H1 histone, polylysine and spermine facilitate nucleosome assembly in vitro

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Nucleosome formation has been studied in a system containing relaxed Col E1 DNA, core histones and an extract of *Drosophila* embryos. The formation of nucleosomes was established by the introduction of supercoils into DNA. The degree of DNA supercoiling was shown to be higher if nucleosomes were assembled in the presence of the H1 histone, polylysine (M_r 20000) or spermine. These agents do not stimulate relaxation and are the more effective the earlier they are added to the reaction. Thus, the H1 histone, polylysine and spermine facilitate nucleosome assembly in vitro.

Nucleosome assembly *Assembly factor* *H1 histone* *Polylysine* *Spermine*

1. INTRODUCTION

The formation of nucleosomes under physiological conditions in vitro, and possibly in vivo, is considerably facilitated in the presence of 'assembly factors': nucleoplasmin [1–3], some RNAs [4] and topoisomerase I [4,5]. In view of the diversity of intracellular conditions, one cannot rule out the existence of other assembly factors operating in vitro and possibly involved in nucleosome assembly in vivo. The idea of the present study was to look for such agents. The transition of relaxed DNA into the superhelical form upon deproteinization served as a criterion of nucleosome assembly. The appearance of supercoils in DNA is the result and a reliable criterion of nucleosome assembly [1,4–6], especially since the eukaryotic topoisomerases are incapable of supercoiling DNA.

We demonstrate that the H1 histone, spermine and polylysine added during nucleosome formation cause a considerable increase in the superhelicity of DNA after deproteinization, hence these agents facilitate nucleosome assembly in vitro.

2. MATERIALS AND METHODS

2.1. *Histone, DNA and extract preparations*

H1 histones of 85–98% purity were obtained from *Drosophila* and mouse cell cultures [7]. The mixture of core histones from *Drosophila* embryos and Ehrlich carcinoma was prepared from the total histone preparations from which the H1 histone was removed with $HClO_4$.

Relaxed Col E1 DNA was isolated as in [8].

The extract of *Drosophila* embryos was obtained from 0–12-h-old embryos of *Oregon R* flies [9].

2.2. *Nucleosome assembly assay*

Nucleosome formation was carried out for 10–180 min at 25–27°C in 40 μl samples containing 0.185 M NaCl, 9 mM sodium phosphate (pH 6.0), 20 mM potassium phosphate (pH 7.4), 5 mM EDTA, 15–25% glycerol, 0.2 μg of relaxed Col E1 DNA, 0.1–3.2 μg of the core histones mixture and 50 μg of *Drosophila* embryo extract protein. The reaction was stopped by adding a mixture of sodium paraaminosalicylate, sarcosyl and sodium dodecyl sulphate (SDS) up to 3, 0.4 and 5%, respectively, then the samples were heated for

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1 min at 65°C. We added Ficoll to a concentration of 3%, 3 mM EDTA and bromphenol blue, then the DNA was analysed by electrophoresis in 1% agarose.

3. RESULTS AND DISCUSSION

3.1. *Effect of the H1 histone on the degree of DNA supercoiling upon nucleosome formation*

The incubation of relaxed DNA with core histones and the *Drosophila* embryo extract under conditions similar to [6] led to a transition of the relaxed Col E1 DNA to the supercoiled form after deproteinization, indicating the formation of nucleosomes. The increase in DNA superhelicity was insignificant in the absence of histones and lacking entirely without the extract. Nucleosome formation was observed only if the nucleosome core histones were added after the DNA had been mixed with the extract, as in [6]. Even a 16-fold excess of histones over DNA did not inhibit nucleosome assembly (not shown).

Preincubation of relaxed Col E1 DNA with the H1 histone for 1 or 10 min at 25–27°C with the subsequent addition of the extract and core histones or a simultaneous addition of all 5 histones considerably increased the superhelicity of DNA isolated after deproteinization, compared with the reaction in the absence of the H1 histone (fig.1a). In different assays with the same amount of DNA (0.2 μg), the amount of core histones varying from 0.1 to 0.8 μg and the incubation time from 10 min to 3 h, the stimulating effect of the H1 histone manifested itself in the presence of 0.1–0.4 μg of this protein, and the maximum stimulation was observed with 0.2–1.6 μg of H1. In conformity with other data [10,11], the H1 histone had no effect on DNA superhelicity in the absence of core histones (not shown).

The ability of the *Drosophila* and mouse H1 histones to increase the superhelicity of DNA on nucleosome formation was the same with homologous or heterologous core histones.

