scattering intensity of sample solutions at 90° (I_s) was determined by setting the two monochromators at 385 nm.

Laboratory-ware and filtration of stock solutions of triethanolamine chloride (TEACl) buffer (pH 7.0) and EDTA were according to [10]. Sample solutions were adjusted to pH 7.0 with 5 mM TEACl (or 1 mM TEACl for 0 mM salt) plus 0.2 mM EDTA. Temperature was maintained at 20°C.

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3. RESULTS

Titration of a fixed concentration of chromatin (c_p) with increasing total concentration of H1 (c_A^0) was followed by means of the fluorescence intensity (F) and light scattering intensity (I_s) at 90° (with unlabelled H1). The results using 10 μg/ml solutions at 0 and 40 mM NaCl are shown in fig.1. The fluorescence data gave straight lines at low H1 concentrations (first stage during titration), indicating that all of the H1 added bound to the chromatin chains, as confirmed by sucrose density gradient analysis. Further additions of H1 led to an asymptotic approach to a line parallel to the control trace (titration in the absence of chromatin). Saturation of the binding sites on the chromatin was thus implied.

Fluorescence titration at 0 mM salt (1 mM TEACl) showed behaviour similar to that at 40 mM NaCl but much more H1 is required to approach the control trace in the case of the former.

Plotting of I_s as a function of c_A^0 showed the occurrence of marked changes in the curves for a narrow range of values of c_A^0. Around this range, saturation of binding sites was indicated in the fluorescence titration. Over the first half of the course of titration, no aggregation was observed on electron microscopy (not shown).

The plateau values of I_s remained constant for at least 5 h, indicating a considerable degree of hydration of the aggregates [13].

The fluorescence quenching efficiency Z was estimated to be 0.5 ± 0.04 at 40 mM NaCl, since the initial straight line has a slope only half of that of the control line; at 0 mM salt $Z \approx 0.9$. These results were confirmed using the second method where the degree of quenching was measured by addition of a large excess of chromatin to the H1 solution. For concentrations of NaCl increasing from 0 to 300 mM, the Z value decreased steeply up to 40 mM from $Z \approx 0.9$ to $Z \approx 0.5$, and remained constant above 40 mM (not shown).

Linear extrapolation to $F = 0$ of the final straight line in fig.1 resulted in intercepts on the abscissa at c_A^0. The stoichiometry n was evaluated from the equation [14,15]:

$$n = Zc_p/c_A^0 \quad (1)$$

The value of n is transformed to the specific stoichiometric number, $\underline{n}$, defined as the number

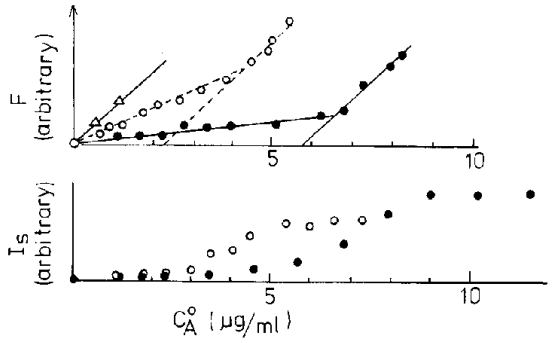

Fig.1. Titration of chromatin with increasing concentrations of H1 (c_A^0) performed at 0 mM with 1 mM TEACl (●) and 40 mM NaCl (○) was followed by monitoring the fluorescence intensity (F) at 520 nm and intensity of light scattering at 90° for light of 385 nm (I_s). Chromatin concentration 10 μg/ml. The control line (△) was obtained in the absence of chromatin.

of H1 molecules per nucleosome, in which a nucleosome is taken to be associated with 200 base pairs of DNA [2]. A value of $\underline{n} \approx 2.2$ was obtained with $Z = 0.5$ at 40 mM NaCl. The n value was dependent on salt concentration. $\underline{n}$ decreased from $\underline{n} \approx 3.6$ at 0 mM to $n \approx 1$ above 80 mM NaCl. This indicates that rat liver chromatin is able to accommodate up to 3–4 H1 molecules per nucleosome.

The fluorescence intensity (F) of a chromatin solution of $c_p = 0.7$ μg/ml was recorded with increasing concentration of H1 (c_A^0) at 80 mM NaCl. As shown in fig.2, the first experimental point is close to the control line obtained for $c_p = 0$. This implies that little H1 binding to chromatin takes

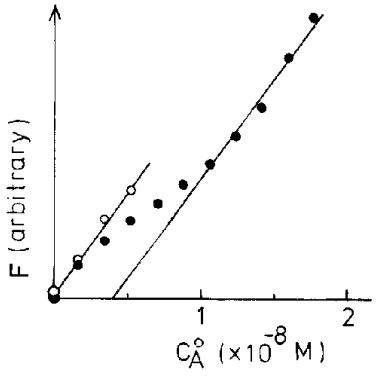

Fig.2. Fluorescence intensity (F) at constant chromatin concentration ($c_p = 0.7$ μg/ml) was measured as a function of total concentration of H1 (c_A^0) at 80 mM NaCl. From the final line $n \approx 145$ base pairs/H1 ($\underline{n} \approx 1.4$) was obtained.

place in this region. On further addition of H1 the titration curve deviated from the control line, subsequently reaching the final line. Therefore, H1 binding to chromatin is cooperative [10,11].

