Folding of single-stranded DNA on the histone octamer

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A complex between the single-stranded DNA of the bacteriophage M13 and the histone octamer was analyzed by electron microscopy, low-angle X-ray diffraction and nuclease analysis. The morphology and the diffraction pattern of the complex strongly resemble those of the nucleosome. These results, as well as the finding of a protected DNA fragment about 100 nucleotides long following single-stranded DNA specific nuclease digestion, indicate that 'a nucleosome-like' complex can be formed between single-stranded DNA and the histone octamer. Competition experiments suggest that under physiological conditions the histone octamer is transferred from single- to double-stranded DNA.

Chromatin *Single-stranded DNA* *Histone octamer* *Electron microscopy* *Low-angle X-ray diffraction* *Nuclease analysis*

1. INTRODUCTION

Differential gene expression appears to be correlated with changes in chromatin structure [1]. Differences between the structure of 'active' genes and that of the bulk chromatin have been mainly revealed by specific nucleases which selectively hydrolyze the phosphodiester bonds at defined regions and yield a characteristic digestion pattern [1,2]. A fine analysis is difficult since the hypersensitive sites represent a small fraction of the total chromatin [2]. Therefore, it appears useful to analyze model systems such as the complexes between histones and single-stranded DNA, which should be involved in replication as well as transcription processes. Recently, denatured 140-nucleotide-long DNA has been shown [3,4] to interact with the histone octamer producing a particle with nucleosome-like hydrodynamic, structural and morphological features. However, partial renaturation cannot be excluded since histones are able to promote the renaturation of the complementary single strands [5]. Moreover, folding of DNA single chain on the histone octamer remains undetermined since a DNA of an a priori fixed length of 140 nucleotides has been used. Here we have extended the previous investigations [3,4] to a DNA lacking the complementary strand: in fact, we have analyzed the complexes between single-stranded DNA of bacteriophage M13 and the histone octamer by low-angle X-ray diffraction and electron microscopy; the DNA length involved in the complex has been determined using single-stranded specific nuclease digestion analysis. An evaluation of association constant in comparison with that of double-stranded DNA has been made in competition experiments.

2. EXPERIMENTAL

The M13 DNA was prepared, using the standard detergent-phenol procedure for deproteinization, from an infected culture of *Escherichia coli* JM103 [6]. The absence of M13 double-stranded DNA was evaluated using gel electrophoresis (not shown). Histone octamer purified of the H1 and H5 histones was prepared from rat liver or chicken erythrocyte nuclei [4].

X-ray diffraction measurements were carried out according to Bradbury et al. [7].

For electron microscopy the complexes were prepared as for X-ray measurements using

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histone/DNA ratio (w/w) of 0.4 and were prepared and stained according to Sogo et al. [8].

Competition experiments were carried out adding an equivalent amount of pBR322 DNA to the solutions of histone octamer single-stranded DNA complexes [histone/DNA ratio (w/w) = 0.8] in 0.2 M NaCl, 10 mM Tris, 1 mM EDTA, pH 7.8, on ice. After 3 h, 1 U/μg of topoisomerase I from chick erythrocyte [9] was added and the solution was incubated for 1 h at 37°C. The enzyme was inactivated by adding 1% SDS and proteinase K. DNA purified by phenol extraction and alcohol precipitation was analyzed by agarose gel electrophoresis.

3. RESULTS AND DISCUSSION

The X-ray diffraction pattern of the single-stranded M13 DNA/octamer complex as compared to that of chromatin is reported in fig.1. Though the pattern is less defined than for chromatin and for reconstituted nucleosomes [7] it reveals the presence of maxima at 55, 37 and 27 Å, about the same spacings as those of chromatin. Single-stranded DNA might wrap on the basic surface of the histone octamer, following the best pathway of neutralization of phosphates, because of its higher flexibility compared to double-stranded DNA. In such a case the scattering pattern would be similar to that of chromatin. The analysis suggests the formation of a particle with a size comparable to that of nucleosome.

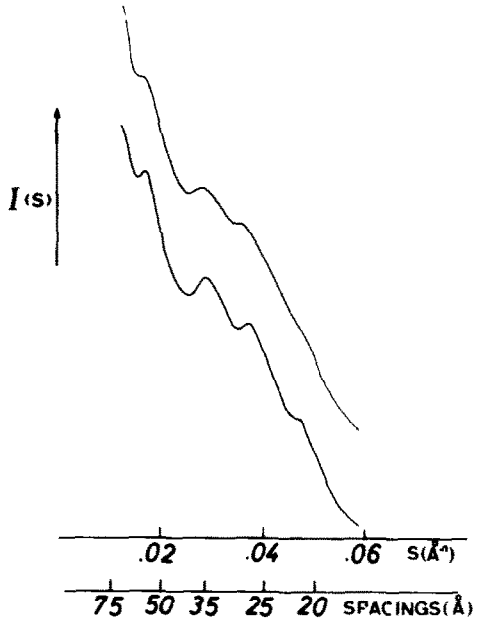

Fig.1. Densitometer profiles of X-ray diffraction patterns of chromatin (below) and M13 DNA-octamer complex (upper).

