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H1a, an *E. coli* DNA-binding protein which accumulates in stationary phase, strongly compacts DNA *in vitro*

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ABSTRACT

We characterize a component of the E. coli bacterial nucleoid H1a, which accumulates in stationary phase. This protein, identical with the major component of a plasmid-protein complex previously isolated in our laboratory, has a pI close to 7.5. Acrylamide gel electrophoresis and sedimentation in sucrose gradient have shown that the protein H1a induces significant compaction into DNA. This compaction is equivalent to that observed in nucleosome core although it introduces only a slight change in linking number. In addition, the structural change induced in the lactose L8UV5 promoter by H1a results in the decrease in the kinetic of formation of the open complex with RNA polymerase.

INTRODUCTION

In all living cells, DNA is highly compacted. For eukaryotic cells, the final state of compaction is the result of several levels of organization. The first stage is the winding of the DNA around an octameric assembly of histone proteins to form the "nucleosome structure". This first step in the process of compaction is supported by a large number of experimental results (1,2,3,4). The second level of structure that has been postulated is the solenoidal organization of the nucleosomes themselves to form a helical network (5). Finally, the chromatin appears to be organized in domains of superhelicity which are independent of one another (6).

In the case of prokaryotic cells, the packaging of DNA is far from being elucidated. The intermediate structures corresponding to a regular organization analogous to the chromatin fiber have not yet been well characterized. It is known however that DNA molecules isolated from prokaryotic cells, with few exceptions, are negatively supercoiled with superhelical densities of the same order of magnitude as those obtained from eukaryotic cells (7). In addition, several observations have suggested that a structure does exist in prokaryotes which is analogous to nucleosomes (8,9,10). These structures however are not solely responsible for the degree of supercoiling because,

contrary to eukaryotic cells where internucleosomal DNA is nearly entirely relaxed (11), the DNA of prokaryotes possesses measurable torsional tension (12). This tension can be attributed to the enzymatic activity of topoisomerases 1 and 2 (13,14). As in the case of eukaryotes, independent domains of superhelicity have been noted (15).

One approach to understanding the structure of the bacterial nucleoids is to identify its protein components and then elucidate their role. Several groups have therefore looked for DNA-binding proteins, acid-soluble and heat-stable, present in high copy number on the E. coli chromoid (for recent reviews, see (16,17,18)). At least four different histone-like proteins have been isolated which fulfill these criteria.

Protein Hu (19,20) has been shown to be composed of two subunits Hu(α) and Hu(β) (21,22), which are associated in vitro as an heterologous dimer. In vitro, Hu can unwind DNA and form nucleosome-like structures (23). The structural gene for another basic protein HLP1 (24) has been located and the phenotype of some mutants of this gene implies that, directly or indirectly, this protein affects RNA polymerase transcription. A further 28K protein, H, presents similarities with histone H2A (25). As early as 1971, another DNA-binding protein, H1, having a pI of7.5, was isolated from E. coli (26,27). The initial observations supported the view that this 15K protein was the major component which remained bound to DNA after lysis at low ionic strength, protamine precipitation and repeated extraction. On the basis of in vitro experiments, it was proposed that H1 could selectively modulate the initiation of transcription in vivo (27). We have further characterized the physico-chemical behaviour of this DNA-binding protein (28). Later, it was shown that, apart from core RNA polymerase,the major protein,stably bound to a pMB9 plasmid (29) after lysis and gentle extraction, was a 15K protein which presumably was H1. Recently, V.V. Bakayev (30), specifying earlier results (31), has reported that, when the E. coli chromosome is isolated under mild conditions, he could prepare a rather stable bacterial deoxyribonucleoprotein complex; its two major components, B1 and B2, were identified respectively as proteins H1 and Hu. On the basis of nuclease digestion experiments, this author concludes that both proteins should participate in DNA folding.

In this article, we first show that the 15K protein of the plasmid protein complex is the protein H1, which in fact is composed of three species H1a, H1b and H1c, differing by their isoelectric points. Secondly, we show that the relative amount of H1a, H1b and H1c depends on the phase of growth, H1a accumulating in the stationary phase. Thirdly, we describe a purification

of the form H1a, allowing in vitro characterization of this protein. H1a binds to DNA, causing effective compaction, which is accompanied by only a very small positive supercoiling of the DNA. Moreover, the binding of the H1a protein to the lactose UV5 promoter, although it does not block polymerase binding, retards the formation of the open complex.

MATERIALS AND METHODS

Preparation of cellular extracts

E. coli MRE600 cells (RNase I^-) were grown in 30 ml of a low sulphate medium containing 6 g/l of glucose (32) and including 500 µCi of carrier-free ^{35}S-sulphate. They were harvested after two hours of stationary phase.

After gentle lysis by lysozyme and low SDS as previously described (29), equal volumes of the 900 µl lysate were treated in the three following ways, before centrifugation 3 hrs at 40 000 rpm:

a) DNase I (1 µg/ml) was added in the presence of 10 mM $MgCl_2$.

b) The final ionic strength was increased to 140 mM by NaCl addition.

c) The final ionic strength was increased to 500 mM.

Supernatants were then analysed by two-dimensional acrylamide gels.

