Interaction of the HMG1 protein with nucleic acids

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Binding constants have been measured for the interaction of the protein HMG1 with native DNA, denatured DNA and a number of polynucleotides at near-physiological ionic strengths, using gel filtration and thermal denaturation. The interaction of HMG1 with DNA is shown to be noncooperative and reversible. Nucleic acids form the following series in order of increasing binding constants: poly(U) ∽ poly(A) < poly(dA) < dsDNA ∽ poly(dA) · poly(dT) ∽ poly(dG) · poly(dC) ≪ poly[d(A–T)] ∽ ssDNA.

Nonhistone protein HMG1 *DNA binding* *Gel filtration* *DNA melting*

1. INTRODUCTION

High mobility group proteins, first described in [1], are a wide-spread class of chromosomal proteins. Little is known about the role of proteins HMG1 and 2 in the structure and functioning of chromatin. It is assumed that these proteins are in some way associated with the active state of chromatin [2–8] and influence the activity of certain nucleases [9–11].

The proteins HMG1 and 2 influence the stability of the DNA double helix, their effect depending on the ionic strength [12]. At near-physiological ionic strength, these proteins preferentially bind to ssDNA [9,12–14] and unwind the DNA double helix somewhat. It has been demonstrated by the kinetic formaldehyde method that the protein does not form locally denatured regions on DNA outside the DNA melting range [15]. We have studied the HMG1–DNA complex using thermal denaturation and gel filtration. The protein–DNA binding parameters have been measured. The affinity of the protein HMG1 to various polynucleotides has been determined.

Abbreviations: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; HMG1, high mobility group protein 1

2. MATERIALS AND METHODS

2.1. HMG1 protein, DNA and polynucleotides

We used HMG1 protein from calf thymus, isolated as in [16]. The purity of the sample, as assessed by electrophoresis [17,18] with the help of standard HMG proteins provided by Dr K. Grade (Zentralinstitut fur Molekularbiologie, Akademie der Wissenschaften der DDR), was at least 95%. The concentration of the protein was determined on the premise that $\epsilon_{280} = 0.82$ ml/mg [19].

Phage T7 DNA was kindly supplied by Dr L.V. Neumyvakin (Institute of Molecular Genetics, USSR Academy of Sciences). We also used chicken erythrocyte DNA, poly(U) (Reanal, Hungary), poly(dA)·poly(dT), poly(dC)·poly(dG) (Boehringer, Mannheim), poly(dA) and poly[d(A–T)] (Sigma, USA). Concentration was expressed in mol base pairs in all cases, including single-stranded polynucleotides. This allows direct comparison of physico-chemical constants. The polynucleotides were purified by gel filtration and, if necessary, fractionated to increase the molecular mass of the specimen before use.

Chicken erythrocyte DNA was denatured by heating (10 min) at 100°C, then quickly cooled. The HMG1–nucleic acid complex was prepared either by direct mixing or by dialysis of samples

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prepared at high ionic strength. Melting curves were essentially the same with both methods of preparation. The amount of HMG1 and nucleic acid in the complex was determined by light absorption at two wavelengths (usually 230 and 260 nm), using the value $\epsilon_{230}/\epsilon_{260}$, individually established for each instrument. Unlike other systems [20,21], complex formation between DNA or polynucleotide and HMG1 does not involve spectral effects, as we have ascertained.

2.2. DNA melting

Melting curves were obtained on Cary 219 and Perkin-Elmer 402 spectrophotometers equipped with thermostated cells. The temperature was raised automatically at a rate of 0.2°C/min and measured by means of a thermocouple.

2.3. Gel filtration

The HMG1–nucleic acid interaction parameters were determined by the 'pulse' method [22] on Sepharose 6B or CL-6B (Pharmacia) 600-μl columns with the help of a Milichrome liquid chromatograph (Special Electronics and Analytic Instruments Design Office, Novosibirsk). The column was saturated with protein solution, then the sample containing the nucleic acid or the nucleic acid–protein complex was applied, and the column was eluted with protein solution at a constant rate (usually 10 μl/min). We also used a modification of the 'frontal' technique [22], when a small amount of protein solution was applied to a column saturated with nucleic acid solution.

3. RESULTS

3.1. The entire protein is active in the binding to dsDNA

Fig.1A presents the elution profiles of free HMG1 protein, dsDNA from chicken erythrocytes, and protein eluted with a DNA solution of constant concentration.

Fig.1A,B shows that, under these conditions, practically the entire protein binds to native and denatured DNA. The complex is reversible, as gel filtration of a preformed HMG1–DNA complex leads to its dissociation (fig.1C,D).