4. DISCUSSION

The validity of the solenoid model can also be examined from the thermodynamic point of view. H1 molecules, which are trapped inside condensed chromatin according to the solenoid model [2], must disrupt the stabilizing forces between the neighbouring turns in concert in order to dissociate from chromatin. Accordingly, the solenoid model suggests the cooperative mode. Some authors have suggested the non-cooperative mode [4,5] to be the correct description, whereas the cooperative form [6,7] was proposed by others. Chromatin samples of less than 30 nucleosomes in length, often shorter than dodecamers, were used in these experiments. Since such short fragments cannot overcome the so-called end effect, the binding mode was examined using chromatin of ≈80 nucleosomes in the present work. The binding mode was found to be cooperative and hence is compatible with the solenoid model.

The results in fig.1 reveal that chromatin accepts H1 molecules until H1-chromatin complexes aggregate. Since the aggregates are considerably hydrated, a certain proportion of the DNA charge remains uncompensated in the aggregates. Mg^{2+} displays a greater effect on this phenomenon than Na^+ [2,3]. These observations suggest that 89% of the charges in the aggregate are neutralized as DNA in [16]. To examine the validity of this hypothesis, the number of H1 molecules having the 55 positive charges required for 89% neutralization of the phosphate charges of DNA of 200 base pairs in length containing one core histone octamer (150 positive charges) was calculated by counting the number of polar residues of H1, lysine, arginine, aspartic acid and glutamic acid [17]. The hypothetical specific stoichiometry $\underline{n} = 4.0$. The agreement between this value and experiment at 0 mM salt, $\underline{n} = 3.6 \pm 0.4$, is satisfactory, especially on taking into account the presence of 1 mM TEACl and deviations in effective positive charges of rat liver histones from those of calf thymus. It is thus reasonable to conclude that critical charge neutralization (≈90%) of the charge on chromatin, induced by bound H1 and cations (Na^+), determines the stoichiometry.

This concept simplifies the explanation of the following observations. Firstly, intact H1 and a fragment consisting of the C-terminal tail and part of the globular domain of H1, on which most of the positive charge of H1 is concentrated, precipitate complexes with chromatin at a similar protein/nucleosome ratio [3]. Secondly, further addition of H1 to the chromatin aggregates does not increase the light scattering intensity, suggesting that a definite number of H1 molecules are associated with a particular size of aggregate [13]. Thirdly, Huang and Cole [5] reported that (i) soluble fragments are partly depleted of H1, while aggregated fragments are enriched in H1, based on the solubility of chromatin samples examined and (ii) the more soluble chromatin becomes, the greater is the degree of acetylation of its core histones for a fixed amount of H1.

In macromolecular systems, intermolecular forces also operate intramolecularly. Consideration of the balance of charges in the chromatin segment would hence provide a first approximation to a picture of the local structure of chromatin. The degree of H1 binding is proportional to that of chromatin condensation [18]. By virtue of the cooperativity, the bound H1 should form clusters in chromatin and condense regions complexed with H1. The binding of non-histone protein (NHP) to chromatin is also electrostatic to a substantial degree (NHP are removed with 0.35 M NaCl). NHP, which is abundant in native chromatin prepared in 10 mM NaCl, is expected to neutralize chromatin DNA to a certain extent. In contrast to H1, however, NHP is unable to condense chromatin due to insufficient charge density [3]. This implies that native chromatin has an irregular but not completely random appearance. Such images of native chromatin have been observed in the electron microscope [2,3,18].

The cooperativity of H1 binding may play an important role in the regional heterogeneity of structure and the function of chromatin. Furthermore, these considerations suggest the possibility of an interaction which is coupled with the mechanical properties of the condensed chromatin. The displacement of H1 from chromatin by NHP will loosen the condensed area,

by which additional DNA-protein interaction is regulated.

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REFERENCES

[1] Igo-Kemenes, T., Hörz, W. and Zachau, H. (1982) Annu. Rev. Biochem. 51, 89–121.
[2] Thoma, F., Koller, T. and Klug, A. (1979) J. Cell Biol. 83, 403–427.
[3] Thoma, F., Losa, R. and Koller, T. (1983) J. Mol. Biol. 167, 619–640.
[4] Caron, F. and Thomas, J.O. (1981) J. Mol. Biol. 146, 513–537.
[5] Huang, H.-C. and Cole, R.D. (1984) J. Biol. Chem. 259, 14237–14242.
[6] Renz, M., Nehls, P. and Horzier, J. (1977) Proc. Natl. Acad. Sci. USA 74, 1879–1883.
[7] Kumar, N.M. and Walker, I.O. (1980) Nucleic Acids Res. 8, 3535–3551.
[8] Thoma, F. and Koller, T. (1981) J. Mol. Biol. 149, 709–733.
[9] Losa, R., Thoma, F. and Koller, T. (1984) J. Mol. Biol. 175, 529–551.
[10] Watanabe, F. (1986) Nucleic Acids Res. 14, 3573–3585.
[11] Watanabe, F. (1984) FEBS Lett. 170, 19–22.
[12] Harborne, N. (1987) Thesis, King's College, University of London.
[13] Manning, G. (1978) Q. Rev. Biophys. 11, 179–246.
[14] Schwarz, G. and Watanabe, F. (1983) J. Mol. BIol. 163, 467–484.
[15] Watanabe, F. and Schwarz, G. (1983) J. Mol. BIol. 163, 485–498.
[16] Wilson, R.W. and Bloomfield, V.A. (1979) Biochemistry 18, 2192–2196.
[17] Mirzabekov, A.D. (1980) Q. Rev. Biophys. 13, 255–295.
[18] Allan, J., Harborne, N., Rau, D.C. and Gould, H. (1982) J. Cell Biol. 93, 285–297.