Biophysical Chemistry, 39 (1991) 145–152

BIOCHE 01534

Cooperative interaction of the C-terminal domain of histone H1 with DNA

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Received 5 June 1990
Revised manuscript received 27 August 1990
Accepted 29 August 1990

Histone H1; C-terminal domain; DNA binding; Binding cooperativity; DNA charge neutralization

We have studied the interaction of the isolated C-terminal domain of histone H1 with linear DNA using precipitation curves and electron microscopy. The C-terminal domain shows a salt-dependent transition towards cooperative binding, which reaches completion at 60 mM NaCl. At this salt concentration, the C-terminal domain binds to some of the DNA molecules, leaving the rest free. A binding site of 22 base-pairs can be calculated from the stoichiometry of the precipitated fractions. The C-terminal domain condenses the DNA in toroidal particles. The average inner radius of the particles is of the order of 195 Å. Consideration of the value of the inner radius of the toroids in the light of counterion condensation theory suggests that in these complexes the isolated C-terminal domain is capable of nearly full electrostatic neutralization of the DNA phosphate charge.

1. Introduction

Histone H1 is required for the formation of chromatin higher-order structures [1–6] and also interacts with nucleosomes stabilizing two turns of nucleosomal DNA [5,7]. Histone H1 contains three structural domains: a globular central region flanked by highly basic and hydrophilic C- and N-terminal tails [8,9]. Evidence has been accumulated supporting the view that the structural domains of H1 are involved in multifunctional roles in chromatin structure, as also seems to be the case for core histones. At low salt, nucleosomes are thought to be loosely associated with the globular H1 domains at the entry and exit points of the spacer DNA [10,11]. On the other hand, previous results from circular dichroism studies showed that the condensation of DNA by H1 at physiological salt is mainly due to the C-terminal domain [12,13]. It has also been shown that the higher-order folding of the nucleofilament does not require the involvement of the N-terminal domain [14]. It has been recently proposed that the C-terminal domain might be structured into α-helical segments when bound to the DNA [15].

The C-terminal domain possibly contributes to the bending of the spacer DNA in the 30 nm filament [15–17]. The study of the mode of binding of the C-terminal domain of H1 to DNA is thus likely to be relevant to the role of H1 in the folding of the nucleofilament. Several authors have described a salt-dependent transition from non-cooperative to cooperative binding of H1 to either linear or supercoiled DNA in the range 20–50 mM NaCl [18–21]. We have studied the interac-

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tion of the isolated C-terminal domain of H1 with linear DNA using precipitation curves and electron microscopy. The isolated C-terminal domain shows a salt-dependent transition towards cooperative binding, which reaches completion at 60 mM salt. At this salt concentration the C-terminal domain binds to some of the DNA molecules, leaving the rest free. The electron-microscopic results show that the isolated C-terminal domain condenses the DNA in toroidal particles. The consideration of the inner radii of the toroids in the light of counterion condensation theory [22,23] suggests, with possible implications for the role of H1 in chromatin higher-order structure, that most of the DNA phosphate charge might be neutralized in the complexes.

2. Materials and methods

2.1. Preparation of the C-terminal peptide

Histone H1 was prepared from calf thymus by the method of Johns and Butler [24]. It was further purified by carboxymethylcellulose chromatography as described previously [25]. The C-terminal peptide (residues 123–C-terminus) was obtained by cleavage at Lys-123 with thrombin, following the method of Chapman et al. [26].

2.2. Protein and DNA concentrations

For both soluble and precipitated complexes protein concentrations were determined by amino acid analysis of protein hydrolysates on a Durrum B500 amino acid analyzer. DNA concentrations were measured spectrophotometrically using $E^{1\%}_{260} = 20$.

2.3. DNA preparation

DNA from Sigma (type I) was further purified by chloroform/isoamyl alcohol extractions after digestion with proteinase K (0.1 mg enzyme/mg DNA) in the presence of 0.5% SDS. The DNA was sonicated at a concentration of 0.1 mg/ml in 2×10^{-3} M phosphate buffer (pH 7.0) for four bursts, whilst keeping the samples cold on ice. This reduces the molecular weight to approx. 7×10^5 as estimated by electrophoresis in 1% agarose gels.

