DNP DOUBLE FIBERS INDUCED BY DNA–H1 HISTONE INTERACTION

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1. Introduction

The chromatin of eukaryotes is organized in a linear array of structural subunits called nucleosomes consisting of DNA and the 4 histone classes H2A, H2B, H3 and H4 [1–4]. The role of the fifth class of histones, the very lysine-rich H1 histones, and their structural relationship to this fundamental unit of chromatin still remains fairly unclear, although implication of a higher order of structural organization is supposed [5–7]. Many efforts have been made in the past to reconstitute chromatin from its components. Reconstitution experiments with DNA and the 4 core histones [1,6,8–10] as well as with H3 and H4 [10,11] have indicated the formation of nucleosomes or smaller nucleosome-like particles. No nucleosome-like particles were found in complexes of DNA with histones H2A and H2B and with H4 or in DNA–H1 complexes [12,13]. The structures observed for DNA–H1 complexes are different depending on the environmental conditions and on the method employed for complex formation [12–15]. We show here that the structural behavior of the DNA–H1 complexes formed depends on ionic strength and leads to the formation of a new type of basic DNP structures consisting of twisted double fibers of the DNA under distinct salt conditions.

2. Experimental

DNA from calf thymus and T4 phage, isolated as in [16], had mol. wt 7×10^6 and 110×10^6, respectively, and $\epsilon(P) = 6600\ M^{-1}cm^{-1}$ and $\epsilon(P) = 6500\ M^{-1}cm^{-1}$, respectively. Histone H1 was isolated and purified from calf thymus as in [17,18]. DNA–histone complexes were formed by a modified stepwise gradient dialysis as in [19]. The procedure involved mixing of the DNA solution at ~35 μg DNA/ml and H1 at 2 M NaCl (pH 7.0) at an input ratio H1/DNA of 0.3–1.2/1.0 (w/w) as given in the text; the salt concentration was then decreased by dialysis to 0.02 M Na^+ (as ~0.1 SSC buffer) or to 0.04 M and 0.06 M Na^+ as desired. Estimation of H1 histone [20] bound to DNA at an input ratio of 0.9 in 0.02 M Na^+ resulted in a real ratio of 0.8.

For electron microscopy, samples were diluted with the same buffer used for complex formation to 0.5–5.0 μg DNA/ml final conc. One drop of the solution containing the DNA–H1 complex was put on a formvar/carbon-coated grid positively charged in a pentylamine atmosphere [21]; after 1 min the drop was washed off by a 1% wet solution of uranyl acetate, air-dried and rotary-shadowed with platinum at 9°C. In some preliminary experiments the slow mixing method in [13] was applied, but in a solution of 0.1 SSC buffer (pH 7.0) with an input ratio H1/DNA of 0.9. The DNA in these experiments was 5 μg/ml final conc. in 0.3–0.4 ml. CD measurements were recorded in a CD attachment of a Cary 60 spectropolarimeter. CD data are presented in terms of the CD per mole of DNA phosphate $\Delta\epsilon = \epsilon_L - \epsilon_R$; ϵ_L and ϵ_R are extinction coefficients for left and right circularly-polarized light, respectively.

