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A sedimentation study of the interaction of superhelical SV40 DNA with H1 histone.

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ABSTRACT

By moving boundary sedimentation it is shown that the interaction of H1 histone with superhelical circular SV40 DNA results in the formation of giant heterogeneous aggregates. The size of these aggregates grows with increasing H1 concentration. $s_{20,w}$ values of some 1o ooo S were measured. As compared with open/relaxed circular DNA a preferential interaction of superhelical DNA with H1 histone is observed, irrespective of the sign of the superhelical turns which was reversed by the addition to DNA of ethidium bromide. The addition to the H1 complexed aggregates of ethidium bromide effects a progressive breakdown of the aggregates. Furthermore, the superhelicity of DNA is not changed by the addition of small amounts of H1 histone.

INTRODUCTION

Recently, the interaction of histones with superhelical circular DNA has attracted increasing interest (1-3). This interest is attributable to the fact that the complete set of histones which is found in the nucleosomes of the chromatin is integrated in the structure of the SV40 virions (4,5).
Furthermore, nucleosomes stringed on DNA rings and termed "minichromosomes" have been demonstrated in the chromatin of SV4o lytically infected cells (6,7). Such structures can also arise by the interaction of histones with DNA in vitro, irrespective of its linear or circular forms (3,7). On the other hand, in vitro interactions of histones with circular superhelical DNA also provide insight into changes of the DNA secondary structure of the complexes. Germond et al. (3) were able to demonstrate that the formation of one nucleosome on a negatively

superhelical DNA results in an unwinding of one superhelical turn. This is equivalent to an unwinding of the DNA double helix by one turn. Such small changes of the DNA secondary and tertiary structure which can simply be measured with superhelical DNA because of the equivalence of superhelical and double helical turns could possibly play an role in the regulation of chromatin functions. H1 histone is not part of the nucleosome structure but is assumed to be located on the extranucleosomal DNA and involved in the crosslinking and condensation of chromatin fibrils (8,9). Furthermore, H1 histone could not be demonstrated in some papova virions (SV40, polyoma). In two recent papers Vogel and Singer (1,2) have studied the trapping of complexes of superhelical DNA and histones on nitrocellulose filters. They have shown, among others things, that H1 histone exhibits a preferential interaction with circular superhelical DNA and that the extent of this interaction increases with increasing superhelicity. Furthermore, the interaction of H1 with superhelical DNA alone and in the presence of ethidium bromide which alters the degree of superhelicity seems to be reversible.
We want to present here sedimentation experiments which essentially confirm the results mentioned (1,2). Moreover, we will concentrate on further aspects which can specifiely be derived from sedimentation measurements.

MATERIAL AND METHODS

The CV-1 line of monkey kidney cells (10) was grown in Eagle's minimal essential medium, supplemented with 10 % Tryptose phosphate broth and 5 % calf serum. The plaque purified wild type SV40, strain 776, was used for production of SV40 DNA. Confluent monolayers of CV-1 cells were infected at low multiplicity ($5 \cdot 10^{-4}$ PFU/cell) and the culture terminated when 75 % of cells exhibited a cytopathic effect. Infected cells were lysed and viral DNA extracted by the method of Hirt (11). The Hirt supernatant was extracted with 1 vol. redistilled phenol and then twice with a mixture of 0.5 vol. redistilled phenol and 0.5 vol. chloroform and, thereafter, with 1 vol.

chloroform. The DNA was precipitated with 2 vol. ethanol at - 20° C for 24 hours. Both SV40 DNA components were separated by centrifugation in CsCl (ρ = 1.56 g/cm^3) containing 200 µg/ml ethidium bromide (EB) for 48 hours at 42 000 rev./min. in the SW56 Spinco rotor at 20° C (12). After careful collection of the DNA bands EB was immediately removed by extracting 3 times with redistilled isopropanol. The DNA was dialysed against SSC for 48 hours at 4° C.
Histone H1 was prepared from calf thymus by extraction with 5 % perchloric acid and reprecipitation with guanidine chloride as described by Johns (13,14).