A DNA fragment of 1631 bp was obtained by digestion of pBR322 with *Hin*f1 [27]. The fragment was recovered from 0.8% agarose gels by electroelution using an ISCO model 1750 concentrator.

DNA from chicken erythrocyte core particles was obtained as described [28].

2.4. Protein-DNA complexes

For the precipitation curves complexes were prepared by direct binding in 1 mM phosphate, 0.8 mM Na_2 EDTA (pH 7.4) and a variable amount of NaCl from 0 to 60 mM. The protein solution was added under continuous gentle stirring to the DNA solution. The final DNA concentration was 0.03 mg/ml. The complexes were incubated for 1 h at 20 °C and centrifuged for 25 min at $15\,000 \times g$. Supernatants and sediments were recovered and the concentration of both protein and DNA determined. The complexes for the experiments of electron microscopy were prepared by binding in either 1 mM phosphate, 0.8 mM Na_2EDTA (pH 7.4) or in the same buffer plus 0.14 M NaCl. The final DNA concentration was 0.01 mg/ml.

The (+/−) ratio of the complexes was defined as Lys/nucleotide for C-terminal domain complexes and Lys + Arg/nucleotide for H1 complexes. It was calculated on the basis of the analysis of the amino acid composition.

2.5. Electron microscopy

For visualization, samples were adsorbed on carbon-coated grids previously rendered hydrophilic by serum albumin treatment [29] and contrasted by either negative or positive staining with 0.5% uranyl acetate. For the magnification calibration, catalase crystals negatively stained from Taab Laboratories were used.

In some experiments the samples were fixed at 4 °C for 15 h by adjusting the solution to 0.1% formaldehyde.

3. Results

3.1. Precipitation curves

The complexes of the C-terminal domain of H1 and DNA were obtained by direct mixing in low concentration buffer and varying amounts of salt from 0 to 60 mM. DNA of 147 base-pairs from chromatin core particles was used to avoid aggregation, which was already significant with sonicated DNA of an average size of 0.7 kb. Fig. 1A shows the precipitation curves for the C-terminal domain at different salt concentrations. It can be seen that with independence of salt concentration, the totality of the DNA is precipitated at an r value of 0.7 (r, protein/DNA weight ratio). For the C-terminal domain of the H1 from calf thymus this r value is roughly equivalent to a +/− ratio of 1 as deduced from the amino acid analysis. However, different shapes of the precipitation curves are obtained depending on the salt concentration. In the absence of salt, no appreciable precipitation is observed below an r value of 0.4, whereas at 60 mM NaCl the DNA is gradually precipitated from the beginning of the titration. At 60 mM NaCl the amount of C-terminal peptide left in the supernatants was negligible all along the precipitation curve. This behaviour indicates that the DNA molecules are saturated in turn by the C-terminal peptide. The absence of protein in the

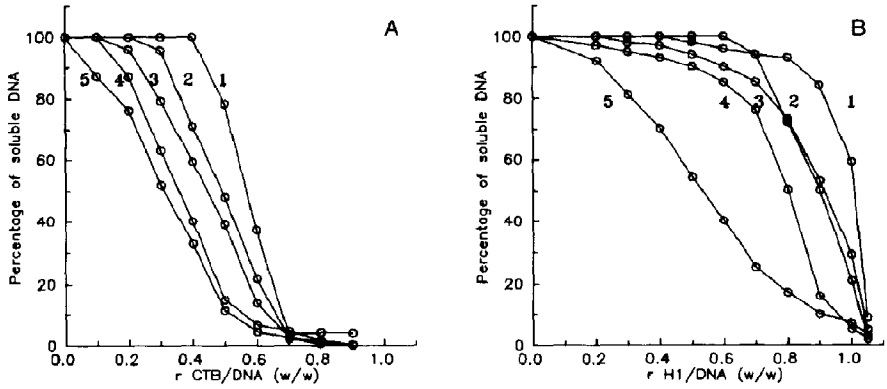

Fig. 1. Precipitation curves of mononucleosomal DNA by the isolated C-terminal domain of histone H1 (A) and whole H1 (B). Titrations were carried out in 1 mM phosphate, 0.8 mM Na_2EDTA (pH 7.4), plus a variable amount of salt: (1) 0; (2) 10; (3) 20; (4) 30 and (5) 60 mM NaCl. r: protein/DNA weight ratio.

