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Theoretical analysis of gel electrophoretic data for interaction of lysine rich histone with supercoiled DNA

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Abstract

We demonstrate the possibility of using gel electrophoresis as a technique for the quantitative analysis of interaction of lysine rich histone with DNA. On the basis of theoretical framework for extended ligand binding to one-dimensional lattices such as DNA we have set up systems of equations which relate the ligand-to-DNA ratio to the observed gel migration distance of the complex. From the analysis of experimental data for gel electrophoresis of supercoiled DNA in the presence of lysine rich histones we have found that the observed variation of electrophoretic mobilities of the histone–DNA complexes at low histone-to-DNA ratios can be described by a non-cooperative binding behaviour. At this limit we have estimated the intrinsic binding constant to be of the order of $10^3\ M^{-1}$.

Keywords: Supercoiled DNA; Lysine rich histone; DNA binding constant; Gel electrophoresis

1. Introduction

Histone H1 (a lysine rich protein) has been proposed to take part in the function and maintenance of higher order chromatin structure in eukaryotes. Evidence that H1 histone primarily interacts with DNA has led to extensive studies of artificial H1–DNA complexes *in vitro*. Using the method of electron microscopy H1 complexes with linear DNA were described as double fibres [1] at low ionic strengths and toroids [2] at high ionic strengths, whereas aggregates with superhelical DNA were found to be double fibre and cable like at both low and high ionic strengths [3,4]. However, it was ascertained later that the interaction depends on the histone-to-DNA input ratio and the ionic strength. Gel electrophoresis and electron microscopy have revealed that at low input ratio of H1 to DNA individual soluble complexes are formed [5,6]. Above a critical ratio of H1 to DNA, aggregation of the nucleoprotein complexes occurs. This critical point may be shifted to lower H1-to-DNA ratio by increasing the ionic strength of the reaction mixture. It was proposed that the soluble complexes can be considered as a model for the interaction of H1 in chromatin fibres. On the otherhand, there also exists the viewpoint that the aggregation induced by H1 is a model of the compact state of the chromatin.

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Another feature of the interaction between H1 and DNA which has attracted attention is the preference of H1 for supercoiled DNA. A series of filter binding experiments [7–9] has revealed that H1 possesses a considerably higher affinity for superhelical DNA than for linear or relaxed circular DNA. Moreover, the affinity increases with both increasing negative and positive supercoiling which seems to indicate that H1 histone binding to supercoiled DNA is not connected with alteration of supercoiling. However, these experiments were carried out in the presence of high NaCl concentration (100 mM) sufficient to generate aggregates of histone and DNA. It remains yet to be checked in how far the above results are valid for soluble complexes, both qualitatively and quantitatively.

Formation of individual soluble complexes and aggregates at different regimes of histone-to-DNA ratio and its dependence on the ionic strength indicates the existence of a hierarchy of interactions involving histone–DNA, histone–histone and DNA–DNA parts. Surprisingly, in spite of a large number of experimental results, there are very but few theoretical analyses of the complex nature of interaction of histone H1 with DNA. To the best of our knowledge no estimate of the intrinsic binding affinities of H1 histone for various forms of DNA is available and the same is also true of histone–histone interaction, the parameter which would be responsible for the degree of cooperativity in modelling the aggregation phenomenon. However, recent investigations employing the gel electrophoretic technique, which studies the retardation of H1–DNA complexes and their retention in the wells at increasing histone-to-DNA ratio, have opened up the possibility of applying quantitative methods.

Application of gel electrophoretic methods for detecting and analyzing specific protein–DNA interactions is based on the differential electrophoretic mobilities of protein DNA complexes relative to the more rapidly migrating uncomplexed DNA [10,11]. It has the advantage over the filter binding method in that it can detect different stoichiometry of binding. Site specific binding of repressor proteins to DNA operator sequences is one such problem which is currently being investigated quantitatively by employing this technique [12,13]. However, the analysis of the present problem differs from the above in the complete absence of ladder formation by protein–DNA complexation. A closely analogous situation to the present problem that has been examined recently is the agarose gel electrophoresis of synthetic homopolyribonucleotides in the presence of lysine derivatives [14].

In the present paper we have shown that the distance of migration of ligand–DNA complexes can be related to the ligand concentration or the ligand-to-DNA ratio through a set of equations based on the theory of extended ligand binding to infinite one-dimensional lattices [15,16]. The theoretical results derived have been applied subsequently to explain the variation of electrophoretic mobility of supercoiled plasmids complexed with different amounts of lysine rich histone. It was observed that the present experimental data can be quite accurately described by a non-cooperative mechanism of histone binding to DNA below the aggregation point. We have also estimated the intrinsic binding constant of histone for supercoiled DNA. For the experiments considered the binding constant came out to be of the order of $10^3\ M^{-1}$.