Direct visualization by electron microscopy of histone-octamer single-stranded M13 DNA complexes reveals a beaded structure whose morphology and size are very similar to those of the complexes obtained with double-stranded DNA (fig.2). Further, the contour length of the complexed molecules is shorter than that of naked DNA. The decrease in size is a proportional function of the bead number, indicating that the single-stranded DNA is wrapped around the histone octamer (fig.2g).

The nuclease protection of single-stranded DNA-octamer complexes was assayed by the single-stranded specific P1 nuclease. A DNA limit fragment of about 100 nucleotides was found (fig.3).

The results show that specific interactions between single-stranded DNA and the histone octamer produce a 'nucleosome-like particle' as can be assayed by X-ray, electron microscopy and P1 nuclease analysis. The flexibility of a single polynucleotide chain probably allows the neutralization of all the histones positive charges of the octamer, organizing a complex which is almost isoelectric as can also be derived from its very low solubility.

The complex association constant, that can be supposed to be lower than that for the nucleosome also from the lack of well defined protected fragments, has been assayed in competition experiments with double-stranded circular DNA (fig.4). Or adding to the complex between single-stranded DNA and the histone octamer, in 0.2 M NaCl, an equivalent amount of supercoiled or relaxed pBR322 DNA and treating with topoisomerase I, the histone octamer has been found to be transferred from single- to double-stranded DNA. In fact, in these ionic conditions the histone octamer isolated is dissociated and no longer able to induce double-stranded DNA supercoiling. This point has been checked by carrying out the same experiments with histones previously dissociated. In this case, the supercoiled as well as