Table 1

Stoichiometries of the soluble (S) and precipitated (P) fractions of the complexes of the isolated C-terminal domain of histone H1 with DNA of 147 bp

The complexes were formed in 1 mM phosphate, 0.8 mM Na_2EDTA (pH 7.4) and a variable amount of NaCl. r is the protein/DNA weight ratio. All the values of the stoichiometry are expressed as protein/DNA weight ratio. NP represents the absence of the corresponding fraction.

Input r	0 mM NaCl		10 mM NaCl		20 mM NaCl		30 mM NaCl		60 mM NaCl	
	S	P	S	P	S	P	S	P	S	P
0.2	0.20	NP	0.20	NP	0.18	0.69	0.17	0.70	0.04	0.73
0.3	0.30	NP	0.28	0.70	0.19	0.70	0.09	0.70	≈0	0.68
0.4	0.40	NP	0.28	0.68	0.20	0.68	≈0	0.68	≈0	0.65
0.5	0.49	0.60	0.26	0.72	0.19	0.70	≈0	0.70	≈0	0.70
0.6	0.54	0.70	0.28	0.69	0.18	0.69	≈0	0.69	≈0	0.68
0.7	0.63	0.70	0.29	0.73	0.21	0.70	≈0	0.70	≈0	0.69

supernatants from the 60 mM salt complexes was confirmed by melting after dialysis against 1.4 mM NaCl, 0.1 mM citrate (pH 7.0). A single transition corresponding to naked DNA was observed in all cases (data not shown). Similar results were obtained at salt concentrations higher than 60 mM NaCl and with higher molecular weight DNA. In all cases the supernatant fractions consisted of free DNA.

Table 1 lists the stoichiometries of the soluble and precipitated fractions obtained in 0, 10, 20, 30 and 60 mM NaCl, for complexes of different input r value. The stoichiometry of the precipitated fractions is always around 0.7 and independent of both salt concentration and input r value. As mentioned above, at 60 mM there is no protein in the supernatant. At 30 mM small amounts of protein are found at the lowest r values. Below 30 mM the amount of protein found in the supernatants increases significantly with decreasing salt.

The transition from non-cooperative to cooperative DNA binding of whole H1, widely described by others [18–21], can also be clearly observed by means of precipitation curves. The transition towards binding cooperativity is more abrupt than with the C-terminal domain, but again at 60 mM NaCl the precipitation of DNA is proportional to the amount of added protein (fig. 1B). The stoichiometry of the precipitated fractions is always about 1.0 at all salt concentrations.

Beyond an r value of 0.7 for the C-terminal peptide and 1.0 for whole H1, added protein is found as free protein in the supernatants. This property allows the calculation of the size of the DNA-binding sites for the C-terminal domain and whole H1 from the stoichiometries of the precipitated fractions. Values of 22 and 33 base-pairs are found for the C-terminal domain and H1, respectively.

3.2. Electron microscopy of complexes of the C-terminal domain of histone H1 with DNA at physiological salt concentrations

The complexes of the C-terminal domain of H1 and DNA were usually obtained by direct mixing in 140 mM NaCl, 1 mM phosphate buffer, 0.8 mM Na_2EDTA (pH 7.4). The complexes were occasionally prepared by stepwise dialysis with identical results. Sonicated DNA from calf thymus (average molecular weight 7×10^5) or a fragment of 1631 bp obtained by digestion of pBR322 with *Hin*fI was used in the preparation of the complexes. Similar results were obtained with both types of DNA.