2. Materials and methods

The plasmid pSV2 neo [17] was a gift from Prof. J. Justin McCormick, Carcinogenesis laboratory, Michigan State University, USA. It is a recombinant SV40 based vector having promoter and enhancer elements. It was grown in *E. coli* HB101 strain and isolated and purified by the alkali lysis method as described by Maniatis et al. [18]. The purity of the plasmid was checked in 0.8% agarose gel. The densitometric scanning of the gel photographs showed that the purified plasmid contained about 60% in the supercoiled form and 40% in the nicked circular form. The concentration of DNA was determined from the absorbance at 260 nm, assuming that 1 mg DNA/ml has an absorbance of 20.

Lysine rich histone subgroup f_1 (histone H1) was purchased from Sigma Chemicals Company,

USA and was dissolved in 1 m*M* phosphate buffer (pH 7.40), 0.2 m*M* sodium EDTA, 0.25 m*M* phenylmethylsulphonylfluoride (PMSF). The concentration of histone was checked spectrophotometrically, assuming that 1 mg/ml of histone has an absorbance of 1.85 at 230 nm [18].

For histone–DNA interaction, plasmid and histone were added to the 1 m*M* phosphate buffer (pH 7.4) containing 0.2 m*M* sodium EDTA, 0.25 m*M* PMSF. The concentration of histone was varied such that the histone-to-DNA ratios ranged from 0 to 1.2. The reaction cocktail was kept on ice for 2 hours. The nucleoprotein complexes thus formed were fixed by adding 0.1% glutaraldehyde and kept at 4°C for 16 hours. The samples were mixed with dye and loaded on the 0.8% agarose gel and the samples were run at 2–3 volts/cm. The gel electrophoretic mobility was checked after staining with 1 mg/ml ethidium bromide (EtBr) and photographs were taken in UV light. The negatives were scanned in a densitometer (LKB 2202) and the distances of migration of supercoiled DNA molecules at different concentrations of histone were determined from the distances of the corresponding peaks from the well. The relative distances of migration of histone–DNA complexes compared to that of control plasmid was measured similarly from the tracing obtained from the densitometric scanner.

3. Theory

We consider the distance of DNA migration d to be a linear function of the fractional saturation y of the DNA molecule (number of bound ligands per base pair)

$$d = ay + D \tag{1}$$

The constant D is the distance of migration of the free DNA (i.e., in the absence of ligands). The other constant can be obtained by measuring the distance of migration D_s in an excess of ligand when the DNA is completely saturated with ligand. If the ligand is an extended one and excludes l base pairs from other ligands on binding, eq. (1) becomes

$$D - d = (D - D_s)ly \tag{2}$$

The fractional ligand saturation y for non-cooperative binding in the case of extended ligands has been shown to be expressed by the relation [15,16]

$$y = KC_f f(y) \tag{3}$$

where K is the binding constant of the ligand for DNA and C_f is the free ligand concentration in the equilibrium mixture and

$$f(y) = (1 - ly)\left[\frac{1 - ly}{1 - (l-1)y}\right]^{l-1} \tag{4}$$

which for $ly \ll 1$ reduces to [19]

$$f(y) = 1 - (2l - 1)y \tag{5}$$

Since binding of lysine rich histone to DNA is predominantly electrostatic in character [20], at complete saturation of the DNA with ligands most of the negative charges of the DNA molecule will be neutralised. In that case D_s can be assumed to be negligible compared with the distance of migration D of the free DNA. Expression (2) then becomes

$$d = D(1 - ly) \tag{6}$$

for a dilute DNA solution and at low fractional saturation of ligand we can obtain a simple relation between the distance of migration and the free ligand concentration by eliminating y from eqs. (3), (5) and (6).

$$d = D\left[1 + \frac{lKC_f}{1 + (2l - 1)KC_f}\right] \tag{7}$$

For binding of histone H1 to DNA it is customary to give the histone-to-DNa ratio R in the reaction mixture. Since for the usual values of R the concentrations of histone and plasmid become comparable, C_f, the free ligand concentration is obtained from the histone-to-DNA ratio R in the solution as

$$C_f = C_d(\mu R - Ny) \tag{8}$$

where C_d is the DNA concentration in the solution, μ is the ratio of the molecular weights of DNA and histone and N is the number of base pairs in the DNA molecule.

