Condensation of polynucleosome by histone H1 binding

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The cooperative binding of histone H1 to polynucleosome was studied quantitatively. The equilibrium and kinetic data were satisfactorily described in terms of the large ligand model. The binding constant K and the cooperativity parameter q showed remarkable salt effects: $K = 7.5 \times 10^7\ M^{-1}$ and $q = 1.3 \times 10^4$ at 0.2 M NaCl, pH 7.5 and 20°C. This considerably strong cooperativity reveals that the polynucleosome state, condensed or not, is sensitive to small changes in the free histone H1 concentration around the value of $1/K$. The association rate constant was of the order of $10^7\ M^{-1} \cdot s^{-1}$ and had a small salt concentration dependence, indicating a diffusion-controlled association process.

Polynucleosome *Histone H1* *Cooperativity* *Kinetics*

1. INTRODUCTION

The structure of chromatin has been studied by various methods to obtain detailed information about the molecular mechanism of genetic regulation in eukaryotic cells. It has been confirmed that the binding of histone H1 causes polynucleosomes to take a condensed solenoid structure [1]. The salt concentration effect on the solenoid structure is known in some detail [2].

To understand how the chromatin structure is regulated by histone H1 binding, it is necessary to describe quantitatively thermodynamic and kinetic aspects of the binding. However, no such report has been published. Authors in [3] have suggested a cooperativity in the binding. It is therefore reasonable to analyse the binding data based on the standard large ligand model [4,5]. The present work reveals the utility of the model and presents new knowledge of the system studied.

2. MATERIALS AND METHODS

Calf thymus histones purchased from Sigma (type II-S) were purified as in [6]. Lambda DNA was kindly donated by Drs Bickle and Kuroiwa of the institute. Histone H1 was labelled with fluorescein isothiocyanate at the N terminus [7]. Histone H1-depleted polynucleosomes were reconstituted by the method of stepwise salt concentration decrease from a 2.1:1 (w/w) mixture of core histones and DNA [8,9].

Characterization of the polynucleosome was performed as follows. The electron microscopic (EM) image was taken using a Philips 300 [2,10]. The number of beads was counted in the EM images of samples which showed no obvious prolonged regions of a nicked DNA. Only samples of more than 160 spots (which corresponds to 300 bp/H1) were titrated with the labelled histone H1 at 0.1 M NaCl, pH 7.5 and 20°C. Samples which could show the stoichiometric number below 250 bp/H1 were used here. Sample solutions were adjusted to pH 7.5 with 5 mM Tris–HCl buffer.

Fluorescence measurements were performed using a Farrand MK1 fluorometer. Excitation and emission wavelengths were 495 and 513 nm, respectively. A stopped-flow device described in [10] was used for the kinetic measurement. Plastic laboratory-ware was exclusively used to minimize the adsorption effect.

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3. RESULTS

3.1. *Equilibrium study revealing a considerably large cooperativity*

The fluorescence of the labelled histone H1 was largely quenched upon binding to the histone H1-depleted polynucleosome. This allows us to assume complete quenching as a first approximation, since the experimental error was fairly large in the present system. This facilitated determination of the concentration of the free histone H1 (c_A) in sample solutions.

The fluorescence intensity (F) was measured as a function of the total concentration of histone H1 added (c_A°) at a constant concentration of the polynucleosome (c_p) (in unit of DNA base). An example of such titrations is shown in fig.1. The final straight line indicates saturation of available binding sites on the polynucleosome: the degree of saturation, θ, is unity ($\theta = 1$). The initial slope, being parallel to the final one, implies no binding ($\theta = 0$): this finding confirms the existence of cooperativity [2]. The degree of saturation is graphically determined as shown in the diagram. The final line was extrapolated to the abscissa, as shown in fig.1, where the stoichiometry of binding (n) was evaluated to be $n = 175 \pm 45$ bp per histone H1. This value corresponds well to the DNA length of a unit composed of one nucleosome and the linker DNA [1,2,9].

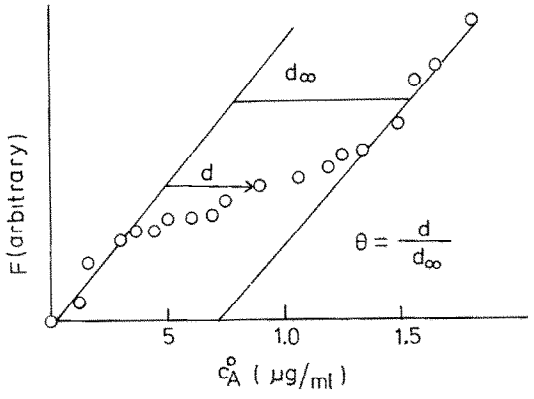

Fig.1. Fluorescence intensity F at constant polynucleosome concentration of $c_p = 4.1$ μg/ml *vs* total histone H1 concentration, c_A°, at 20°C, 0.2 M NaCl and pH 7.5. The degree of saturation θ is determined by d/d_∞. The stoichiometry n is determined from the intercept of the final line with the abscissa.

The standard large ligand binding model was introduced to describe the observed cooperative binding quantitatively [4,5], regarding the bead-string unit as the binding site of the polynucleosome. In the case of strong cooperativity, the following equation was derived in [5]:

$$(2\theta - 1)/\sqrt{\theta(1-\theta)} = \sqrt{q/n}\,(Kc_A - 1) \qquad (1)$$

for $q/n \geqslant 4$ and $\theta \sim 1/2$

where K and q refer to the cooperative binding constant and the cooperativity parameter, respectively.