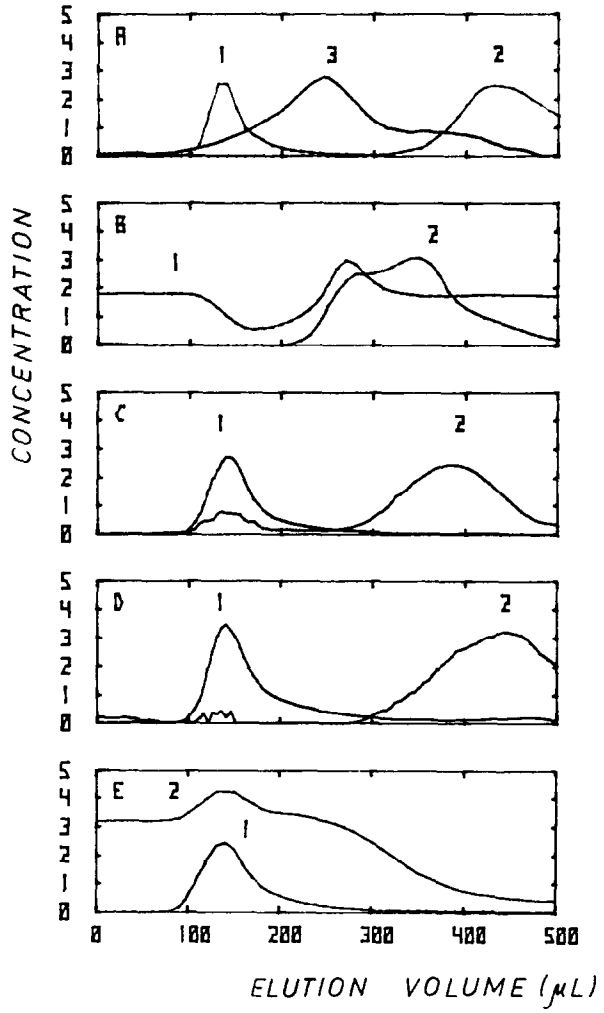

Fig.1. (A) Elution profiles for chicken erythrocyte native DNA (1), the protein HMG1 (2) and HMG1 eluted by a 30 μg/ml solution of native DNA (3). (B) Elution profiles for a solution of chicken erythrocyte denatured DNA after HMG1 solution was applied (1) and for HMG1 eluted by DNA solution (2). (C) Elution profiles for native DNA (1), and the HMG1 protein (2) after a preformed complex was applied. (D) The same for denatured DNA. (E) Elution profile for DNA (1) eluted by a constant-concentration HMG1 solution (2). Buffer: 10^{-3} M sodium phosphate, 10^{-4} M EDTA, 10^{-3} M dithiothreitol, 0.075 M NaCl (pH 7.6, T = 20°C). Graduations on the ordinate: A, (1), (2) arbitrary units, (3) 5×10^{-7} M; B, (1) 4×10^{-4} M, (2) 2×10^{-5} M; C, (1), 10^{-5} M, (2) 5×10^{-7} M; D, (1) 4×10^{-6} M, (2) 5×10^{-8} M; E, (1) 2×10^{-4} M, (2) 2.5×10^{-5} M.

3.2. *Determination of the binding constants for DNA and polynucleotides*

Fig.1E shows typical elution profiles obtained by the 'pulse' method [22]. The results of such assays for native and denatured DNA are presented in fig.2 in Scatchard coordinates: ν/L_f *vs* ν, where $\nu = L_{bound}^{HMG1}/C^{DNA}$, L_{bound}^{HMG1} is the concentration of bound HMG1, C^{DNA} is the concentration of DNA, assessed at the maximum of the DNA peak, and L_f is the concentration of free protein. The solid curves were computed according to the theory in [23] for the interaction of long ligands with homogeneous templates. The data obtained at 0.075 M NaCl (for dsDNA, fig.2) and at 0.15 M NaCl (not shown) suggest that the interaction of HMG1 with both native and denatured DNA is noncooperative. The best agreement of the experimental points and theoretical curves is achieved with the cooperativity factor $\omega = 1$. At 0.075 M NaCl the binding constant of HMG1 and ssDNA is too large to be evaluated in this way: the Scatchard plot is practically vertical. Thus, a rigorous thermodynamic determination of the binding parameters demonstrates that at near-physiological ionic strength the protein binding constant is higher for ssDNA than for dsDNA (see table 1).