Polylysine with molecular mass of 20 kDa proved an even more active stimulant than the H1 histone (fig.1b). Like the H1 histone, polylysine did not introduce supercoils into DNA in the absence of core histones. Lysozyme and RNase added to the nucleosome assembly system did not

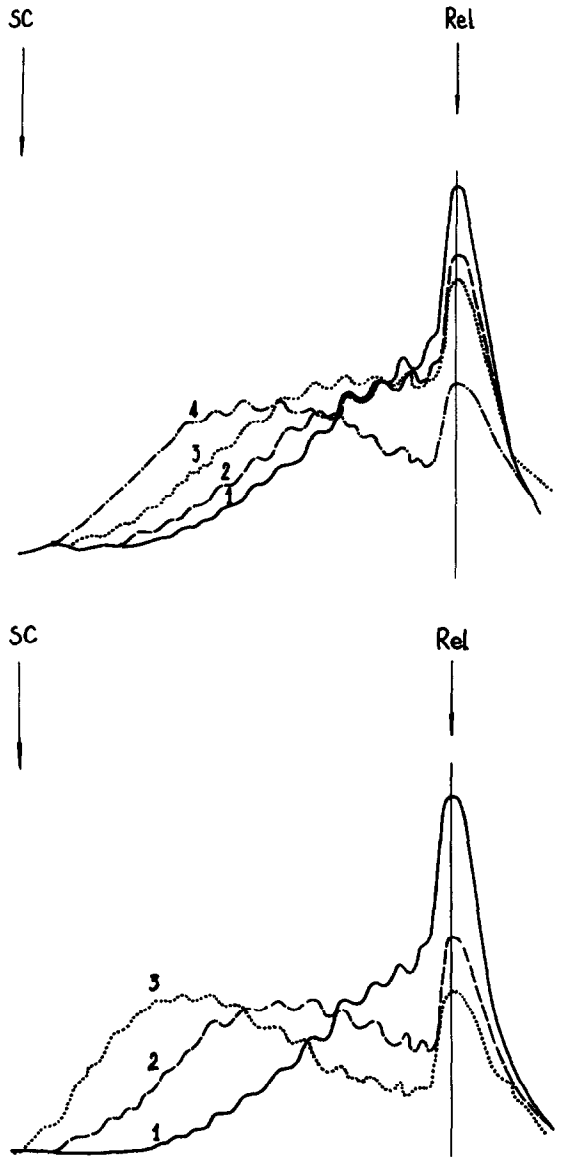

Fig.1. Change in DNA superhelicity on nucleosome assembly. 0.72 μg of *Drosophila* core histones was added along with: (a) 0, 0.2, 0.4 and 0.8 μg of mouse H1 histone in no.1,2,3 and 4, respectively; (b) 0, 0.2 and 0.4 μg of polylysine in no.1,2 and 3, respectively (10 min incubation).

increase the degree of DNA superhelicity, i.e., the effects of polylysine and the H1 histone are relatively specific.

The increased supercoiling of DNA during nucleosome formation in the presence of the H1

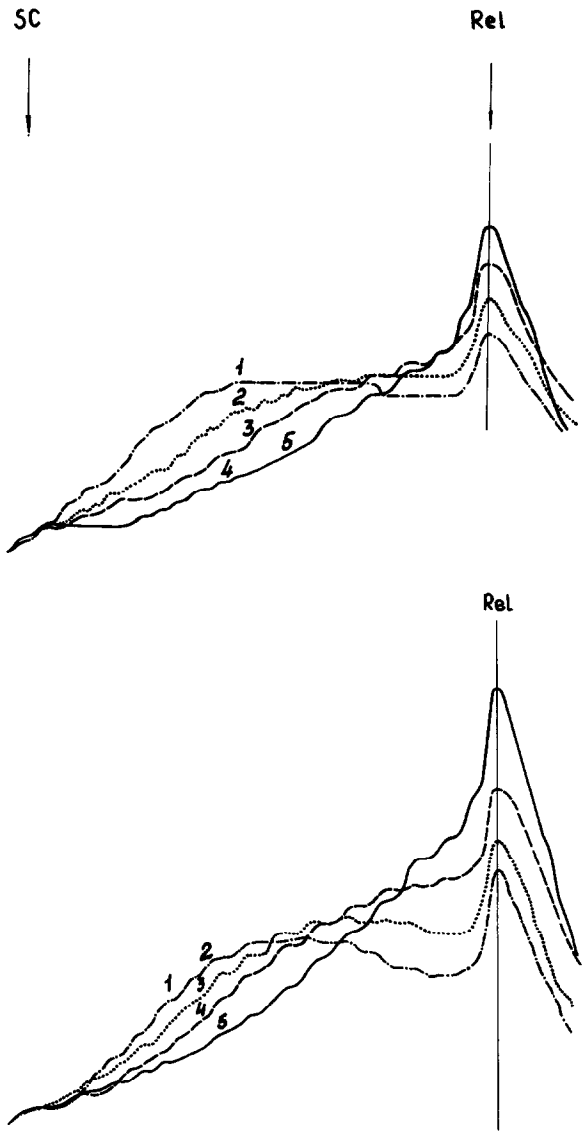

Fig.2. Change in DNA superhelicity on nucleosome assembly in the presence of 0.72 μg of *Drosophila* core histones: (a) with 0.8 μg mouse H1 histone added at different times during incubations; (b) the same as (a) but with 0.4 μg polylysine. The polypeptide was added simultaneously with the core histones (1), 2 min later (2), 5 min later (3), 8 min later (4), no H1 or polylysine (5). All samples were incubated for 10 min from the moment the core histones were added.

histone or polylysine may be the result of the nucleosome assembly stimulation by these polypeptides or it might be due to other causes, e.g., activation of the topoisomerase which relaxes internucleosomal DNA.