Moving boundary sedimentation was performed in an analytical ultracentrifuge Spinco E equipped with absorption optics, monochromator, and photoelectric registration. At low-speed runs (900 - 3 000 rev./min.) the heavy AnJ rotor was used. Most of the runs were performed at 30 000 - 36 000 rev./min. To avoid convection disturbances the temperature regulating system was not used. The temperature was about 20° C after acceleration of the rotor and did hardly change during the run. Sedimentation coefficients obtained by evaluating the 50 % point were corrected for standard conditions where mentioned. Partial specific volumes of H1-DNA complexes were calculated additively (15). H1-DNA complexes were prepared both by direct mixing of H1 and DNA solutions in standard saline citrate (SSC) (0.15 M NaCl, 0.015 M Na citrate, pH 7) in test tubes or by adding small volumes of H1 solutions in the centrifuge cell by means of a Beckman micropipette. H1 concentration was known by weighing, DNA concentration by its absorbance ($E_{260}^{1cm,1\%}$ = 200). The DNA concentration used was 20 - 40 µg/ml. The incubation time was at least 15 min. Control experiments after 2 hours intensive shaking provided the same results. SSC was used as the solvent where a maximum of H1 histone-DNA interaction should be expected.

Ratios Mol H1/Mol DNA were calculated using a molecular weight of SV40 DNA of 2.6 . 10^6 (16) and a histone molecular weight of 21 000 (17).

RESULTS

After mixing SV40 DNA solutions containing about equal amounts of the superhelical covalent closed ring Component I and the open ring Component II with increasing amounts of H1 histone containing buffer solutions the reaction product was analyzed by moving boundary sedimentation in the analytical ultracentrifuge. Whereas at high rotor speed (30 000 rev./min.) surprisingly a progressive diminution tentatively originating from pelleted material with increasing H1 histone concentration of Component I was observed, the amount of Component II decreased only slowly. Only after disappearing of Component I with further increasing H1 concentration Component II progressively disappeared. Visually the increase in H1 histone concentration was accompanied by a slowly increasing turbidity of the mixtures. The $s_{20,w}$ values at 30 000 rev./min. of both components in the presence of H1 did hardly change just as long as they could be seen, as will be shown later. As sedimentation measurements at low rotor speeds (900 - 3 000 rev./min.) have shown, this diminution of the SV40 DNA-H1 histone complexes is attributable to the formation of giant complexes or aggregates. After reaching the high rotor speed the aggregates have already been sedimenting to the bottom of the centrifuge cell. Fig. 1 shows the aggregate formation for

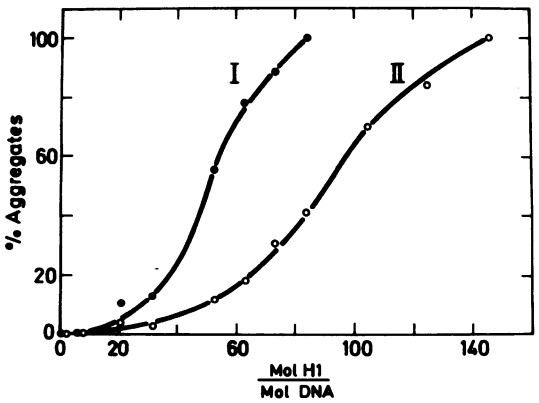

Fig. 1: Aggregate formation of the superhelical circular DNA Component I and the open circular DNA Component II of SV40 after addition of H1 histone (input) in SSC as derived from sedimentation velocity measurements. 30 000 rev./min., total DNA concentration 30 µg/ml.

both circular DNA components derived at high rotor speed. The decrease of concentration of both DNA components was measured on the ultracentrifuge scans and corrected for radial dilution. It seems clear from this figure that a preferential interaction of H1 histone with the superhelical ring Component I occurs. Fig. 2a shows the sedimentation behaviour of the aggregates at low rotor speeds. There are growing amounts of heterogeneous aggregates with increasing sedimentation rates which reach enormous values in dependence on the increasing H1 concentration expressed as Mol H1/Mol DNA. At an input ratio H1/DNA of about 1 on a mass basis (corresponding to Mol H1/Mol DNA =124) complete precipitation occurs.

It is well known that the intercalating dye ethidium bromide (EB) effects an unwinding of negative superhelical turns because of its ability to decrease the duplex winding number of DNA (18,19). Therefore, we expected to gain information on the nature of these aggregates by making them react with EB. As is shown in Fig. 2b EB leads to a progressive breakdown of the aggregates. The ratio Mol H1/Mol DNA was in this experiment about 124. It is noteworthy that after the EB titration

