Experimental evidence for asymmetrical shielding of nucleosomal DNA by histones

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ABSTRACT

Electron spin resonance study of Mn (II) binding to chromatin and derivatives, including core particles, shows that Mn (II) is a good probe for testing the overall electrostatic balance of the nucleoproteic complex as well as DNA accessibility.

Experimental results are in good agreement with a recent model proposed (Mirzabekov A. D. and Rich A. (1979) Proc. Natl. Acad. Sci. USA 76, 1118-1121), for the core particle, in which an asymmetrical shielding of DNA by the protein core is assumed.

Furthermore, it was found that the histone H1 hinders a number of charges on the linker DNA in a proportion equal to the net positive charge of the histone itself. This result is interpreted as due to a tighter interaction between the linker DNA and the core histones in the presence of histone H1.

INTRODUCTION

The nucleosomal subunit structure of chromatin is now widely accepted (for review, see reference 1). Precise information concerning the structure of the core particle containing an octamer of histones and 145 base pair long DNA segment come from both crystallographic data (2) and the analysis of digestion products using deoxyribonuclease I (3-5). The former show that histones and DNA are tightly packed in a disk-shaped particle of 110 Å x 110 Å x 55 Å dimensions (2). Similar values for these parameters have been determined by model calculations from neutron scattering data (6) except that in this model 24% of the histone core, probably corresponding to the more basic N-terminal amino-tails, are supposed to be in close association with the DNA. Thus, these two models would predict significantly different accessibility of DNA to various probes as a consequence of the electrostatic shielding. On the other hand, the properties described for the core particle are not necessarily valid for the nucleosome itself. Indeed, in addition to the 145 base pair DNA fragment of the core particle, the nucleosome has a variable fragment of DNA, the linker, to which histone H1 and
probably a few non-histone proteins are bound. Due both to the variability of the linker length (7) and the variability among histone H1 molecules, it has not yet been possible to get crystallographic data from the whole nucleosome. Nevertheless, it can be easily conceived that the presence of histone H1 on the nucleosome may affect the structure of DNA in both linker and core particle region. Because of the highly heterogeneous charge distribution in histone H1 (8), such a structure can be assumed to be sensitive to the ionic environment of the particle giving rise to a variety of conformations of the chromatin fiber observed in electron microscopy.

The function of chromatin involves controlling interactions between a variety of macromolecules as well as small chemicals and DNA at the nucleosomal level. A number of such reactions can be assumed to be initially governed by the electrostatic state of the DNA. The aim of the present study is to get more informations about the accessibility of DNA in respect to this hypothesis.

We have used manganese ions as a probe for DNA accessibility in chromatin. Earlier studies (9) involved the use of sheared chromatin, prepared in the presence of EDTA. In these, equilibrium dialysis procedure gave values for Mn (II) binding sites which seem abnormally high. It is now well known that Mn (II) binds to DNA through a chelation process between a phosphate group and the N7 of the guanine (10) and such a binding indicates the existence of an outside site for Mn (II) as has been shown by proton relaxation enhancement studies (11,12). As Mn (II) is a paramagnetic ion, it is possible to use electron paramagnetic resonance (EPR) as a tool for studies on its interactions with biological macromolecules like proteins, nucleotides, nucleic acids (13-17).

MATERIAL AND METHODS

Chemicals and buffers

Manganese chloride, MnCl2, 4H2O (analytical grade from Merck or Prolebo) was used. As this chemical is subject to hydration changes, stock solutions were titrated by mean of atomic absorption spectrometry. All experiments were performed in 1 mM triethanogelamine (TEA)-HCl buffer, pH = 7.4.

Chromatin and derivatives

Calf thymus or chicken erythrocyte DNA were used. Native chromatin was prepared from rat liver nuclei (18), using mild micrococcal nuclease digestion (19). Non-histone proteins (NHP)-depleted chromatin and chromatin depleted of H1 and NHP were prepared according to a modification...
Core particles were prepared following Lutter's method (22), slightly modified by the use of a centrifugation step instead of the final column on Sepharose 6B to fractionate the nucleosome cores from residual dinucleosomes, nucleotides, etc.

**Controls**

The length of DNA in core particle was measured in the DNA electrophoresis system of Maniatis et al (23). It was found to be 145 ± 3 base pairs using either a digest of φ X 174 (RF) by Hae III (New England Biolabs) as marker in non-denaturating gels, or a DNase I digest of calf thymus core particle in denaturating gels; in this latter case, the periodicity was assumed to be 10.4 bases (4).

Protein concentrations in the various samples were estimated by the Lowry's method (24), and analyzed in the sodium dodecyl sulfate (SDS) gel system of Laemmli (25) (Fig. 1).