Two-dimensional analysis of proteins

Isoelectric focusing was performed as described by O'Farrell (3.2% ampholines)(33). The second dimension was performed on 20% acrylamide SDS gel (N-N methylene bis-acrylamide/acrylamide ratio of 1/60), as described by Laemmli (34). Proteins were stained with Coomassie brilliant blue R-250. ^{35}S-labelled proteins were revealed by fluorography according to Bonner and Laskey (35).

Quantitation of H1a, H1b and H1c

^{35}S cellular extracts prepared as above were analysed by two-dimensional gel electrophoresis. ^{35}S spots corresponding to H1a, H1b and H1c were identified by superimposition with Coomassie blue-stained non-labelled purified H1a, H1b and H1c. H1a, H1b and H1c spots were cut from the gel and counted for ^{35}S content, after solubilization in a mixture of 90 ml 5 g/l PPO in toluene, 10 ml tissue solubilizer I (Koch-Light) and 2 ml of 4M NH_4OH. The specific radioactivity of proteins was calculated from direct measurements of sulphur radioactivity in the extract and protein concentration determined by the Lowry method using bovine serum albumin as a standard. The number of bacteria was determined from the absorbance (assuming that one absorbance unit at 600 nm corresponds to 5.10^8 bacteria/ml). Calculations of the copy number per cell of H1a, H1b and H1c imply that the relative amount of sulphur in each of these proteins is not significantly different from total protein in E. coli

and that there is no significant loss of protein during the quantitation steps.

DNA purification and labelling

203 bp UV5 lactose fragment used in this study was purified as previously described (36) and 5' end-labelled by kination with γ^{32}P ATP (Amersham). Labelled fragments were diluted with excess unlabelled fragment of known concentration. The specific activity generally used was about 10,000 cpm/µg of DNA.

The detailed construction of the DNA plasmids will be reported elsewhere (A. Spassky, unpublished results). pRB17 is a shorter derivative of pBR322 where the DNA region between the unique EcoRI and PvuII sites of the vector (bp 1 to 2068) was replaced by a 17 bp insert corresponding to the C-terminal region of the lac i gene.

Protein labelling

H1a was purified as described in Results. Its concentration was determined spectrophotometrically using ε_{278} = 0.86 l $g^{-1}cm^{-1}$ (28). For stoichiometric determinations, purified H1a was labelled with ^{125}I using Bolton and Hunter reagent (37). The specific activity of iodinated H1a was determined after running the sample on SDS polyacrylamide gel calibrated with unlabelled H1a of known concentration, stained by the silver nitrate procedure (38). The labelled protein was as active as the unlabelled one in the DNA binding assays. E. coli RNA polymerase was isolated by the procedure of Burgess and Jendrisak (39). Holoenzyme was separated from core enzyme according to Lowe et al. (40) and its concentration determined by absorbance measurements (ε_{278} = 0.62 l g^{-1} cm^{-1}).

Polyacrylamide gel electrophoresis

Gel electrophoresis method has been previously described (41,42). Samples (10 µl), containing different concentrations of H1a and DNA, were incubated for 15 mn at 37°C in standard buffer 40 mM Tris pH 8, 100 mM KCl, 1 mM dithiothreitol, 100 µg/ml bovine serum albumin. Immediately before loading on the gel, 5 µl of the same buffer containing 50% glycerol, 0.01% xylene cyanol blue were added. Electrophoresis was performed at 17 V/cm at room temperature in 7.5% polyacrylamide slab gels (105 x 105 x 1.5 mm) equilibrated with TBE buffer (90 mM Tris, 90 mM borate, 2.5 mM EDTA pH 8). After 2 hr of electrophoresis, the position of the bands was revealed, on one hand, by ethidium bromide staining, and on the other hand, by autoradiography. To quantitate the radioactivity in the bands, the gel was sliced up. ^{32}P counts were determined by Cerenkov counting and ^{125}I radioactivity was determined on a gamma counter.

Filter retention assays

Samples were prepared as described above, except that the final volume was 100 µl. Filter assays were then performed exactly as previously described (43). At the end of the incubation period, 0.5 ml of standard buffer was added to the sample and the mixture was immediately passed through a 25 mm diameter cellulose nitrate filter (pore size 0.4 µm). ^{32}P counts on the filter corresponding to retained radioactive DNA were determined by Cerenkov counting.

Sucrose gradient sedimentation

Samples prepared exactly as for filter retention assays were placed on 11 ml 5-20% sucrose gradients and centrifuged at 40,000 rev/mn in a Beckman SW41 rotor for 4 hr at 20°C. The position of DNA was detected by radioactivity after fractionation into 30-35 aliquots of similar volume.

Unwinding experiments

The method has already been described (44,45,46). 1 µg of pRB17 plasmid DNA was incubated in 40 µl of standard buffer (similar buffer as above) with:

- on one hand, 10 µl of purified topoisomerase 1 (5-50 units) for 2 hr at 37°C. Protein H1a was then added (final concentration ranging 25 µg/ml to 100 µg/ml) and incubation was continued overnight;
- on the other hand, protein H1a in the same concentration range for 2 hr at 37°C. Purified topoisomerase 1 (5-50 units) in the same buffer was then added and incubation was continued overnight.

In the two cases, incubation was stopped by addition of SDS (1% final) and EDTA (final concentration 25 mM) prewarmed at the same temperature.