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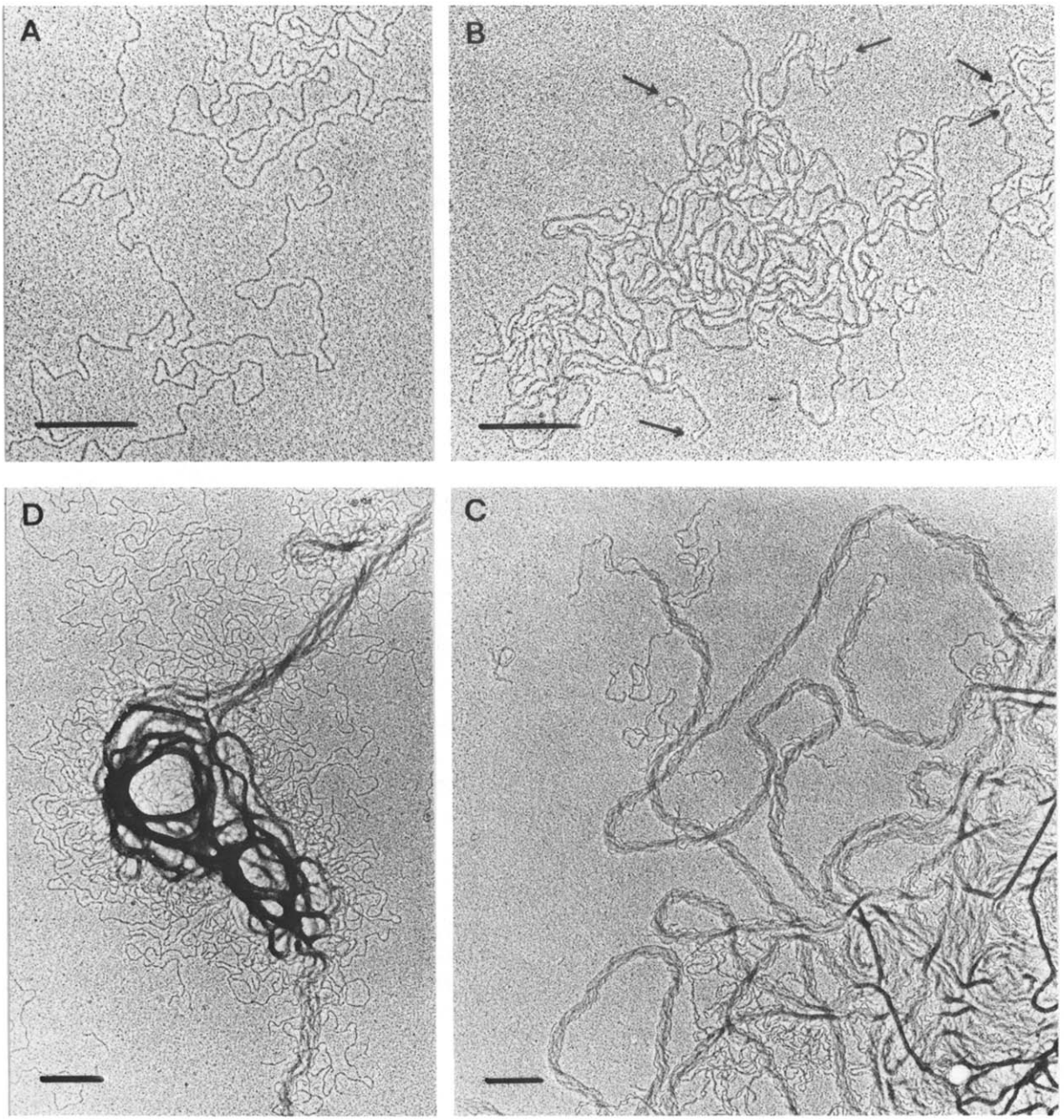

Fig.1. Electron micrographs of DNA–H1 histone complexes under different conditions. (A) Control thymus DNA with mol. wt 7×10^6 at 0.02 M Na^+, pH 7.0. (B) DNA–H1 complex at 0.02 M Na^+ (pH 7.0) with an input ratio H1/DNA of 0.9; arrows indicate looplike backfold regions of double helical molecules of DNA. (C) DNA–H1 complex under the conditions of (B) but at an input ratio of 1.05; the preferential formation of loose cables and dense stemlike structures consisting of twisted double fibers are clearly visible. (D) DNA–H1 complex with an input ratio of 0.9 at 0.06 M Na^+ (pH 7.0); no double fibers are visible; the dense stemlike structures exhibit the same diameter as in (C). The bars indicate 200 nm.

3. Results

In the control preparations of calf thymus DNA and T4 phage DNA exhibiting an equal distribution of the material over the whole grid, free DNA is always visible as single fibers (fig.1A). Samples of the DNA-H1 complex at an input ratio of 0.3 are not different from the controls. DNA–H1 complexes at an input ratio of 0.6 vary to some extent; ~70% of the material appears similar as in the controls while ~30% looks like connected fibers at an input ratio of 0.9. The DNA–H1 complexes at an input ratio of 0.9 in 0.02 M Na^+ (pH 7.0) preferentially contain double fibers of DNP in the whole material (fig.1B). As indicated by arrows, loops of backfolded double helical DNA are very often visible. These loops demonstrate that most probably the H1-induced formation of double fibers is basically an intramolecular process resulting in the backfolding of the DNA molecule. Several double fibers adhere together or form loosely-arranged complexes. Sometimes, but particularly at higher input ratios (1.05 and 1.2) and higher concentrations of the DNA–H1 complex (5 μg DNA/ml) electron microscopic results clearly indicate the presence of double fibers twisted together thus forming higher-ordered loose cables (fig.1C). The loosely-twisted cables show continuous transitions into densely-compact structures (stemlike structures) of ~100 Å diam. when stained with uranyl acetate only. They are preferentially composed of 4–6 and sometimes 7 double fibers. DNA–H1 complexes with T4 phage DNA show the same structures as with calf thymus DNA. When the H1/DNA input ratio is increased, the presence of cables and stemlike structures predominate (fig.1C). The whole material adhering to the grid is arranged in such complexes while well-separated double fibers are no longer equally distributed but appear rather seldom. Input ratios of 1.3 and higher resulted in precipitation of the material during the dialysis and have therefore not been further studied. Naked DNA in 0.04–0.06 M Na^+ has the same appearance as at 0.02 M Na^+. DNA–H1 complexes in 0.04 M and 0.06 M Na^+ at input ratios of 0.6 and lower do not differ from the controls. At 0.9 input ratio DNA–H1 complexes in 0.04 M and 0.06 M Na^+ preferentially show more or less large aggregates of stemlike structures surrounded by single fibers of DNP. The stemlike structures consist of 10–14 single fibers of DNP and have the same diameter as at 0.02 M Na^+. Double fibers prevailing at 0.02 M Na^+ were very rare at 0.04 M Na^+ and never seen at 0.06 M Na^+ (fig.1D). Donuts were sometimes present independent of the sodium concentration, but occured very seldom in all specimens.