The C-terminal-DNA complexes are seen in the

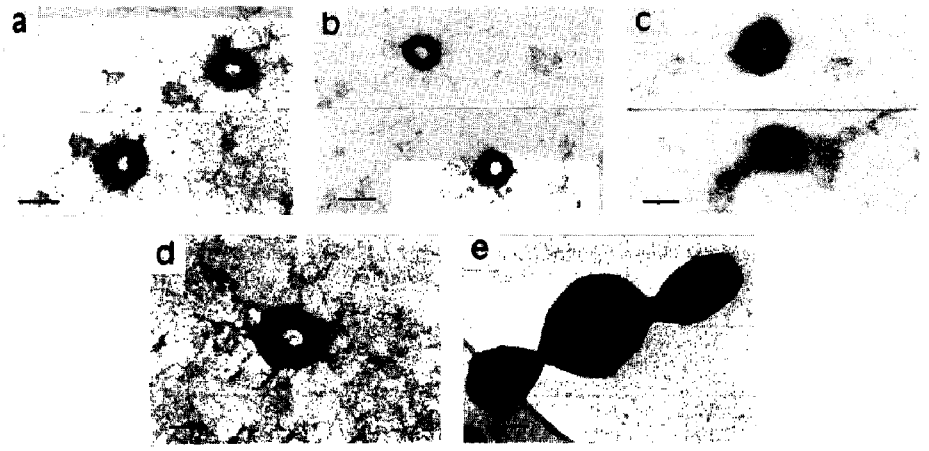

Fig. 2. Electron micrographs of complexes of the isolated C-terminal domain of histone H1 with DNA in 0.14 M NaCl, 1 mM phosphate, 0.8 mM Na_2EDTA (pH 7.4). The samples were contrasted with uranyl acetate. (a) $r = 0.1$, positive staining; (b) $r = 0.2$, positive staining; (c) $r = 0.4$, negative staining; (d) $r = 0.8$, positive staining; $r = 1.0$, negative staining. r: protein/DNA weight ratio. The bar represents 0.1 μm.

electron microscope in the form of annular rings, or toroids. Fig. 2 shows complexes at different r values stained with uranyl acetate. In negatively stained samples the particles show circular striations which support the continuous circumferential DNA winding of torus organization (fig. 2c) [30,31].

Toroids were observed at r values as low as 0.1 (equivalent to an input +/− ratio of 0.13). (fig. 2a) and as high as 1.0 (fig. 2e). The measurement of a set of 46 particles gave an average value of 196 ± 44 Å for the inner radius of curvature. The value of the inner radius of the particles is independent of r. In contrast, under standard conditions of preparation, the outer radius of the particles tends to become larger with increasing r. At $r = 1.0$, some particles are so thick that the hole in the middle is barely visible (fig. 2e). In these extreme cases toroids could be confused with spheres.

Experiments with fixed and unfixed samples have shown that fixation is not essential for specimen preservation as the morphology and the particle dimensions were the same in both types of samples.

3.3. Electron microscopy of complexes of the C-terminal domain of histone H1 with DNA at low salt

Complexes were also formed in 1 mM phosphate, 0.8 mM Na_2EDTA (pH 7.4). Under these ionic conditions, toroids were observed at $r = 0.8$ or higher, but not at lower ratios. Although toroids are already present at $r = 0.8$, at this ratio aggregates of uncertain morphology are predominant. At $r = 1.0$, toroids are already abundant (fig. 3). This r value is equivalent to a +/− ratio of 1.3. An excess of protein over the input for a +/− ratio of 1.0 is therefore necessary for proper toroid formation at low salt. The inner radius of curvature of the particles is similar to that observed at 0.15 M salt. A value of 195 ± 42 Å was obtained from the measurement of a set of 69 particles. At $r = 1.0$ the thickness of the particles is about 1/2 of their inner radius.