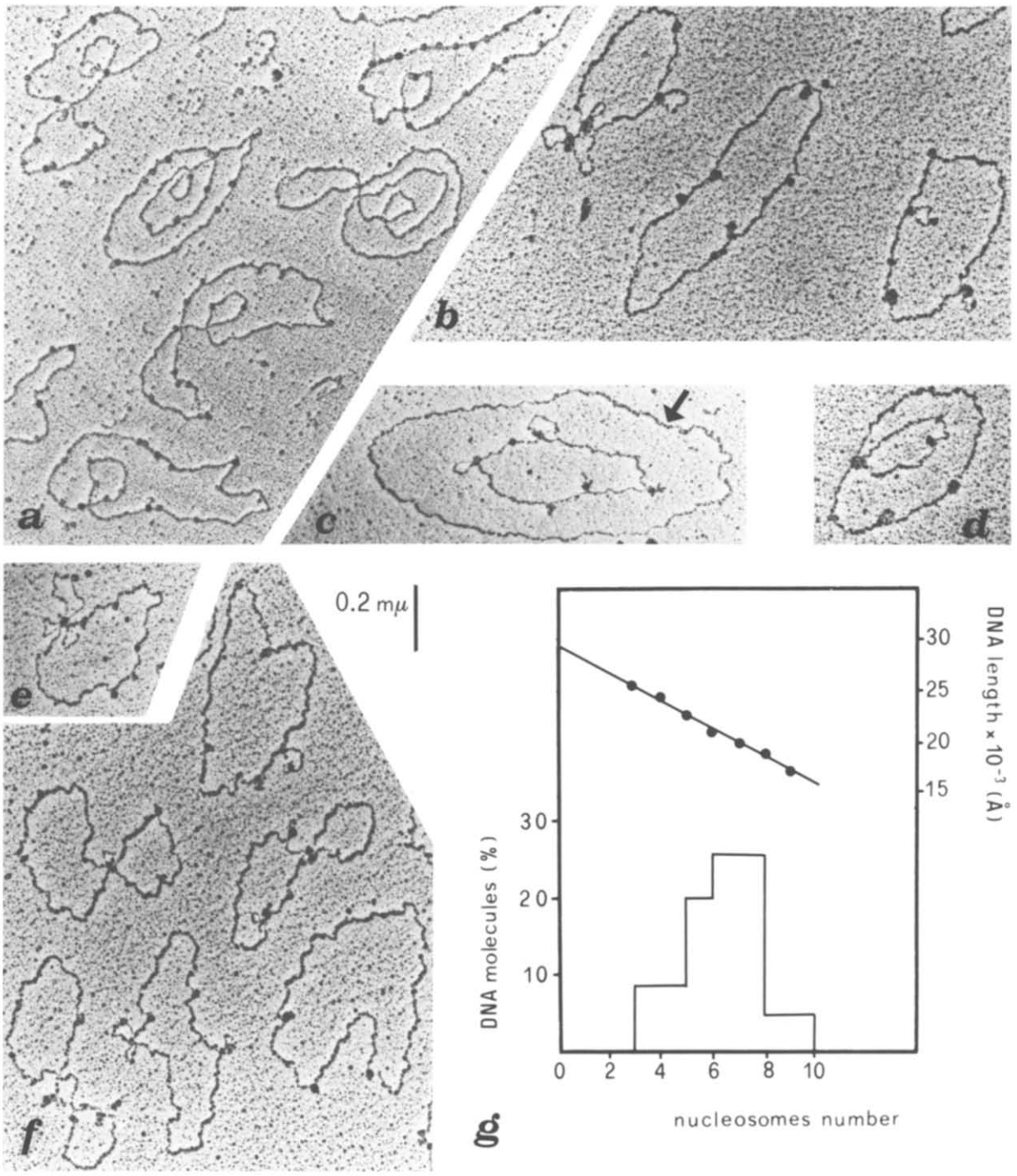

Fig.2. Electron microscopy visualization of complexes of histone octamer and M13 single-stranded and RF DNA. Magnification: 56000. (a) M13 RF DNA/histone-octamer complexes; (c) M13 single-stranded DNA, naked; (b,d,e,f) M13 single-stranded DNA/histone octamer complexes; (g) histogram of nucleosome distribution per molecule (below) and complexed molecule DNA length (upper).

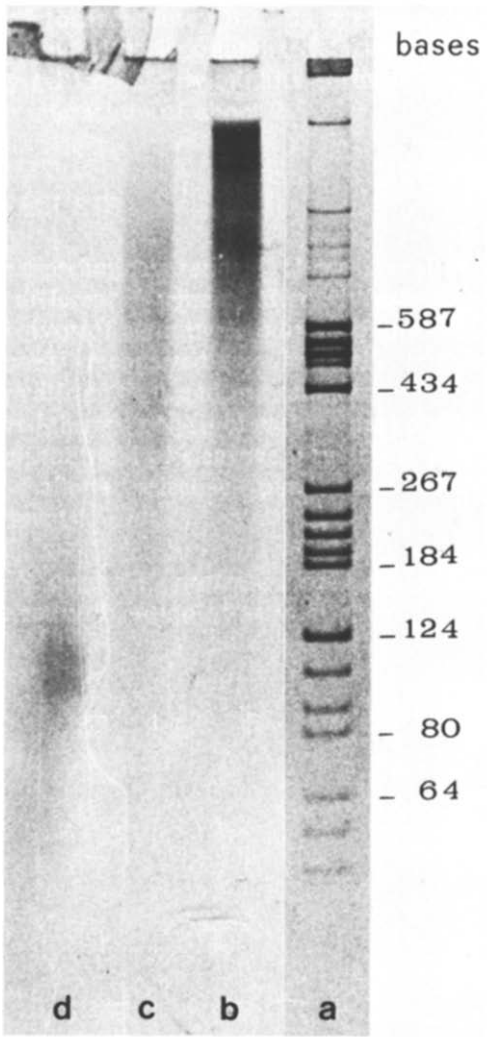

Fig.3. 6% acrylamide-urea gel electrophoresis of DNA fragments obtained after digestion of histone octamer-M13 DNA complex (protein/DNA ratio 1.0, w/w) with P1 nuclease. The complexes in 10 mM Tris-HCl, pH 7.5, 50 mM NaCl were incubated with P1 nuclease (5×10^{-2} U/μg DNA) at 37°C. The samples were then phenol extracted, alcohol precipitated and dissolved in 98% formamide. (a) pBR322 DNA restricted with *Bsp*; (b,c,d) complex digested for 2′, 5′ and 10′, respectively.

the relaxed DNA, after treatment with topoisomerase I, were found to be relaxed (fig.4d).

The results show that the single-stranded DNA can be folded on the histone octamer, forming a nucleosome-like complex that can be a transition state in biological processes such as chromatin replication and transcription. However, the

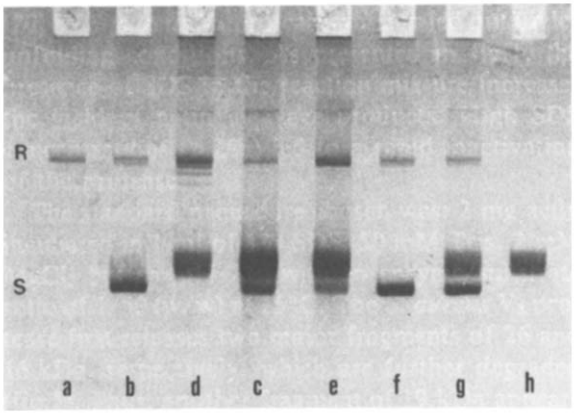

Fig.4. 1.2% agarose gel electrophoresis of competition experiments. (a) Relaxed pBR322 DNA marker; (b) supercoiled pBR322 DNA marker; (c) sample obtained after adding an equivalent amount of pBR322 DNA to histone octamer/single-stranded M13 DNA complex in 0.2 M NaCl and subsequent treatment with topoisomerase I; (d) experiment performed as in (c) but with dissociated histones used; (e) sample treated as described in (d) except that pBR322 was previously relaxed; (f) histone/octamer pBR322 DNA complex at a ratio of 0.8 (w/w) after topoisomerase treatment; (g) sample obtained after adding an equivalent amount of single-stranded M13 DNA to histone/octamer pBR322 DNA complex in 0.2 M NaCl and subsequent treatment with topoisomerase I; (h) single-stranded M13 DNA marker.

possibility of isolating this complex could be prevented by its lower stability with respect to nucleosomes, suggesting the use of foot-printing reactions in vivo [10].

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