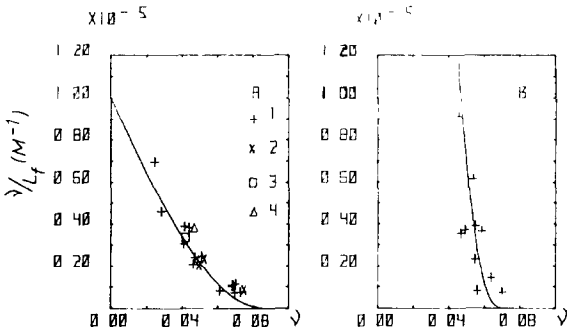

Fig.2. Scatchard plots for the binding of HMG1 to various polynucleotides. The solid curves were calculated according to the theory of [23]. (A) Chicken erythrocyte native DNA (1), phage T7 native DNA (2), poly(dG)·poly(dC) (3), poly(dA)·poly(dT) (4); 0.075 M NaCl; solid curve: $K = 10^5\ M^{-1}$, $n = 10$, $\omega = 1$. (B) Denatured DNA from chicken erythrocytes, 0.075 M NaCl; solid curve: $K = 10^6\ M^{-1}$, $n = 13$, $\omega = 1$.

The binding of HMG1 largely depends on the template. The results of the measurements are listed

Table 1

Binding parameters

Template	Extinction coefficient $\epsilon_{260} \times 10^{-3}$ ($M^{-1} \cdot cm^{-1}$)	[NaCl] (M)	Binding constant K (M^{-1}) at 20°C	Site size n (bp)	Cooperativity factor ω
Phage T7 native DNA	13.2	0.075	$(1 \pm 0.2) \times 10^5$	10 ± 2	
Chicken erythrocyte native DNA	13.2	0.075	$(1 \pm 0.2) \times 10^5$	10 ± 2	1
		0.15	$(1.5 \pm 0.3) \times 10^4$	12 ± 2	1
Poly(dA)·poly(dT)	12.3 [28]	0.075	10^5		
Poly d(A–T)	13.3 [29]	0.075	$>10^6$		
Poly(dG)·poly(dC)	14.0 [30]	0.075	10^5		
Chicken erythrocyte denatured DNA	15.44	0.075	$>10^6$	13 ± 2	1
		0.15	$(4 \pm 0.8) \times 10^4$	11 ± 2	1
Phage T7 ssDNA		0.075	10^9	20	1
Poly(dA)	18.9 [22]	0.075	$(1 \pm 0.3) \times 10^4$	12 ± 2	
Poly(A)	19.2 [30]	0.075	$<10^4$		
Poly(U)	18.86 [31]	0.075	$<10^4$		

in table 1. Where a complete binding isotherm was not obtained the binding constant was evaluated on the premise that $n = 12$ bp, $\omega = 1$, where n is the size of the binding site. Thus, according to the binding constant the polynucleotides form the following series: poly(U) ∽ poly(A) < poly(dA) < ds-DNA ∽ poly(dA)·poly(dT) ∽ poly(dG)·poly(dC) ≪ poly[d(A–T)] ∽ ssDNA.

3.3. Melting

The protein HMG1 stabilizes DNA against thermal denaturation at low ionic strengths and destabilizes it at near-physiological ionic strengths [12]. The melting curves are of a two-phase kind. There are two theoretical approaches to the melting of DNA in a complex with long ligands. In the theory of [24] the semigrand canonical partition function of the homopolymer-ligand system is calculated. The theory in [25] uses the fact that ligand binding effects the helix–coil transition independently of the template type, hence the melting curve of a real DNA can be introduced into the theory. The melting curves predicted by the two theories coincide with sufficient accuracy.

Both theories predict that the melting curves are two-phase in character when the binding constants for single-stranded (K_c) and double-stranded (K_h) DNA are significantly different. Besides, steps may arise as a result of a structural transition in the protein, aggregation or other events.

We have shown earlier that the protein HMG1 undergoes a structural transition in the melting range of protein-bound DNA [15]. A small light scattering, distorting the melting curves, is observed in the transition interval. This effect, however, is slight for the protein/DNA weight ratio $P/D < 1$ (as shown by the increase in extinction at $\lambda = 320$ nm). Henceforth no correction was made for light scattering. The reversible denaturation of the protein was allowed for in the following way. It was assumed that the protein followed the all-or-none melting transition and that only native protein could bind to DNA. After the model [25] was modified appropriately and the assumption that the entire protein was bound to DNA [25] was rejected, the constants K_h and K_c came to be replaced by $K_h f_0(T)$ and $K_c f_0(T)$, respectively, where $f_0(T)$ is the protein denaturation curve. Calculations according to the thus modified formulas [25] on the premise that $K_h = 0$, $\omega = 1$, led us to the following results: the first step in the DNA melting curve did not disappear but became flattened and shifted towards higher temperatures. Further allowance for the fact that $K_h \neq 0$ did not change the curves in any signficant way (fig.3). The coincidence of the experimental and theoretical curves is qualitative only. The value of n was chosen for the best fit of the first phase of the theoretical and ex-