After phenol extraction and ethanol precipitation, samples were rinsed, dried, dissolved in 30 µl of 10 mM Tris-HCl pH 8, EDTA 1 mM, 6% ficoll 0.025% bromophenol blue, 0.025% xylene cyanol blue and applied to 1.5% agarose gel (16 x 16 x 0.3 cm). Electrophoresis and determination of centers of masses of DNA species are carried out exactly as previously described (46).

Transcription assays

The properties and technical aspects of the abortive initiation assay have been described by McClure et al. (47).

2 nM DNA (203 bp promoter UV5 fragment) were incubated in the standard buffer where $MgCl_2$ 10 mM was added with 25 µg/ml H1a, 15 mn at 37°C. The assay solution contained $_{\alpha}{}^{32}P$ UTP (10 µCi/ml final concentration) UTP 50 µM and ApA 500 µM. 50 to 300 nM RNA polymerase was then added and, in every case, aliquots of reaction were removed from the assay mixture at appropriate time (30 sec to 40 mn) and analysed as previously described. τ_{obs} was deter-

mined using lag assays as described by McClure (48) adapted to the case of the UV5 promoter as in (49). The final steady state rates of abortive initiation were determined using a non linear least squares analysis.

Isotopes, enzymes and reagents

Carrier-free (^{35}S) sulphate, ($\gamma^{32}P$) ATP and ($\alpha^{32}P$) UTP were obtained from Amersham. Egg white lysozyme, bovine pancreatic RNase-free DNase and DNase I were obtained from Worthington. Ampholines, corresponding to a pH range of 5 to 8, were purchased from LKB. Nonidet P-40 (NP 40) was purchased from BDH Chemicals Ltd (England). Restriction enzymes and polynucleotide kinase were purchased from Boehringer or BRL Ltd and used under the conditions specified by the supplier. Other reagents were of the highest quality available.

RESULTS

The 15K protein present on the pMB9 plasmid-protein complex is H1

H1 protein was purified by the method originally described by Jacquet et al. (26,27) from non-labelled E. coli bacteria. In parallel, the strain HB101 harboring the pMB9 plasmid was grown in a low sulphate medium. In this medium, proteins were labelled with ^{35}S and the complex between pMB9 and proteins was isolated (29). Labelled proteins and unlabelled H1 were mixed and analysed on the two-dimensional gel (33) shown in figure 1.

Figure 1 shows firstly that both the 15K protein from the Jacquet purification (top) and from the plasmid-protein complex (bottom) consists of several spots. Secondly, the superimposition of Coomassie blue staining and radioactive spot demonstrates that the isoelectric point of each spot is identical. The most important one, called H1a, had a pH_i of 7.5. The two main minor spots are called H1b and H1c.

The amount of H1a varies with the growth phase

It is possible to identify and to evaluate the amounts of protein H1 in crude cellular extracts. ^{35}S crude extracts have been prepared from labelled cells as described in Materials and Methods and, after addition of unlabelled purified H1, directly analysed by two-dimensional gel analysis and fluorography (the position of spots associated with H1a, H1b and H1c was located by Coomassie blue staining).

As expected for proteins bound to the chromoid, they are found in the supernatant when the lysate is treated by DNase (see Fig. 2) or when the ionic strength is increased to 500 mM (data not shown). On the contrary, if the ionic strength is only increased to 140 mM NaCl, they are found in the preci-

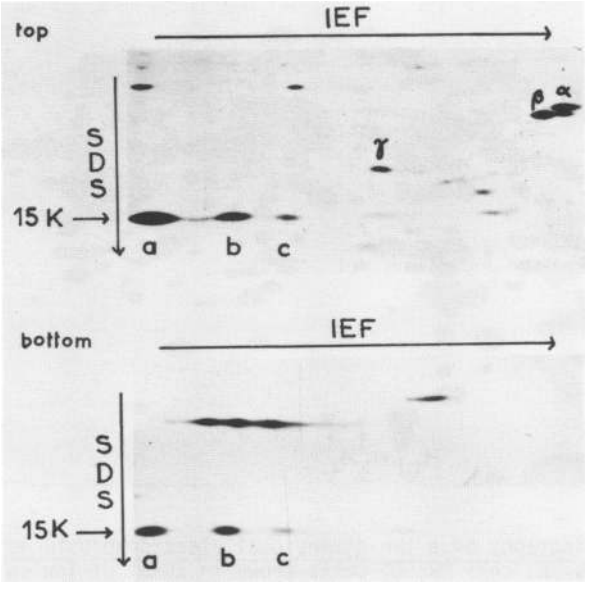

Fig. 1 : Two-dimensional gel electrophoresis. Comparison between unlabelled purified H1 protein (top) and the ^{35}S-labelled neutral proteins present in a plasmid protein complex (bottom). 10 ml of E. coli strain HB101 containing pMB9 plasmid were grown to an absorbance of 0.6 at 600 nm in low sulphate medium containing 0.1 mCi ^{35}S sulphate. Cells were lysed and plasmid-protein complexes were purified by the procedure described previously (29). 20 µl of plasmid-protein complex (1.3 µg protein/µg DNA) were mixed with pure unlabelled H1 protein and a set of non-radioactive markers (α, β, γ part of a set of muscle protein extract obtained from R. Whalen, Institut Pasteur). This mixture was analysed by isoelectric focusing in the first dimension (cf. Materials and Methods). Unlabelled proteins are revealed by Coomassie blue staining (top) and ^{35}S-labelled ones by fluorography (bottom). It has been verified that all the 15K protein initially present in the mixture is accounted for by the spots present on this gel.

pitate and not in the supernatant (data not shown).