**FIG. 1** - SDS polyacrylamide slab gel electrophoresis.
From left to right: chromatin depleted of H1 and NHP; NHP-depleted chromatin; native chromatin.
Turbidity measurements were performed in order to check that precipitation of chromatin does not occur in the range of Mn (II) concentration used.

**Electron Paramagnetic Resonance Spectra**

EPR spectra were recorded on a Varian E 104, or a Varian E 12 spectrometers at 20°C and a frequency of 9.45 GHz. Scanning range was 1000 G, and modulation was 4 G.

**RESULTS AND DISCUSSION**

**EPR Spectra**

A solution of free Mn (II) ion gives an EPR spectrum, made of 6 lines due to the hyperfine coupling between the magnetic moment of the odd electron and the nuclear magnetic moment \( S = 5/2 \). (Fig. 2A).

Binding of Mn (II) to DNA induces a drastic fading of the spectrum so that it is possible to neglect this "bound contribution". (Fig 2B) shows such a spectrum, in the case of a large excess of DNA.

**Mn (II) titrations**

Figure 3 shows how to estimate, for any given sample, the respective amounts of free and bound Mn (II), from the EPR spectra.

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**FIG. 2 - EPR spectra of Mn (II) ions in solution**

(A) free Mn (II)

(B) bound Mn (II) in presence of a large excess of DNA.

Both spectra are runned at the same conditions: frequency = 9.45 GHz; field = 3500 G; scan range = 1000 G in 8 min; power = 10 mW; gain = 5000; modulation = 4 G; time constant = 0.64 s.
In a first step, the height of the first peak, $h$, (at higher field) of the 6-lines spectrum is measured for different Mn (II) in the buffer. These experimental data give a linear variation of $h$ versus Mn (II) concentration, which could be considered as the reference.

Experiments are performed in order to determine an eventual binding of Mn (II) to isolated histones (H1 and octamer histones separately). This assay fails to reveal any measurable binding.

In presence of chromatin, with phosphodiester groups binding the manganese ions, the height of the peak is proportional to the remaining free Mn (II) in the solution. As shown on Fig. 3, for a given Mn (II) concentration, it is possible to estimate the amount of free Mn (II) and the difference with the peak height of the reference allows estimate of the amount of Mn (II) bound.

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**FIG. 3** - Experimental determination of the binding isotherms:

- Peak height of Mn (II) in the reference buffer.
- Peak height in presence of native chromatin for OD = 2

The continuous curve is the theoretical binding curve representative of $K_h^2 + (K_N P - K_C + 1) h - C = 0$.

where $h$ is the peak height, in arbitrary units.

- $C$ is the total Mn (II) concentration
- $P$ is the amount of chromatin expressed in phosphorus content (here, for O.D. = 2).
- $K$ is the binding constant (here, $1.06 \times 10^5$ M$^{-1}$).
- $N$ is the number of binding sites (here, 0.14).
bound Mn (II).

The experimental values of the titration curve shown on fig. 3 correspond to the study of native chromatin (O.D. 258 = 2).

Such a titration curve is represented in a Scatchard plot \([R/C = f(R)]\) (Fig. 41, leading to the values of \(N\) (number of binding sites) and \(K\) (binding constant) (26).

Table 1 gives the values found for \(N\) and \(K\) for the samples under investigation; these values are deduced from experimental results by means of a least square fit. Indeed, for such low concentrations, the electrostatic interaction between the bound manganese are weak enough, especially in the low ionic strength medium used in this study: the Scatchard plot described in this area can be considered as linear, while a significant curvature would be observed for higher values of \(R\) where electrostatic interaction between neighbor bound Mn (II) would occur (15). The binding parameters determined in this study are related to the sites where the apparent binding constant is the highest. The parameters displayed in table 1 refer to these

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**FIG. 4 - Binding isotherms (Scatchard plots)**

- Native chromatin
- NHP depleted chromatin
- Chromatin depleted of H1 and NHP

\(R\) is the ratio of bound Mn (II) per phosphate group and \(C\) the concentration of free Mn (II) at the equilibrium.

Insert: core-particles
Table 1. Binding constants $K$ and number of binding sites (per phosphate) $N$

<table>
<thead>
<tr>
<th>Sample</th>
<th>$K$</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naked DNA</td>
<td>$5 \times 10^3 \pm 1.0 \times 10^3$</td>
<td>$0.45 \pm 0.03$</td>
</tr>
<tr>
<td>Chromatin depleted of H1 and NHP</td>
<td>$7 \times 10^3 \pm 2.5 \times 10^3$</td>
<td>$0.34 \pm 0.04$</td>
</tr>
<tr>
<td>NHP-depleted chromatin</td>
<td>$6 \times 10^3 \pm 1.5 \times 10^3$</td>
<td>$0.20 \pm 0.02$</td>
</tr>
<tr>
<td>Native chromatin</td>
<td>$1 \times 10^5 \pm 1.5 \times 10^5$</td>
<td>$0.14 \pm 0.01$</td>
</tr>
<tr>
<td>Core particle</td>
<td>$1 \times 10^6 \pm 1.5 \times 10^5$</td>
<td>$0.28 \pm 0.02$</td>
</tr>
</tbody>
</table>