According to eq.1, a straight line should be expected for strong cooperativity, when $(2\theta - 1)/\sqrt{\theta(1-\theta)}$ is plotted *vs* c_A. An example of this type of plot is shown in fig.2. The intercept on the axis of c_A is equal to $1/K$, yielding $K = 7.5 \pm 2.2 \times 10^7\ M^{-1}$ at 0.2 M NaCl, pH 7.5 and 20°C. On the other hand, the ordinate axis is intersected at $-\sqrt{q/n}$. With $n = 350$ (bases per H1) q was evaluated to be $q = 1.3 \pm 0.6 \times 10^4$.

In this way the relevant parameters of the model were evaluated at various salt concentrations. The binding constant (K) decreased by a factor of 3 as NaCl increased from 0.1 to 0.3 M. On the other hand, the cooperativity parameter (q) increased

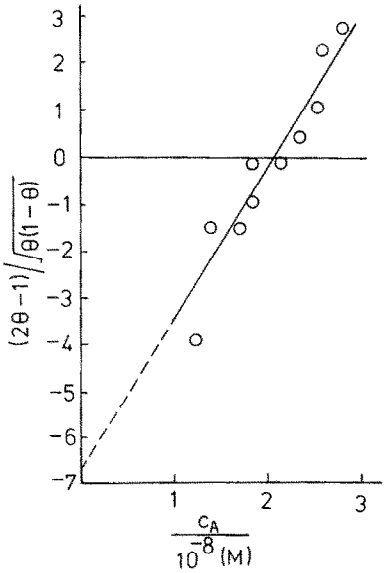

Fig.2. Data of fig.1 plotted according to eq.2. Evaluations of the binding constant K and cooperativity parameter q are made in this way (see text).

steeply (by a factor of 6), when NaCl was increased from 0.1 to 0.3 M. As a whole the results of equilibrium studies were quite similar to those of the protamine–calf thymus DNA system [10], except for its considerably strong cooperativity.

3.2. *Kinetic study showing a diffusion-controlled association process*

A sudden increase of salt concentration reduces K as stated in section 3.1 so that some of the bound histone H1 will dissociate from polynucleosomes. Consequently the fluorescence intensity (F) will increase transiently. Its time course was measured to study the kinetic aspect of the binding process.

To analyse this initial relaxation time (τ) the simplest kinetic model was introduced as in [10]. The two ends of the cluster of the bound species were considered as reaction sites. The following expression has been derived, assuming strong cooperativity.

$$1/\tau = k\sqrt{\theta(1-\theta)}c_p/\sqrt{qn} \quad (2)$$

where k is the association rate constant and θ and q refer to the solution before the perturbation.

In fig.3 $1/\tau$ is plotted *vs* c_p. The linear relationship between $1/\tau$ and c_p in eq.2 was justified. From its slope the association rate constant (k) was evaluated to be $k = 3.5 \pm 2.0 \times 10^7\ M^{-1}\cdot s^{-1}$ at 20°C, 0.35 M NaCl and pH 7.5 and K was less

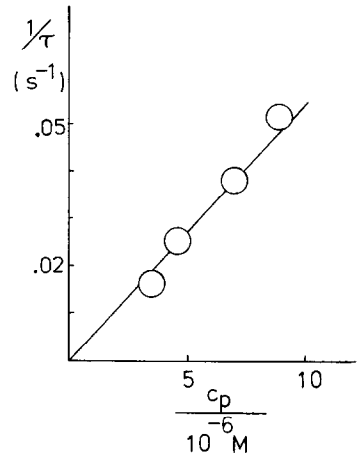

Fig.3. Reciprocal initial relaxation time $1/\tau$ plotted as a function of the polynucleosome concentration c_p. Salt concentration jump was performed from 0.30 to 0.35 M NaCl at 20°C and pH 7.5. The concentration of histone H1 was adjusted to give $\theta = 1/2$ at 0.30 M NaCl.

dependent on salt concentration. These results indicated that the association process is controlled by diffusion of the histone H1 molecule [11].

4. CONCLUDING REMARKS

The first question is whether we can apply the large ligand model [4,5] to the present system. The results obtained showed that:

(i) The measured stoichiometry n was in agreement with the DNA length of one nucleosome plus linker region [1,2,9];
(ii) The linear relationship of eq.1 was satisfied as shown in fig.2; and
(iii) The kinetic result was also satisfactorily described in terms of the model.

Therefore, it was concluded that the model is useful at least as a first approximation to describe the binding properties, when the bead-string unit is regarded as the binding site of polynucleosome.

Second, there are arguments about the fidelity of the reconstitution method, especially about the linker DNA length and the irregularity of its structure. Even if this is the case, the present kinetic approach offers new information about the properties of polynucleosomes in solution. The diffusion-controlled association process suggests that the condensation process following histone H1 binding does not need activation energy in practice. This result may be correlated with the finding in a similar system of chromatin that the interaction is essentially electrostatic [12].

Third, the present results show remarkable salt concentration dependences of the binding constant K and the cooperativity parameter q, although the evaluated parameters have a fairly large error due to unavoidable precipitation and adsorption of the complex around physiological salt concentration. These dependences are quite similar to those of the protamine–calf thymus DNA system [10]. According to the Finch-Klug model, the histone H1-polynucleosome can take a solenoid structure at sufficiently high salt concentration [1,2]. In this case interactions of histone H1-histone H1 and nucleosome-nucleosome between successive turns in the solenoid may be responsible for the strong cooperativity. This relationship should be interesting in connection with the question of the physical basis of the cooperativity in nonspecific DNA–protein interactions [10].

Moreover, it should be noted that the observed large cooperativity may be significant for an understanding of the regulation of the chromatin structure by histone H1 binding. The polynucleosome changes its state over a small region of change of the free histone H1 concentration around $c_A = 1/K$: the polynucleosome adopts the conformation of an extended coil for c_A below the region, while it takes a condensed structure for c_A above this region.

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