3.4. Electron microscopy of complexes of whole H1 with DNA

Other authors have previously shown that toroidal particles form when H1 is complexed

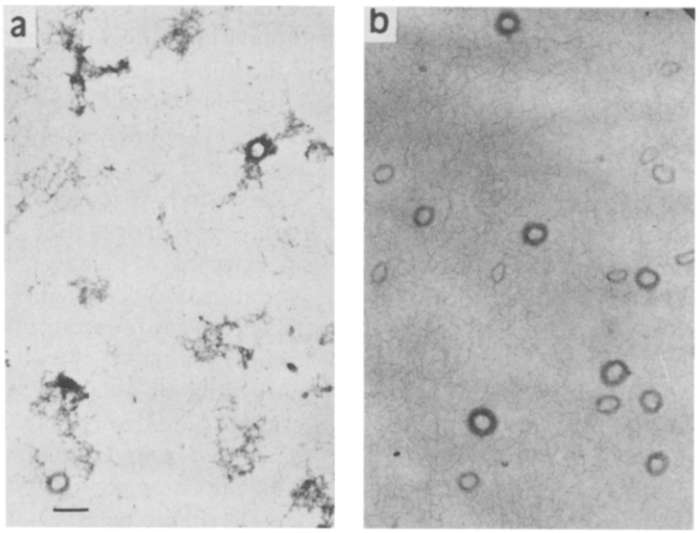

Fig. 3. Electron micrographs of complexes of the isolated C-terminal domain of histone H1 with DNA in 1 mM phosphate, 0.8 mM Na_2EDTA (pH 7.0). (a) $r = 0.8$; (b) $r = 1.0$. r: protein/DNA weight ratio. The bar represents 0.1 μm.

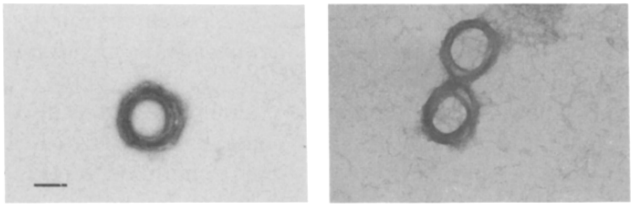

Fig. 4. Electron micrographs of complexes of histone H1 with DNA in 0.15 M NaCl, 1 mM phosphate, 0.8 mM Na_2EDTA (pH 7.4). The r value was 1.1. The bar represents 0.1 μm. r: protein/DNA weight ratio.

with DNA [32,33]. These experiments were performed for the purpose of comparison of particle dimensions under the same conditions used in the experiments with the C-terminal domain. Toroidal particles were readily observed with complexes formed at r values between 1.0 and 1.5. The inner radius of the particles was about 600 Å, i.e., 3–3.5-times larger than that obtained with the C-terminal peptide (fig. 4).

4. Discussion

Precipitation curves have been used to demonstrate salt-dependent binding cooperativity of the isolated C-terminal domain of histone H1 to linear double-stranded DNA. The transition towards cooperative binding occurs in the range 30–60 mM NaCl. At this salt concentration free DNA and fast-sedimenting fully saturated complexes co-exist. The protein/DNA ratio (by mass) of the insoluble fractions is always 0.7, which is equivalent to a +/− ratio of 1.0. When protein is added in excess over the amount necessary for a protein/DNA ratio of 0.7 it remains in solution as free protein. The transition towards cooperative binding is sharper for whole H1 and also occurs in the range 30–60 mM. Below this salt concentration the binding of H1 to linear DNA is non-cooperative. The maximum r value that can be achieved in the H1 complexes is 1.0.

The size of the DNA-binding sites for the C-terminal domain and whole H1 can be calculated from the stoichiometry of the precipitated fractions. Values of 22 and 33 base-pairs are found for the C-terminal domain and H1, respectively. The latter value coincides with that reported by Watanabe [19] using fluorescently labeled H1. It should be emphasized that these values are obtained from the stoichiometries of the insoluble fractions and are not therefore necessarily valid for the soluble complexes.

The isolated C-terminal domain of H1 condenses the DNA in toiroidal particles. Toroidal particles are observed at physiological salt concentrations and also at very low salt (10 mM). In the latter case, a small excess of C-terminal peptide over that necessary for an input +/− ratio of 1.0 is required for toroids to become the predominant structure, whereas at physiological salt toroids are observed r values far below charge saturation. The average inner radius of the particles is of the order of 195 Å at both low and physiological salt conditions.