sites only. The number of binding sites shown in Table 1 deserve several comments: the binding parameters found with the EPR method are in good agreement with those found with other methods (27,28). The value of 0.45 found for $N$ in naked DNA can be considered as the reference value of maximum accessibility, which will be referred as 100% accessibility. In these conditions, the accessibilities of DNA in chromatin depleted of H1 and NHP, in NHP-depleted chromatin and in native chromatin are 75%, 45% and 31% respectively.

Figure 5 displays a schematic diagram of the nucleoproteic arrangement in nucleosome and derivatives, in terms of an hypothetical electrostatic balance.

Assuming that the chromatin is composed of a repetitive subunit containing 195 base pairs of DNA, the total number of observed Mn (II) binding sites can be expressed in terms of phosphodiester groups. These correspond to $195 \times 2 \times 0.45 = 175$ phosphorous atoms per subunit from fully protein depleted chromatin, 132 P-atoms in H1 and NHP-depleted chromatin, 78 in only NHP-depleted chromatin and 55 in native chromatin.

From figure 5, the number of phosphodiester groups available on the linker is $132 - 81 = 51$, which corresponds to a number of binding sites $N = \frac{51}{50 \times 2} = 0.51$. This value is, within a 10% experimental error, identical to that found for pure DNA: it means that the linker, depleted of H1 and NHP, behaves like naked DNA.

According to calculations of Mirzabekov and Rich (29) on the electrostatic balance in nucleosome, it is possible to consider the DNA core
FIG. 5 - Schematical assumption for the nucleoproteic arrangement related to a repetitive subunit of 195 base pairs

- octamer
- H1
- NHP

particle as a 145 phosphodiester group "outer part", free of any protein interaction, and with 20 phosphodiester groups each at 5' DNA-end consisting of the "histone-free-region", i.e. 185 DNA phosphodiester groups altogether. The 0.45 ratio applied to these considerations leads to the number of phosphodiester groups available for binding to be 83, which is in very good agreement with 81 that we find experimentally for the core particle.

In the same way, the difference in the available phosphodiester groups between chromatin depleted of NHP alone and both H1 and NHP, corresponds to that due to H1 binding; this value is experimentally found to be 54. This agrees well at least for the fraction 3 of rabbit thymus H1 which has an overall positive charge of 53 (30). However, this agreement needs to
be tested for other H1 molecules including those from rat liver.

Finally, it is noticeable that NHP-depletion makes a 23 phosphate groups accessible to Mn (II) although we do not understand the precise implication of this result on the chromatin structure. At present it cannot be excluded that these NHP contribute in some way to the binding of Mn (II).

It seems very difficult to interpret the meaning of values of $K$ unambiguously; the relative uncertainty about $K$ determination is rather important, due to the concentration of the experimental points near the R axis in the Scatchard plot (for the very low Mn (II) concentrations, the signal-to-noise ratio is drastically decreasing). Thus, there is no evidence available for a significant difference between the $K$ values for DNA and the various derivatives of chromatin. The surprisingly high value found for the core particles still remains unclear.

**CONCLUSION**

The results concerning the stoechiometry of Mn (II) binding to chromatin and derivatives show that this method leads to a fairly good estimate of the DNA accessibility and to a determination of the overall electrostatic balance. Enzymatic studies on the involvement of histone H1 in the structure of the nucleosome usually lead to the conclusion that this histone protects a DNA fragment of 30 (31), or 40 to 50 (32) base pairs. Our manganese binding studies give rise to an evaluation of the DNA protection in terms of electrostatic balance. We have found that 54 phosphate groups are no more available for binding manganese when histone H1 is present on the nucleosome.

This number is strikingly related to the net positive charge of histone H1 but it does not necessarily mean one-to-one neutralization between the positive charges of the histone and the negative charges of the linker DNA. It is more likely related to a secondary process which would lead to an organization of the linker around the nucleosome core in the presence of H1 and not inconsistent with data (33) obtained from enzymatic digestion of integral nucleosome as compared to H1 depleted nucleosome. Concerning the core particle, the Mn (II) binding study has the advantage that it allows an estimate of the DNA accessibility to a very small ionic probe. Our present result with the core particle is in good agreement with the recent model by Mirzabekov and Rich (29).

The DNA surrounding the "octamer" can be divided in two parts: the phosphates of the outer part are completely accessible to solvent i.e.
the cations; the phosphates of the inner part are partially neutralized electrosstatically by the positive charges of the histone core: our Mn (II)
binding studies give experimental evidence supporting this model.

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