Though the main species, H1a, H1b and H1c are found both in the plasmid-protein complex and in the crude cellular extract, their relative amount appears to be different. We reasoned that this could be due to the phase at which cells were harvested (exponential phase in Fig. 1, bottom, stationary phase in Fig. 2). Therefore, crude extracts were made from bacteria grown until the middle of exponential phase (OD_{600}=0.6) and late stationary phase. Approximation of the levels of H1a, H1b and H1c (see Table I) has led to the conclusion that while the amount of H1b and H1c varies little during cellular growth, the amount of H1a per cell increases dramatically between the exponential phase (4,000 copies per cell) and the late stationary phase (18,000

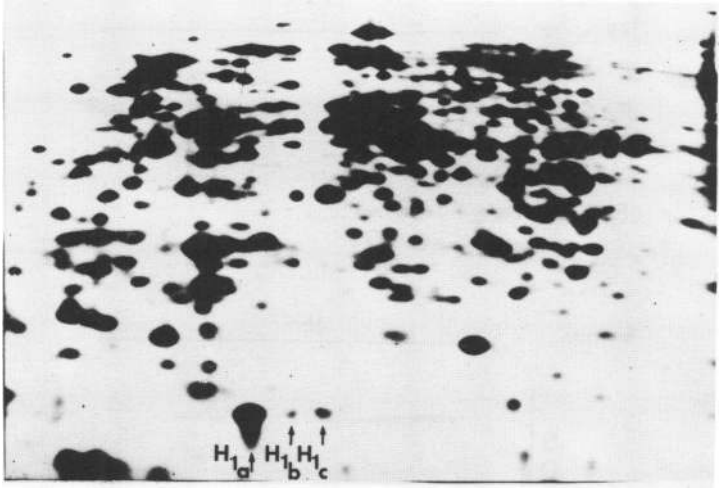

Fig. 2: Autoradiography of a two-dimensional electrophoresis gel of ^{35}S crude extract proteins. E. coli MRE600 cells grown in 10 ml of low sulphate medium including 10 µCi carrier-free ^{35}S sulphate are lysed as described in Methods, and centrifuged after 1 µg/ml of DNase 1 treatment.

copies per cell). A pulse-labelled experiment was performed during the exponential phase and the stability of the newly synthesized H1a was checked (N. Guiso, personal communication). Hence, the relative accumulation of H1a during stationary phase does not result from any particular instability of the protein during exponential phase.

Purification of H1a

A purification of H1a, which avoids contamination by the other variants, was required. The first steps of the H1 preparation devised by Jacquet et al. were maintained (preparation of the crude extract, precipitation by protamine sulphate, extraction by potassium succinate and ammonium sulphate precipita-

Table 1. Content of H1a, H1b, H1c in crude cellular extracts at three times of growth (see Materials and Methods).

Time after inoculation (hours)	OD (600 nm)	Copy number per cell		
		H1a	H1b	H1c
3	0.2	2000	2000	1800
6 (exponential phase)	0.8	4000	5000	5000
12 (stationary phase)	1.8	18000	4000	4000

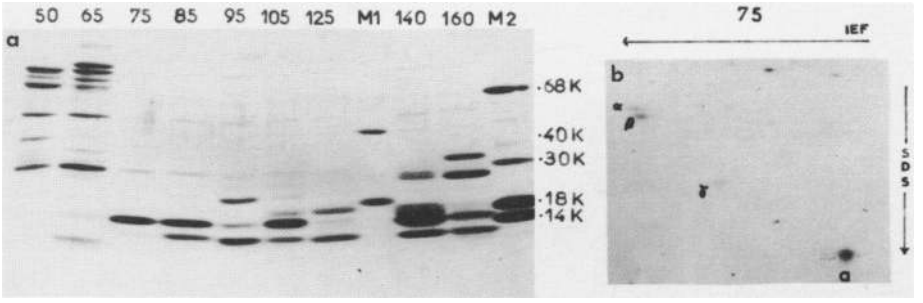

Fig. 3: Purification of H1a. a: Analysis of the different fractions by SDS 20% acrylamide gel; b: Two-dimensional analysis gel of fraction 75 corresponding to 0.35-0.45 M NaCl.

tion). The drastic treatment of heating proteins at 100°C for 15 mn was replaced by a chromatography on phosphocellulose developed by a linear salt gradient. Several fractions, analysed by one dimensional SDS electrophoresis, contain 15K proteins (see Fig. 3A). Two-dimensional analysis shows that only the fraction 75, eluted between 0.35 M and 0.45 M KCl contains H1 mainly constituted by H1a species (see Fig. 3B). This fraction was absorbed on a DEAE cellulose column in Tris 20 mM pH 8, NaCl 0.1 M, EDTA 10^{-3} M, DTT 10^{-4}M, and eluted by a NaCl salt gradient. Protein H1a, at least 98% pure, was eluted between 0.25 M and 0.3 M.

Binding affinity of H1a linear or superhelical DNA

Using the nitrocellulose membrane filter assays (43), we have compared the ability of H1a to retain a given amount of plasmid α^{32}P pBR322 either linear or negatively supercoiled.