It is currently accepted that toroidal DNA is wound circumferentially in a single direction with a minimum radius of curvature bounding the hole in the middle [29,31]. Manning [22] calculated that if the phosphate charge is neutralized, the DNA molecules spontaneously adopt a bent conformation with a radius of curvature of about 170 Å. Wilson and Bloomfield [34], using Manning's counterion condensation theory, calculated a critical value of 90% charge compensation for toroid formation and observed that saturation with Mg^{2+} (88% charge neutralization) does not induce toroids. Therefore, the observation of toroids with inner radii of about 195 Å suggests that the C-terminal peptide is capable of neutralizing most of the charge of its DNA-binding site.

In the context of counterion condensation theory, the stable bending of the DNA molecules in toroidal particles requires the neutralization of the phosphate charge along its entire length. The ob-

servation of toroids at protein/DNA ratios as low as 0.1 in physiological salt is therefore consistent with the results from the precipitation curves showing cooperative binding at 30 mM NaCl or higher. The fact that in the absence of salt toroids are only observed in the presence of an excess of ligand is also consistent with the results of the precipitation curves indicating that without salt the binding is not cooperative. Low Mg^{2+} concentrations (0.4 mM $MgCl_2$) were as efficient as 140 mM NaCl in inducing toroid formation at low protein/DNA ratios. This situation parallels that found in chromatin condensation (data not shown).

The inner radii of toroids of whole H1 are 3–3.5-times larger than those of the C-terminal domain. A possible explanation for the larger inner radius of H1 toroids is that the packing requirements of the globular domain and perhaps also of the N-terminal domain prevent the extensive charge neutralization achieved with the isolated C-terminal domain. The increased local rigidity of the DNA coil in H1 toroids would result in a larger radius of curvature. The quantitative difference in the capacity of phosphate neutralization probably also explains why the C-terminal domain forms toroids in the absence of salt and whole H1 does not.

It is not clear to what extent the C-terminal domain contributes to the cooperative binding of the whole molecule in chromatin. It has been suggested that the globular domains of H1 could interact with each other in condensed chromatin and that these interactions could explain the cooperative binding of H1 in chromatin [20]. In the case of the C-terminal domain, binding cooperativity is probably mediated by local changes of the DNA conformation in the vicinity of bound ligands.

It is firmly established that histone H1 is involved in the higher-order structure of chromatin. A possible binding area for the C-terminal domain is the spacer DNA. The capacity of the C-terminal domain of nearly full electrostatic neutralization of the DNA charge could have a profound effect on the conformation of the spacer. The minimum radius of curvature of about 150 Å obtained when the DNA phosphate charge is extensively neutralized in in vitro complexes is obviously too high for the requirements of the models of the higher-order structure incorporating supercoiled or looped spacers [16,35]. However, in the nucleosome core particle the DNA is bent with a radius of curvature of only 45 Å, on average. It has been suggested that this much lower radius of curvature is achieved through the asymmetrical neutralization of the phosphates along one side of the helical surface of the DNA molecule [36]. In addition, the DNA double helix does not follow a regular superhelical path around the histone octamer, but is bent sharply at several positions [37]. A highly basic cluster in the sequence of histone H4 is in close contact with one of the positions of sharp bending [38].

It cannot be concluded that our results necessarily imply that the spacer cannot bend at radii lower than 170 Å through interaction with the C-terminal domain. It should be considered that in vitro complexes differ from chromatin in several respects. In the first place, in chromatin the H1 stoichiometry is one molecule/nucleosome or less [39]. Furthermore, the position of the C-terminal domain relative to its binding area might be fixed by the interactions of the other domains of the molecule. In addition, the secondary structure of the DNA-bound domain might also play a role in combination with the two aforementioned factors in determining the distribution of the shielded phosphate charges. The constraints imposed by fixed stoichiometry and position, and also perhaps by the conformation of the DNA-bound domain could thus prevent the symmetrical neutralization of the DNA charge and favour the sharp bending of the spacer in condensed chromatin.

Acknowledgement

This work has been supported by Grant PB85-0096 from the Comisión Asesora de Investigación Científica y Técnica.