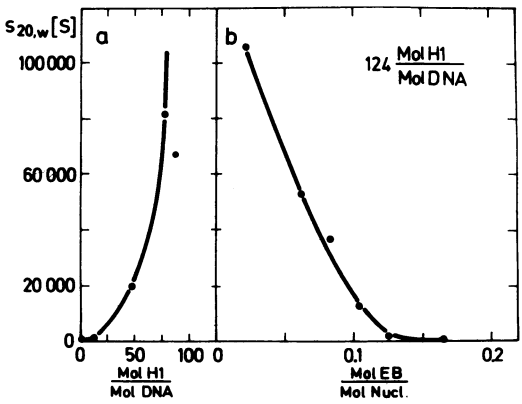

Fig. 2: Effect of H1 histone on the sedimentation behaviour of superhelical DNA (about 35 % Component II present) a) and of ethidium bromide on the aggregates at 124 Mol H1/Mol DNA b). 900 - 3 000 rev./min., total DNA concentration 38 µg/ml, SSC, input ratios given.

of the aggregates both DNA components reappeared, although at this high H1 concentration whole the DNA was originally complexed. Moreover, Component II reappeared first. No indications for a new formation of aggregates at ratios of Mol EB/Mol DNA beyond the equivalence point i.e. the value expected for the transition to open ring DNA have been obtained. According to Vogel and Singer (2), new aggregates should arise with the formation of positive superhelical turns beyond this point. The equivalence point in the absence of H1 histone under our experimental conditions amounts to 0.04 - 0.05 Mol EB/Mol Nucl. in accordance with refs. (18,19), as shown in Fig. 3a. As is demonstrated in Fig. 3b no aggregate formation occurs if Component I has been first transferred into Component II by addition of EB till the equivalence point is reached and then H1 histone is added. However, after having induced positive superhelical turns by further addition of EB, aggregate formation arises once more by addition of H1 (not shown here). The size of the aggregates, however, does not increase so rapidly as shown in Fig. 2a.

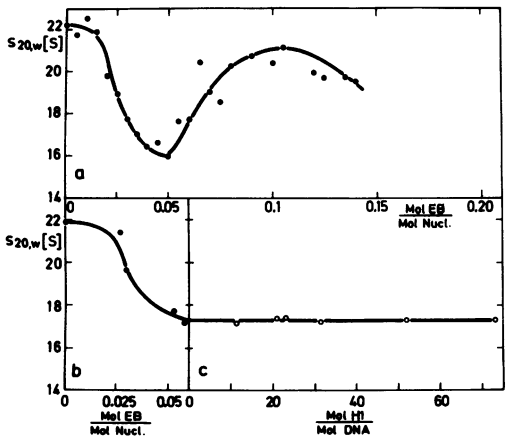

Fig. 3: Variation of $s_{20,w}$ values of superhelical SV40 DNA (about 30 % Component II present, not evaluated) with Mol EB/Mol Nucl. input a). Equivalence point adjusted with EB b) and H1 histone added c). 30 000 - 36 000 rev./min., total DNA concentration 30 µg/ml, SSC.

These experiments indicate that the formation of the giant superhelical DNA-H1 histone complexes is dependent on the presence of superhelical turns irrespective of its negative or positive sign. Additionally, however, the question arises whether a change of the secondary structure of the superhelical DNA is involved in the aggregate formation described. An inspection of the $s_{20,w}$ values of Component I DNA in the presence of H1 histone prior to the massive aggregate formation should possibly provide a first indication for an unwinding or winding of the double helix. Fig. 4a shows the $s_{20,w}$ values of both DNA components slowly to increase with increasing H1 concentration, as long as they could be measured at high rotor speed. This increase should be due to the increase of the molecular weight effected by the H1 histone added. Furthermore, also the equivalence point did not change at 18.2 Mol H1/Mol DNA, which means that the superhelicity is not changed at this H1 concentration (Fig. 4b). We conclude that a change of the DNA secondary structure by H1 histone can only be very small.

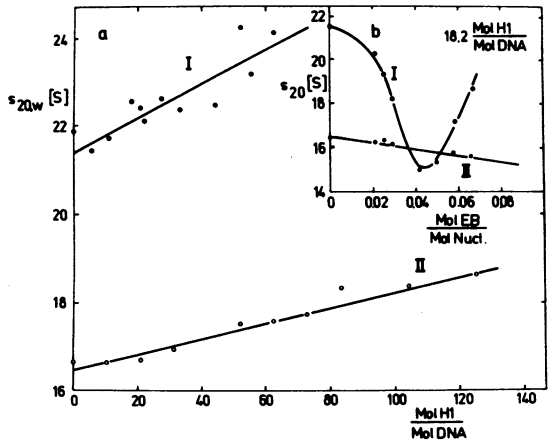

Fig. 4: $s_{20,w}$ values of both circular DNA components as a function of the input ratio Mol H1/Mol DNA a). Variation of s_{20} of the not aggregated part of circular SV40 DNA with Mol EB/Mol Nucl. at 18.2 Mol H1/Mol DNA b). 30 000 - 36 000 rev./min., total DNA concentration 30/ug/ml a) and 38/ug/ml b), SSC.