The concentration of DNA in the assays was low enough to insure that the retention reflected the affinity of the protein for these templates, and not the stoichiometry of the binding process (end point of titration deduced from other experiments (28, this paper) are indicated by arrows in Fig. 4. They are well below the concentration of protein required to completely retain the DNA). From the data displayed in Fig. 4, we conclude that no significant difference is found between the two templates.

The filtration method has also been used to check the stability of the DNA-H1a complex. H1a was first bound to DNA so that the ratio H1a/radioactive DNA = 20 in weight, and an excess of unlabelled competitor DNA was subsequently added (43). The loss of the uptake of the radioactivity by the protein was followed. It appears that the dissociation of the complex is biphasic, re-

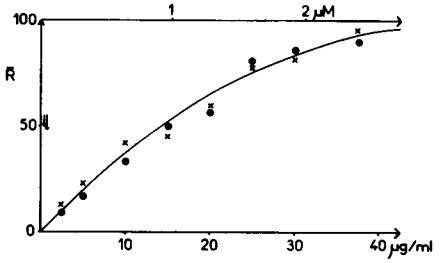

Fig. 4: Comparison of retention on nitrocellulose filters of linear or superhelical DNA complexed with H1a. 1 µg/ml ^{32}P pBR322 plasmid linear (x) or supercoiled (•) are incubated in 100 µl standard buffer with increased amounts of H1a from 1 µg/ml to 50 µg/ml, 15 min at 37°C. The fraction $\bar{R}$ of the radioactivity retained on the filter is plotted as a function of H1a concentration.

vealing probably two types of complexes of unequal lifetime. The first phase is faster than 30 sec while the other has a much longer half time (7 min) (data not shown).

Sucrose gradient sedimentation of linear and supercoiled DNA saturated with H1a

Since a significant fraction of the protein-DNA complexes has a long residence time, one can use sucrose gradient methods to obtain their sedimentation coefficients. Such profiles have been obtained for linear and supercoiled DNA complexed with H1a (cf. Fig. 5).

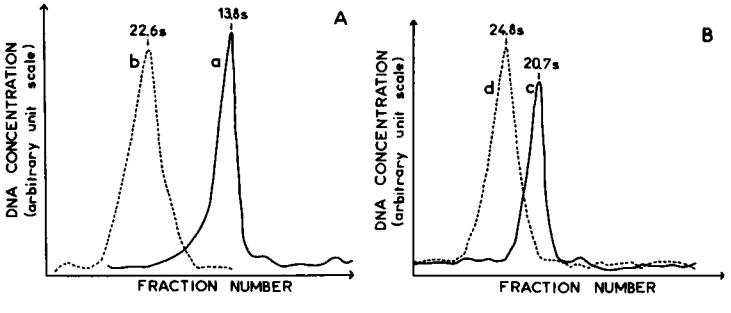

Fig. 5: Sedimentation profiles of H1a-DNA complexes. 0.1 ml samples containing 100 ng of radioactive ^{32}P pBR3222 linear (a) or superhelical (c) alone or complexed with 4 µg H1a (b) and (d) in standard buffer were incubated 15 min at 37°C before loading onto 5-20% sucrose gradients. After 4 hours of centrifugation and fractionation, radioactivity was counted in each fraction. Sedimentation coefficients of the complexes $S_{20,w}$ were estimated, using the known values of the sedimentation constants for free DNA. Supercoiled state of DNA in the complex (d) has been checked on agarose gel (data not shown).

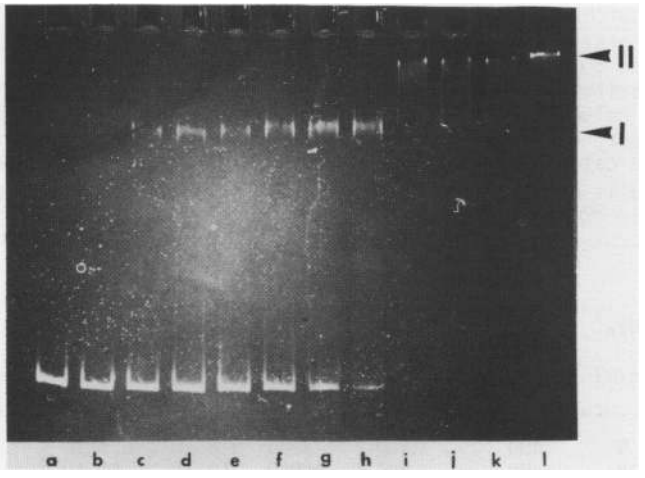

Fig. 6: Visualisation of the two complexes I and II obtained by binding H1a to 203 bp lactose fragment. 15 µl samples containing 18 µg/ml DNA and increasing concentrations of H1a (lane a: no H1a; b: 17 µg/ml; c: 22 µg/ml; d: 25 µg/ml; e: 28 µg/ml; f: 32 µg/ml; g: 36 µg/ml; h: 43 µg/ml; i: 46 µg/ml; j: 51 µg/ml; k: 56 µg/ml; l: 63 µg/ml) were applied to a polyacrylamide gel run as described in Materials and Methods. In this figure, DNA has been revealed after staining by ethidium bromide.

In both cases, there is a very significant increase in the sedimentation coefficient of the DNA after complexation by the protein. This observation suggests a large change in frictional coefficient, especially for the linear DNA.