References

1 F. Thoma and T. Koller, Cell 12 (1977) 101.
2 J.T. Finch and A. Klug, Proc. Natl. Acad. Sci. U.S.A. 73 (1976) 1897.

3 K. Brasch, Exp. Cell Res. 101 (1976) 396.
4 M. Renz, P. Mehls and J. Hozier, Proc. Natl. Acad. Sci. U.S.A. 74 (1977) 1879.
5 F. Thoma, T. Koller and A. Klug, J. Cell Biol. 83 (1979) 403.
6 F. Thoma and T. Koller, J. Mol. Biol. 149 (1981) 709.
7 R.T. Simpson, Biochemistry 17 (1978) 5524.
8 E.M. Bradbury, P.D. Cary, G.E. Chapman, C. Crane-Robinson, S.E. Damby, H.W.E. Rattle, M. Boublik, J. Palau and F.X. Avilés, Eur. J. Biochem. 52 (1975) 605.
9 I. Isemberg, Annu. Rev. Biochem. 48 (1979) 159.
10 J. Allan, P.G. Hartman, C. Crane-Robinson and F.X. Avilés, Nature 288 (1980) 675.
11 F. Thoma, R. Losa and T. Koller, J. Mol. Biol. 167 (1983) 619.
12 F. Morán, F. Montero, F. Azorín and P. Suau, Biophys. Chem. 22 (1985) 125.
13 F. Morán, A.T. Rodríguez, P. Suau and F. Montero, Biophys. Chem. 33 (1989) 133.
14 J. Allan, T. Mitchell, N. Harborne, L. Bohm and C. Crane-Robinson, J. Mol. Biol. 187 (1986) 591.
15 D.J. Clark, C.S. Hill, S.R. Martin and J.O. Thomas, EMBO J. 7 (1988) 69.
16 P.J.G. Butler, EMBO J. 3 (1984) 2599.
17 J. Widom, J.T. Finch and J.O. Thomas, EMBO J. 4 (1985) 3189.
18 M. Renz and L.A. Day, Biochemistry 15 (1976) 3220.
19 F. Watanabe, Nucleic Acids Res. 14 (1986) 3573.
20 D.J. Clark and J.O. Thomas, J. Mol. Biol. 187 (1986) 569.
21 D.J. Clark and J.O. Thomas, Eur. J. Biochem. 178 (1989) 225.
22 G.S. Manning, Q. Rev. Biophys. 11 (1978) 178.
23 G.S. Manning, Biopolymers 18 (1979) 2929.
24 E.W. Johns and J.A.V. Butler, Biochem. J. 82 (1962) 15.
25 L. Franco, F. Montero and J.J. Rodríguez-Molina, FEBS Lett. 78 (1977) 317.
26 F.E. Chapman, P.G. Hartman and E.M. Bradbury, Eur. J. Biochem. 61 (1976) 69.
27 J.C. Sutcliffe, Nucleic Acids Res. 5 (1978) 2721.
28 H. Weintraub, K. Patter and F. Van Lente, Cell 6 (1975) 85.
29 M. Bustin, D. Goldblatt and R. Sperling, Cell 7 (1976) 297.
30 K.A. Marx and T.C. Reynolds, Proc. Natl. Acad. Sci. U.S.A. 79 (1981) 6484.
31 K.A. Marx and G.C. Ruben, Nucleic Acids Res. 22 (1983) 1839.
32 R.D. Cole, G.M. Lawson and M.W. Hsiang, Cold Spring Harbour Symp. Quant. Biol. 42 (1977) 253.
33 D.E. Olins and A.L. Olins, J. Mol. Biol. 57 (1971) 437.
34 R.W. Wilson and V.A. Bloomfield, Biochemistry 18 (1979) 2192.
35 J.D. McGhee, J.M. Nickol, G. Felsenfeld and D.C. Rau, Cell 33 (1983) 831.
36 A.D. Mirzabekov and A. Rich, Proc. Natl. Acad. Sci. U.S.A. 76 (1979) 1118.
37 T.J. Richmond, J.T. Finch, B. Rushton, D. Rhodes and A. Klug, Nature 311 (1984) 532.
38 K. Ebralidse, S.A. Grachev and A.D. Mirzabekov, Nature 331 (1988) 365.
39 D.L. Bates and J.O. Thomas, Nucleic Acids Res. 9 (1981) 5883.