DISCUSSION

The results presented here confirm and supplement those obtained by Vogel and Singer (1,2) by means of a filtration technique which, however, does not allow conclusions as to the size of the arising complexes and the way of the complex formation. First of all we can conclude from our experiments that superhelical DNA progressively forms heterogeneous complexes or aggregates of increasing size tending to enormous $s_{20,w}$ values in the presence of increasing amounts of H1 histone. This aggregation finally leads to precipitates which may also be considered as being complex coacervates, as has been observed with linear DNA (20). However, it should be emphasized that these heterogeneous aggregates allowed to measure reproducible $s_{20,w}$ values. Furthermore, a preferential aggregate formation with the superhelical Component I DNA occurs, which is independent on the sign of the superhelical turns. It has been stressed (2) that the extent of the interaction between superhelical DNA and H1 histone is dependent on the superhelix density or superhelicity. This result can also be derived from Fig. 3b which shows that the open ring form without superhelical turns does not tend to form aggregates and from experiments which indicate that the aggregate formation does not lead to so high $s_{20,w}$ values as shown in Fig. 2a in the presence of EB beyond the equivalence point (not shown here). A conclusion which we failed to reach is the reversibility of the order of the addition to DNA of EB and H1 or vice versa which resulted in the same effect, namely in a breakdown of the complexes with decreasing negative superhelicity and in a reformation of complexes after passing the equivalence point (2). This behaviour was obtained only if EB had been added to the DNA before H1 histone. As shown in Fig. 2b the addition of EB to Component I - H1 histone aggregates results in a breakdown of the complexes. Thereafter, both DNA components reappear. The reason for this different behaviour is not clear. However, it should be noted that the ratio of H1 to DNA as given in Fig. 2b was almost ten times higher than that reported by Vogel and Singer (2). Furthermore, the DNA concentration used here was very much high-

er. At 60 Mol H1/Mol DNA (not shown here) we observed the same breakdown behaviour. At 18.2 Mol H1/Mol DNA which is comparable to that of Vogel and Singer we only found a small amount of aggregates which could not be analyzed quantitatively. It seemed to us, however, that no aggregated material was present at the equivalence point in accordance to the results mentioned (2). Hence, it is possible that the giant aggregates induced at high H1 to DNA ratios behave in a way different from that of the small complexes of only a few H1 complexed DNA molecules. We assume that they arise by an extensive network like crosslinking of DNA molecules effected by H1 bridges, which could specificly interact with the superhelical turns themselves. It should be mentioned that the H1 effected crosslinking was already proposed to occur in the condensation of chromatin (8,9). Furthermore, these huge crosslinked structures could be more resistant against EB induced conformation changes of its DNA. For the unwinding of superhelical turns more EB could be required because the number of binding sites for EB could be additionally reduced (15). We propose that the intercalation of EB resulting in an unwinding of double helical and superhelical turns leads to a release of H1 histone and concomitantly to the breakdown of the aggregates observed. Thereafter, the DNA heavily loaded with EB should hardly react with H1 and bring about new aggregates. It should be mentioned in this context that, recently, in this laboratory an EB induced release of H1 histone in chromatin was observed (21) which supports the conclusion reached here. With this respect we regard the experiments described as model studies for chromatin.

One last point to be discussed here is the finding that only a very small, if any, change of the DNA secondary structure after interaction with small amounts of H1 histone seems to occur. Small changes of the duplex winding number which are relevant for DNA conformation changes can be measured with high sensitivity with superhelical DNA. Therefore, we assume that the DNA retains the B form, at least at small H1 concentrations. Interaction studies of linear DNA with H1 histone at similar ionic conditions using circular dichroic spectra

have been performed recently (22 and further references contained therein). From such studies the view was advanced that the H1 complexed DNA is in the so-called Ψ state, which is possibly characterized by aggregates of compact and ordered DNA molecules with strong interhelical interactions. It has been proposed that the DNA in the Ψ form retains the B form (23,24) which is possibly supported by our measurements. Therefore, we assume that the giant complexes observed should bring about a state similar to the Ψ form of the linear DNA which, however, remains to be proved. A first indication for the correctness of this assumption comes from viscosity measurements which point to a very compact structure of the aggregates observed (25). Further physicochemical studies on this problem are in progress.

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