Visualisation of H1a-DNA complexes by the method of gel electrophoresis. Determination of their stoichiometry.

These stable complexes can also be detected by polyacrylamide gel electrophoresis method already used to characterize the operator-repressor interaction (42) or CRP-DNA interaction (41,51). Fig. 6 shows the effect of increasing concentrations of H1a incubated with ^{32}P labelled 203 bp lactose fragment, on the migration of the DNA fragment. A striking feature is that from lane a to h, disappearance of free DNA band is accompanied by the progressive appearance of a single band of complex, no intermediate band can be seen. At higher concentrations of H1a, a second band progressively replaces the first one.

In order to know if these discrete bands correspond to complexes of well defined stoichiometry, we mixed ^{125}I-H1a with ^{32}P-labelled DNA fragment, loaded then on a gel which was subjected to electrophoresis as indicated in Fig. 6. Then we cut out the bands I and II; from the number of ^{125}I and ^{32}P counts

Table II : Stoichiometry of binding of H1a to DNA fragment

Experimental conditions are the one given in Materials and Methods and in Fig. 6. DNA in complex I is radioactively labelled and protein is iodinated. Specific activity of DNA is 11650 cpm/µg. Specific activity of H1a varied as shown in the column 3. Bands corresponding to complex I were cut from the gel and counted (cf. column 4 and 5). From the data, the quantities of DNA and H1a in weight are calculated in column 6 and . In the last column, the DNA to protein ratio is computed. It is expressed as the number of base pairs complexed per H1a monomer.

Fragment 203 bp µg/ml	H1a µg/ml	Specific activity of H1a cpm/10^{-3} µg	^{32}P counts in the complex	^{125}I counts in the complex	DNA bound in the complex µg	H1a bound in the complex µg	(DNA)/(H1)
19	9	480	100	3240	$8.8\ 10^{-3}$	$6.7\ 10^{-3}$	32
	18	480	800	21060	$60\ 10^{-3}$	$44\ 10^{-3}$	34
	27	480	890	30560	$78\ 10^{-3}$	$63\ 10^{-3}$	29
17.5	51	360	980	19150	$84\ 10^{-3}$	$53\ 10^{-3}$	37
	51	240	930	11920	$80\ 10^{-3}$	$49\ 10^{-3}$	38
	51	160	880	7400	$76\ 10^{-3}$	$46\ 10^{-3}$	38

in each band, and from the known specific activity of protein H1a and DNA, we could deduce the amount of both DNA and protein in each complex (Table II). Six independent experiments have shown that one monomer of H1a is bound per 35 ± 10 bp in the complex I. We have not been able to determine accurately DNA and protein concentrations in the complex II because the corresponding band is not so well resolved. However, the stoichiometric ratio between protein and DNA is approximately the same in the two bands.

H1a slightly rewinds covalently closed DNA

The insensitivity of the binding properties of H1a to the topological state of the DNA template implies that H1a contrary to other DNA binding proteins (44) should not unwind the DNA. Two types of experiments were undertaken to check this point. Increasing concentrations of H1a were bound to a relaxed pRB17 plasmid DNA and then topoisomerase 1 was added (see Fig. 7). In a second part of the experiment H1a was bound to the same plasmid but in the supercoiled state and relaxation was then performed by addition of topoisomerase 1 (data not shown).

In both cases, it appears that H1a does not noticeably affect the linking number characteristic of the relaxed state of this DNA. More precisely in the

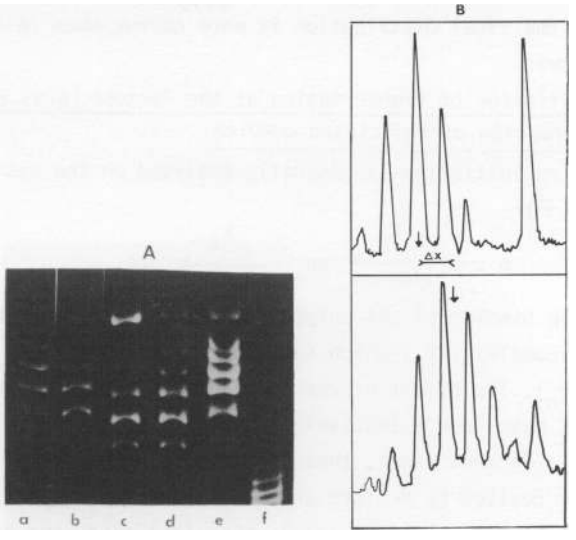

Fig. 7: A/ Photograph of a 1.5% agarose gel run with acetate buffer containing 0.025 µg ethidum bromide. The purified plasmid pRB17 DNA appears as negatively supercoiled species on lane (f). The plasmid relaxed with topoisomerase 1 alone is shown on lane (a) and relaxed in the presence of H1a, 25 µg/ml one lane (b), 50 µg/ml on lane (c), 75 µg/ml on lane (d) and 100 µg/ml on lane (e).