If the DNA used is not a homogeneous sample, e.g., contains admixtures of nicked species along with supercoiled one whose binding characteristics differ, then the free ligand concentration is given by

$$C_f = C_d \mu R - N(C_s y_s + C_n y_n) \qquad (9)$$

where C_s and C_n are the concentrations of the supercoiled and nicked species and y_s and y_n are their fractional ligand saturations respectively. Since the concentration of the nickel species is usually kept small compared to the supercoiled species and also since the binding affinity of nicked DNA for histone H1 appears to be considerably less than that of native supercoiled DNA the second term in the parenthesis in eq. (9) may be neglected. In that case it reduces to eq. (8) which we will use in analysing the mobility variation of histone–supercoiled DNA complexes. However, for analysing situations arising from the possibilities that the binding affinities for the two forms of DNA may be comparable, or when we specifically wish to estimate the binding affinity for the nicked species we have to explicitly consider separate binding isotherms for the two forms of DNA and solve the coupled set of equations to obtain the variation of mobilities of the two forms with ligand binding.

4. Results and discussions

Theoretical study of gel electrophoretic behaviour of histone–DNA complexes was done with model system parameters as in [6]. In this experiment the concentration of DNA was 100 mg/ml and the length of the plasmid pBR2.17 was 4961 base pairs. Taking the molecular weight of lysine rich histone to be 21,000 Da, the value of μ is found to be 156. The size of the histone binding region is taken to be 51 base pairs which is actually the length of the linker regions between consecutive nucleosomes in native calf thymus chromatin [20]. The variation of relative gel electrophoretic mobilities (given by d/D) with different input ratios R and different binding constants K was computed using the set of eqs.

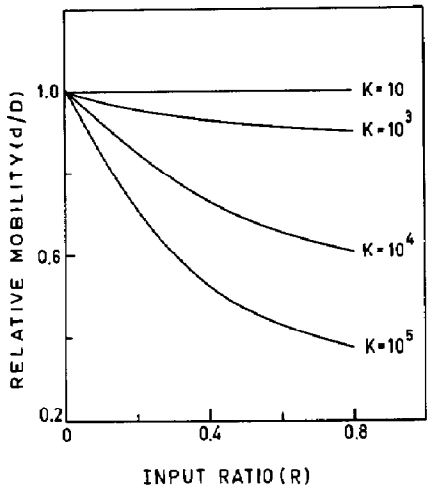

Fig. 1. Computed profiles of relative mobilities of (d/D) histone–DNA complexes as a function of histone-to-DNA ratio (R) for various values of binding constants K in M^{-1}. For details see text.

(3), (4), (6) and (8) and plotted in Fig. 1. It is apparent that for binding affinities of the order of 10^2 M^{-1} and lower, the relative mobility changes are hardly detectable at the range of input ratios studied. However, for binding constants in the range 10^3–10^4 M^{-1} there occurs pronounced changes in the mobilities and consecuently in the distance of migration with changing input ratio. For larger binding constants, ligand saturation effects are detectable at higher input ratios implying the occurrence of histone–histone interaction.

To compare the theoretical results with experiment we studied the gel electrophoretic behaviour of histone–DNA complexes as described in the materials and methods section. With increasing amounts of histone the electrophoretic mobility of supercoiled DNA gradually decreased up to a ratio of 0.9 where aggregation started. This aggregation was recognised by fluorescent material remaining in the well (starting point in the gel). The mobility of the nicked circular DNA remained almost unaffected with increasing input ratio upto $R = 1.2$ (data not shown). The relative mobilities of supercoiled fractions (d/D) obtained were plotted against the input ratios (R). Using these data points the best fit value of the intrinsic binding constant (K) was determined. The best fit curve for a typical experiment is

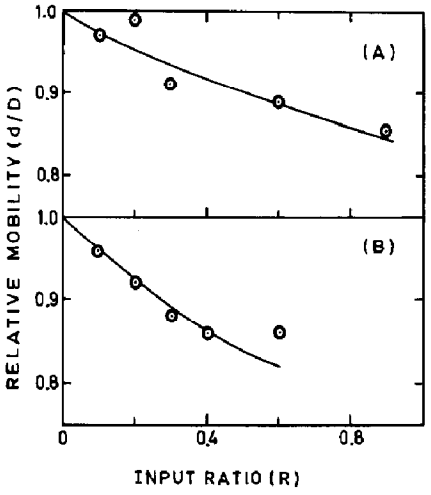

Fig. 2. Plot of relative mobilities (d/D) of histone DNA complexes as a function of histone to DNA ratio (R). The solid curves are theoretical curves best fitted to the experimental points (A) for histone–pSV2 neo complexes with the best fit binding constant $K = 1.8\ 10^3\ M^{-1}$, (B) for histone–pBR2.17 complexes (6) with the best fit value of the binding constant $K = 2.4\ 10^3\ M^{-1}$.

shown in Fig. 2(A). The different parameters used to fit this curve have the following values: $N = 5.6$ kb for the plasmid pSV2 neo; $C = 55$ mg/ml and $\mu = 176$. Keeping the value of l the same as above, the value of binding constant K obtained for the curve from Fig. 2(A) is $1.8\ 10^3\ M^{-1}$.