Using the slowly mixing method in [13] our preliminary results suggest that at 0.02 M Na^+ and an input ratio of 0.9, formation of double fibers of DNP occurs, though less frequently compared to the method of gradient dialysis.

The binding of H1 histone to DNA causes pronounced changes in the CD of DNA. Two different types of DNA–H1 complexes are formed in the salt gradient dialysis depending on the final [Na^+]. At 0.02 M Na^+ a monotonous decrease of the positive band occurs in the CD spectrum of the DNA up to an input ratio of 0.9 without any change to a ψ-like spectrum. In contrast to that, at 0.1 M Na^+ ψ-DNA is formed as indicated by the non-conservative CD spectrum (fig.2A). Increasing the salt concentration induces a gradual change of the first non-ψ-type DNP complex to the second type known as ψ-like DNA (fig.2B). The full results on CD measurements will be given elsewhere.

4. Discussion

Using calf thymus DNA and T4 phage DNA our

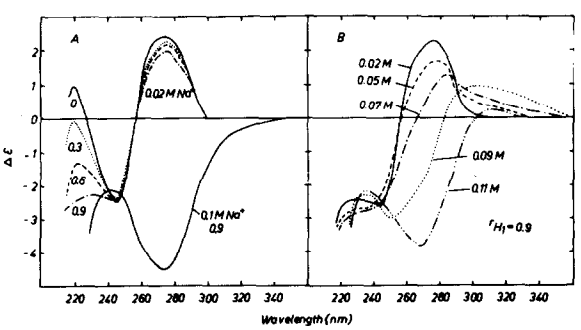

Fig.2. CD spectra of DNA–H1 complexes. (A) DNA–H1 complex formed by salt gradient dialysis to final conc. 0.02 M Na^+ (pH 7.0) input ratios H1/DNA (w/w) 0, 0.3, 0.6 and 0.9, and of 0.1 M Na^+ at 0.9 input ratio. (B) Variation of CD spectrum on addition of NaCl to the concentrations indicated at an input ratio H1/DNA (w/w) of 0.9 (r_{H_1}).

present results demonstrate the formation of characteristic DNP double fibers in soluble DNA-H1 histone complexes with high frequency and reproducibility. The formation of this type of DNA-H1 complexes, not previously described [12–15], is salt dependent and occurs at distinct [Na^+]values. Donuts, stemlike structures and irregularly arranged networks of DNP fibers have been observed [12] while in [13] less reproducible complex formation at lower input ratios and reproducible results at higher input ratios of 1.3 which exclusively showed donuts were observed. The stemlike structures found in our preparations correspond to those in [12] while the twisted double fibers reported herein represent a novel feature for a higher-ordered structure. The structure of DNA complexes containing several polypeptides [22,23] differ in a similar manner since only different types of donuts have been found which are rarely observable in our material. The most striking findings are backfolded structures present in the DNP double fibers. This suggests a primarily intramolecular action of H1 histone on the DNA in the presence of 0.02 M Na^+. A similar intramolecular effect and ionic-dependent complex formation was reported in [15]; however, no double fibers have been observed. This may be due to the different ionic and salt conditions and also the short DNA fragments used in their work. Since rather high H1 concentrations are required that facilitate the formation of double fibers, neutralization of negative charges along the DNA grooves by H1 is involved and is an important factor in maintaining the higher-ordered structures observed. The effect of histone H1 on DNA has been shown [24] to be non-cooperative at NaCl <20 mM, i.e., all the DNA molecules are bound by H1, while at higher [NaCl] the binding becomes cooperative. The most efficient binding of H1 to DNA observed at 20 mM NaCl and at an input ratio H1/DNA of ~1.0 is comparable to our conditions which govern the strongest DNA–H1 interaction associated with the formation of double fibers.

The binding of H1 to DNA results also in a non-conservative, so-called ψ-type CD spectrum preferentially observed at 0.1 M Na^+ or at higher salt concentrations (reviewed [25]). The DNP complex at 0.02 M Na^+ characterized by a normal CD spectrum with reduced positive CD maximum is related to the presence of cables or stemlike structures seen in the electron micrographs. The depression of the CD spectrum of the DNP double fibers (fig.2A) might be attributed to a conformational change due to an increased helical winding and could reflect a higher-ordered structure of twisted DNP double fibers.

Although the large groove of T4 phage DNA is occupied to some extent by glucose residues [26], this does not prevent the binding of H1 and the formation of double fibers, though some differences were observed in the binding behavior as measured by CD [27]. The present findings might be important with respect to the function of H1 histone in chromatin which is as yet not fully understood [5–7,28–33] and suggest that a discrete higher-ordered structure consisting of twisted double fibers and twisted single fibers is induced by H1 under certain conditions.

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