B/ Representative gel scan from lane (a) and (c). The arrows show the median of the Gaussian distribution of topoisomers.

concentration range of protein which corresponds to the formation of complex I, the center of the distribution of relaxed topoisomers is gradually displaced until a maximum shift $\Delta x \simeq +1.5$ is observed (cf. Fig. 7). At higher concentrations of protein, topoisomerase 1 does not seem to have access to the DNA during the time of the experiment and a broader distribution of topoisomers is observed. If H1a was unwinding its template, the linking number of the plasmid, at the time of ring closure by topoisomerase 1 would have decreased. Since this linking number is conserved after deproteinisation, one would have observed a shift in the distribution of products towards lower linking values. The converse is observed. The final products are slightly more positively supercoiled than in control experiments indicating a rewinding of the nucleic acid when H1a is bound. The effect is however very small. At constant writhing number, the rewinding corresponds to a change in the number of base pairs present per helical turn of the order of 7.10^{-3}.

The distribution of relaxed topoisomers around the median value is also affected. At low H1a concentration (corresponding to the formation of the

first complex), the final distribution is more narrow than in the absence of DNA binding protein.

H1a inhibits initiation of transcription at the lactose L8UV5 promoter without hindering the formation of the closed complex

Transcription initiation is generally analysed on the basis of a simple two-step model (47):

$$R + P \underset{}{\overset{K_B}{\rightleftharpoons}} RP_c \xrightarrow{k_2} RP_o$$

A reversible binding of RNA polymerase (R) and promoter (P) leads to an inactive closed complex (RP_c) which subsequently isomerizes to form the active open complex (RP_o). The effect of various experimental conditions on the parameters K_B and k_2 have been extensively studied in the case of the LactoseL8UV5 promoter (49,53). In most cases, these studies took advantage of the abortive initiation assay devised by McClure and collaborators (47,48). We have analysed the effect of H1a on this process using this assay and the formalism of two-step model.

H1a was first added to the DNA, followed by RNA polymerase. The reaction was then started by addition of the nucleotide substrates. Under these conditions, the turnover number of the enzymatic reaction (which characterises the ability to form the correct open complex) is not significantly affected at concentration of H1a lower than 40 µg/ml. This result implies that the formation of complex I between H1a and the DNA carrying the lactose control region does not appreciably prevent the formation of an active complex-preformed at the promoter before substrate addition.

On the other hand, when the reaction was initiated by the addition of RNA polymerase, on a preformed H1a-DNA complex a lag in the approach to the final steady state was observed, and this latency period was clearly longer than in the absence of H1a protein. Such lags were obtained at various RNA polymerase concentrations for a fixed amount of H1a present in the incubation mixture. A TAU plot analysis of the data (48) allows determination of the step affected by the binding of the protein. Data given in Fig. 8 indicate that the initial binding constant is not significantly affected (K_B = 1.3 10^7 M^{-1} in the presence of 25 µg/ml H1 instead of 10^7 M^{-1} in its absence), while the isomerisation rate is decreased by a factor of five (k_2 = 0.02 sec^{-1} instead of 0.1 sec^{-1}). The formation of complex I between the lac (L_8UV5) promoter and H1a does not prevent the reversible association of RNA polymerase leading to a closed complex but slows the second step of the reaction.

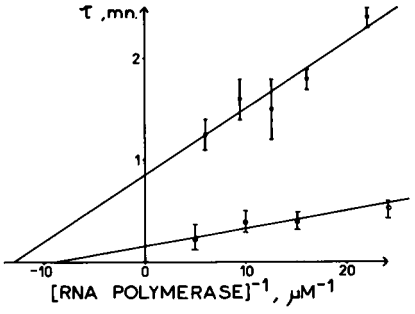

Fig. 8: TAU plots for the 203 L8UV5 promoter in absence of H1a (54) and in presence of 25 µg/ml of H1a. The time required for open complex formation (τ_{obs}) is plotted versus the reciprocal of RNA polymerase concentration. The standard assay conditions were 40 mM Tris HCl, pH 8, 10 mM $MgCl_2$, 100 mM KCl, 1 mM dithiothreitol, 100 µg of bovine serum albumin per ml, 500 µM ApA, 50 µM UTP.

DISCUSSION

As judged by two-dimensional gel electrophoresis, DNA binding protein H1 isolated from E.coli (26,27) appears to be identical to the major protein component found in the plasmid protein complex isolated in our laboratory (29) It is shown here that in both cases the protein is a mixture of three components H1a, H1b and H1c, which differ in their isoelectric point ($7 < pI < 7.5$), the most basic being H1a. The difference in pI between these three species is compatible with a charge difference of one. H1a can also be easily purified from the two other components. Furthermore, as discussed in the introduction and in ref. (56), H1 has been identified as a protein component of the chromoid called B1 (31).

We think that these three proteins are associated with DNA in vivo. Our major argument is that H1a, H1b and H1c are stably associated to the plasmid protein complex previously isolated in our laboratory (29,55). This complex does not contain any appreciable amount of lipid or RNA. It includes proteins involved in the regulation of the E.coli genome when the proper genetic regulatory element is inserted in the plasmid.

Though more indirect, the experiments reported here on the host bacteria point to the same conclusions. We have shown that H1a, as well as H1b and H1c, can be located as distinct spots on O'Farrell gels when E.coli lysates are examined by this method. Crude fracionation of this lysate by centrifugation followed by DNase treatment and/or change in ionic strength has been performed and those three proteins appear in the supernatant only under conditions

such that the chromosome is broken down or after raising the ionic strength to 0.5 M.