We analysed also the data reported [6] for binding of histone H1 to supercoiled plasmid pBR2.17 (Fig. 2B) for a somewhat different experimental condition than that of ours (the reaction buffer contained 36 mM TEA, 30 mM NaH_2PO_4, 1 mM EDTA, pH 7.55). The distances of migration of histone bound supercoiled DNA were measured from the published picture. The best fit value of the binding constant came out to be $2.4\ 10^3\ M^{-1}$. This is of the same order of magnitude to the value determined by our experiment.

It should be realised that for low affinity ligands the present method is not a highly sensitive technique for binding constant determination. To estimate the accuracy of the magnitude of the binding constant we have calculated the maximum possible errors that may come from the experimental measurements and found that these may contribute up to 80% of the determined value of the binding constant. However, another probable major source of uncertainty is the somewhat imprecise knowledge about the size of the binding region of histone H1. It is generally equated with the size of the linker DNA in nucleosomes. In finding the best fit value of K we have taken the length of the linker DNA in nucleosomes as 51 base pairs. However, this value is definitely not universal. In chicken erythrocyte chromatin, for example, the H1 binding site is 67 base pairs [20]. Furthermore, for artificial H1–DNA systems this parameter may have some other value. To find the range through which estimates of K can vary with ligand size we have repeated the same calculations with the value of l varying from 70 base pairs to 30 base pairs and found that K remains within the range $10^3\ M^{-1}$ to $5\ 10^3\ M^{-1}$ for all the cases. This lack of sensitivity of the best fit profiles on the size of the ligand binding region is expected at low binding levels since at this limit the ligands are widely spaced on the DNA and have little knowledge about the presence of other ligands. Consequently, from the foregoing analysis, we find that for the experiments considered the binding constant for H1 histone for supercoiled DNA is of the order of $10^3\ M^{-1}$.

It should be noted that the stabilization of histone–DNA complexes by glutaraldehyde in these experiments may perturb the equilibrium situation assumed in the theory. However, the experiments on the sedimentation patterns and the corresponding gel electrophoretic mobilities of histone–DNA complexes show, that the protein distributions of the fixed and unfixed complexes are almost identical [5,21]. Thus, the estimates of binding affinities in our experimental conditions should not differ much from the true equilibrium values.

One interesting feature of these experiments is the preferrential binding of histone to supercoiled DNA compared to nicked circular DNA. This follows from the gel electrophoresis data which show that the mobility of nicked circular DNA hardly changes with increasing R. According to our theory, this can happen if the histone binding affinity of the nicked DNA is consider-

ably lower than that of the supercoiled DNA, which is consistent with the previous results obtained with filter binding studies [7–9]. However, as is apparent from Fig. 1, gel electrophoresis has a very low sensitivity for low binding affinities and cannot be used to obtain a quantitative estimate for nicked or linear species in the presence of supercoiled form.

The binding mode of H1 histone to supercoiled DNA at low input ratio R was assumed to be noncooperative in a previous work [6]. However, this was a qualitative observation based on the fact that the mobility of the complexes decreased gradually with increasing R. On the other hand, the quite good fit of the experimental data to the theoretical calculations, which are valid only for non-interacting ligand binding, seems to confirm the non-cooperative binding hypothesis quantitatively. This suggests that below the aggregation point histone–histone interaction does not play a significant role. Moreover, the non-cooperatively in binding also indicates that in this range the change in supercoiling energy with histone binding to supercoiled DNA, if any, is negligible.

Since histone H1 is a lysine rich protein it should be interesting to compare its binding affinity for DNA with those of other lysine derivatives. Binding affinity of ethyl ester of lysine for polyribonucleotides have been shown to vary considerably with ionic strength [14]. For 10 mM ionic strength the binding constant for poly(A) is about 120. The binding constants are a few percent less and greater than this for poly(U) and poly(G) respectively. At still lower ionic strengths the binding constants are reported to increase quite sharply.

The higher value of binding affinity for the supercoiled form may be due to structural features peculiar to the protein. It may be advantageous for the protein to bind to a bent DNA structure which is more available when the DNA is supercoiled. This hypothesis may lead to some insight into the structure of a supercoiled DNA by further study of the variation of binding affinity of histone with changing supercoiling of DNA.

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