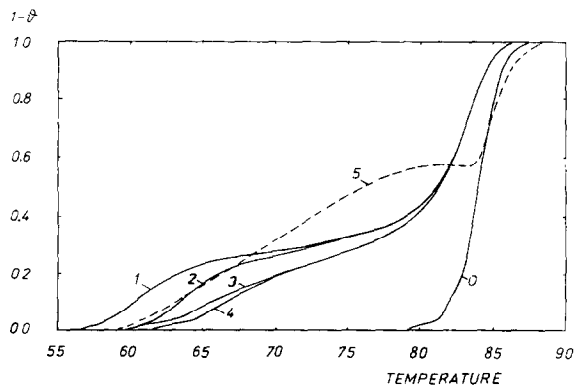

Fig.3. Theoretical (1–4) and experimental (0, 5) melting curves for the DNA–HMG1 complex. $K_c = 10^9\,M^{-1}$, the size of the binding site on melted DNA $n_c = 20$, $\omega = 1$. (0) DNA melting curve, $T_m^0 = 83.8°C$, (1) $K_h = 0$, (2) $K_h = 10^5\,M^{-1}$, the size of the binding site on native DNA, $n_h = 12$, the protein melting temperature $T_m^p = \infty$, (3) $K_h = 0$, $T_m^p = 54°C$, (4) $K_h = 10^5\,M^{-1}$, $n_h = 12$, $T_m^p = 54°C$, 10^{-3} M TEA–HCl, 10^{-3} M dithiothreitol, 10^{-4} M EDTA, 0.075 M NaCl.

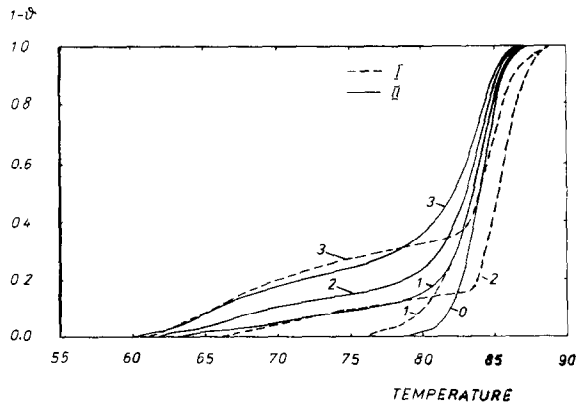

Fig.4. The melting curve for pure DNA (0). Experimental (I) and theoretical (II) melting curves for the DNA–HMG1 complex. $K_c = 2 \times 10^9\,M^{-1}$, $n_c = 20$, $K_h = 10^5\,M^{-1}$, $T_m^p = 54°C$, $\omega = 1$. (1) $P/D = 0.3$, (2) $P/D = 0.5$, (3) $P/D = 0.8$; 10^{-3} M TEA–HCl, 10^{-3} M dithiothreitol, 10^{-4} M EDTA, 0.075 M NaCl.

perimental curves for various P/D values. Some of the theoretical curves obtained are shown in fig.4.

Qualitatively similar results were obtained at 0.1 M NaCl.

4. DISCUSSION

We have furnished conclusive evidence to the effect that the HMG1 protein binds both to dsDNA and to ssDNA at moderate ionic strength. At 0.15 M NaCl the binding constant for denatured DNA from chicken erythrocytes is 3-times as large as that for dsDNA (see table 1). According to melting and gel filtration experiments, this ratio is much larger at 0.075 M NaCl. Thus, HMG1 belongs to the class of ssDNA binding proteins. It has been shown [9,26,27] that the treatment of HMG1–DNA complexes with a cross-linking agent gives rise to protein aggregates. This could be supposed to reflect the cooperativity of the protein–DNA interaction. We have, however, demonstrated the lack of cooperativity in the binding of HMG1 to either native or denatured DNA (see section 3).

We have found the binding of HMG1 to be different not only for ssDNA and dsDNA but also for various polynucleotides. This implies both primary and secondary structure specificity of HMG1 protein. We believe that this template specificity will occasion a search for the natural specific targets of HMG1 and 2. This, we hope, will provide a clue to the problem of their functions.

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