It is then surprising to notice that the relative amount of H1a, H1b and H1c present per bacteria largely depends on the phase of the growth. The numbers given in Table I are subject to large errors, due to the rough approximations done in their computation. Yet, the relative abundance of H1a, HIb and H1c present in a given lysate is much more reliable. Its seems safe to conclude that while the number of copies of H1b and H1c present per bacteria does not vary much, there is a marked accumulation of H1a during stationary phase : under stationary conditions, the number of copies of H1a found per bacteria (2.10^4) corresponds roughly to what was deduced from the experiments done with the plasmid (140 copies of monomer for a plasmid of 18K bp), but this number drops by a factor of 4 at the onset of exponential growth if one takes into account the mass of DNA present per cell under these two conditions

By means of centrifugation in sucrose gradient and acrylamide gel electrophoresis, we have shown that the protein H1a drastically affects DNA structure. The change in sedimentation coefficients observed after the formation of the complex is qualitatively similar to the one observed for the sedimentation of isolated nucleosomes or of minichromosomes depleted of histone H1 (57) and cannot be accounted for by the small increase in the mass of the particle. In order to evaluate the degree of compaction compatible with our observations, we have computed the frictional coefficient of the protein-DNA complexes assuming additivity of the partial specific volumes. Accepting the validity of the Stokes law, we have then deduced the diameter of a spherical object of similar composition which would sediment at the the same rate. Similar computations were performed on the data of Keller et al. (57). These crude computations yield "equivalent diameters" respectively equal to 1500 Å and 1300 Å for the complexes formed by H1a with the linear and supercoiled DNA respectively. Compaction was also estimated by the "packing ratio", which is the ratio between the contour length of the DNA molecule and this "equivalent diameter". We find values equal to 12 to 13 for the H1a-DNA complexes and to 14 to 15 for the SV40 minichromosome depleted of histone H1. We conclude therefore that H1a severely compacts linear as well as supercoiled DNA.

The same conclusions result from the analysis by gel electrophoresis of complex formation as a function of increasing concentration of protein H1a. A single complex is visible on the gel ; it corresponds to the presence of a fixed number of monomers of H1a per fragment. Its migration is slowed substantially more than would be expected based on the increase in molecular weight,

or neutralization of charge since the isoelectric point of the protein is 7.5. This behaviour is analogous to that observed with CRP, the cyAMP receptor protein, complexed at its specific site. In this case the retarded electrophoretic migration has been attributed to the formation of a bend in the DNA induced by the binding of protein (51, 58).

A similar model can be postulated in the case of H1a. In this case, the binding of each molecule could cause a slight change in the DNA curvature until the accumulated deformations provide a major structural transition. This would stabilize the complex and considerably change the migration that can be measured either by centrifugation or electrophoresis.

The fact that large compaction is accompanied by a small topological change was a priori unexpected in view of the results reported for the nucleosome (4) and for Hu (23). However this observation is not without precedent : in the case of chromatin, the formation of a "thick" fiber arising from the helical coiling of the primary nucleofilament is not accompanied by an increase of superhelical turns beyond those introduced into the nucleosomes themselves (57). It is quite possible that the binding of H1a to the DNA results in a change of a pitch of the double helix but that this effect is cancelled by a change in writhing (59-60). Also the wrapping of DNA around H1a could cause alternative positive and negative changes in writhing which translate finally into small variation of the degree of superhelicity. Such compensating effects have been proposed in various cases (57, 59, 60).

Moreover, the formation of a stable, compact complex between H1a and DNA easily accounts for the reduction in the variance of the Gaussian distribution of topoisomers at equilibrium since in a tight complex the free ends of the DNA molecule undergoing relaxation have probably less freedom to rotate around each other. The change in variance which is observed experimentally is probably an underestimation of the effect since the polydispersity of the complexes which are formed (each leading to a slightly different median value of the linking number of the DNA after relaxation) tends to broaden the distribution as observed in the case of nucleosome formation (61).

Such a restriction in rotational freedom could also account for the results of our transcription experiments. We have shown that the presence of H1a slightly favours the formation of a closed complex between RNA polymerase and the lac L8UV5 DNA. Since H1a still influences the rate of a subsequent step (the isomerisation to the open complex), this implies that RNA polymerase and H1a can form a ternary complex at the promoter. The main effect of H1a in this transcription assay is to slow down the rate of isomerisation

from the closed to the open complex. It has been shown elsewhere that, in the absence of Hla, this isomerisation most likely reflects the correct and stable positioning of RNA polymerase at the UV5 promoter (62, 49). The -35 and -10 regions of this promoter are not easily placed in register during open complex formation (62). By hindering the rotational flexibility of the DNA double helix, Hla could make this fit even more difficult to occur.

Last we want to point out some possible physiological implications of the present studies. We assume that in vivo, Hla is complexed on the nucleoid as described here. We have shown that Hla does not prevent RNA polymerase to bind to DNA ; this is consistent with the finding that core RNA polymerase and Hla are the major components of the protein-plasmid complex previously isolated (29). Second Hla compacts the DNA without apparently affecting significantly its topology. This observation is consistent with the hypothesis that the proteins are not primarily responsible for the generation of negative superturns of DNA in prokaryotic systems (18).

Even if the protein counterpart of the chromosome does not hold the genome as tightly as in eukaryotic cells, it can efficiently participate to its packing. We have shown that Hla was able to compact the DNA almost as efficiently as a minichromosome depleted of histone H1. We would therefore suggest that tight packing is not incompatible in vivo with a rather mobile organization of the chromoid.

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