Under standard conditions (see section 2.2), the relaxation of supercoiled DNA occurred very quickly and the effect of the H1 histone or polylysine was not noticeable. When the reaction was slowed down by lowering the temperature or by reducing the amount of extract, it turned out that the H1 histone and polylysine did not stimulate but actually inhibited the relaxation as in [10,12]. Consequently, the additional supercoiling of DNA during nucleosome formation in the presence of these polypeptides is not the result of an activation by them of topoisomerase.

3.2. *Effect of the time of the H1 histone or polylysine addition on the DNA supercoiling during nucleosome formation*

The H1 histone has been claimed to increase DNA superhelicity in reconstructed minichromosomes in vitro in the presence of polyglutamic acid [11]. To see whether H1 should be present during the actual assembly of nucleosomes or might be added after the minichromosomes have been formed and produce the same result, we decided to find out how DNA superhelicity during nucleosome formation depended upon the time at which the H1 histone was added to the reaction mixture. It turned out that the degree of DNA supercoiling is higher, the earlier H1 is added to the nucleosome assembly system (fig.2a). Since the H1–DNA interaction is very rapid [13] and the relaxing activity of topoisomerase in nucleosome formation system is very high under standard conditions (see above), it seems probable that the H1 histone exercises its effect mainly at the time of nucleosome assembly. The binding of the histone might cause some additional coiling of the DNA on nucleosomes, but the contribution of this phenomenon to the observed increase in superhelicity cannot be very important. Similar results regarding the time dependence of the polypeptide's effect were obtained for polylysine (fig.2b).

Thus, according to our data, the H1 histone and polylysine: 1, do not activate but partially inhibit the relaxation of internucleosome DNA; 2, are needed during nucleosome assembly; 3, do not change DNA topology if added to already-formed minichromosomes. These facts suggest that the H1 histone and polylysine increase DNA superhelicity

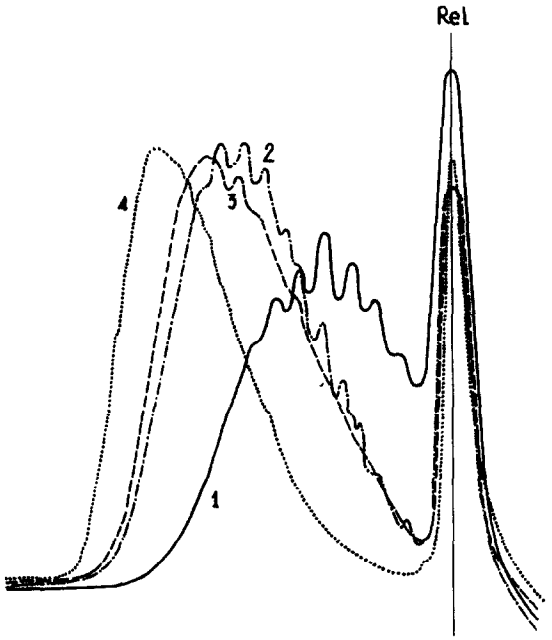

Fig.3. Nucleosome assembly in the presence of 0.72 μg of *Drosophila* core histones with no.2,3,4: 0.8 μg mouse H1 histone, 0.4 μg polylysine, 3.5×10^{-4} M spermine, respectively, added simultaneously with the core histones; no.1, no agent added (20 min incubation).

by accelerating or facilitating the process of nucleosome assembly.

3.3. *Effect of spermine on DNA supercoiling during nucleosome formation*

One can suggest a number of mechanisms to account for the stimulation of nucleosome assembly by the H1 histone and polylysine.

The H1 histone may accelerate chromatin assembly owing to its ability to align and stabilize the mutual disposition of nucleosomes [14]. This, however, seems a remote possibility for polylysine.

We also studied nucleosome formation in the presence of spermine, which is known to condense and aggregate DNA and form complexes with it that are morphologically similar to the DNA–H1 and DNA–polylysine complexes [15–17].

We found spermine to increase DNA superhelicity during nucleosome assembly (fig.3) even at a spermine concentration of 3.5×10^{-5} M, and to a far greater extent at 3.5×10^{-4} M. Like the H1 histone and polylysine, spermine partially inhibited the relaxation of supercoiled DNA in the presence of extract and was needed during the very process of nucleosome assembly (not shown).

Thus, it seems likely that the H1 histone, polylysine and spermine bind themselves to DNA, reduce its charge and condense or alter the DNA structure in such a way that nucleosome assembly is facilitated. However, nonspecific action by all these compounds cannot be ruled out. For instance, they may compete with core histones for the binding to extract components, e.g., RNA, or displace the core histones from such complexes, both of which would accelerate the assembly. Finally, it is possible that the H1 histone, polylysine and spermine operate through different mechanisms.

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