Archaebacterial histone-like protein MC1 can exhibit a sequence-specific binding to DNA

Caroline Teyssier, Bernard Lainé, Alain Gervais, Jean-Claude Maurizot, Francoise Culard

Archaebacterial histone-like protein MC1 can exhibit a sequence-specific binding to DNA

Caroline TEYSSIER,* Bernard LAINE,† Alain GERVAIS,* Jean-Claude MAURIZOT* and Françoise CULARD*‡

*Centre de biophysique moléculaire, rue Charles Saolron, 45071 Orléans-Cedex, France and †U 325 INSERM Institut Pasteur, 1 rue Calmette, 59019 Lille Cedex, France

The binding of MC1 protein, the major chromosomal protein of the archaebacterium *Methanosarcina* sp. CHTI 55, to the region preceding the strongly expressed genes encoding methyl coenzyme reductase in a closely related micro-organism has been investigated. By gel retardation and DNAase I footprinting assays, we identified a preferential binding sequence in an open reading frame of unknown function. The large area of DNA protected against DNAase I is interrupted by a strong cleavage enhancement site on each strand. By circular permutation assays, we showed that the DNA bends upon MC1 binding. Furthermore we observed that the presence of a sequence outside the binding site can induce an unusual electrophoretic behaviour in some complexes.

INTRODUCTION

MC1 is the most abundant chromosomal protein present in various species of Methanosarcinaceae [1,2]. In the *Methanosarcina* sp. CHTI 55 strain, MC1 is a polypeptide of 93 amino acid residues (M_r 11000) which is mainly characterized by a high number of basic and acidic residues (respectively 24 and 12) distributed along the entire length of the protein [3]. With respect to the characteristics of its primary and secondary structure, and particularly the distribution of basic residues and the low α-helix content [4], MC1 differs significantly from eukaryotic histones and from eubacterial and other archaebacterial histone-like proteins (for a review see ref. [5]).

MC1 binds to DNA as a monomer in a non-cooperative way [6]; it can protect DNA against thermal denaturation [7] and against radiolysis by fast neutrons [8]. Its DNA-interacting region has been identified by photochemical cross-linking [9]. As for the protein HU from *Escherichia coli*, MC1 is able to promote the circularization of short DNA fragments by T4 DNA ligase [10,11].

Among the various prokaryotic histone-like proteins, several proteins, such as the protein HU, are believed to bind to DNA only non-specifically [12,13], while others bind both non-specifically and specifically to DNA. This is the case for three proteins, TF1 from the bacteriophage SP01 [14], IHF and FIS from *E. coli* [15,16], the two former being very closely related to HU. In addition, it has been shown recently that the protein H-NS, one of the most abundant nucleoid-associated DNA-binding proteins, exhibits high affinity for fragments carrying promoter sequences *in vitro*; in this case the protein seems to recognize a structural feature of the DNA [17,18].

This led us to examine whether, in addition to its strong non-specific binding [6,11], MC1 interacts specifically with some DNA regions. To study DNA sequences involved in gene regulation, we exploited the fact that the region preceding the genes encoding for the enzyme methyl coenzyme reductase (methyl CoM reductase) involved in the final step of methane production in a large number of methanogenic bacteria [19], has been characterized [20,21]. Within this sequence, a 400 bp intergenic region has a 26% GC content versus the 42% GC content of the whole chromosome [20]. This low GC content is a noticeable feature shared by sequences necessary for transcription in front of archaebacterial genes [20,22]. It is therefore of interest to compare the binding of the major chromosomal protein MC1 to the various sequences of this region.

Here, we report that MC1 has a preferential binding site located in an open reading frame (ORF) of unknown function and that this binding induces DNA bending. Furthermore we observe an unusual electrophoretic mobility of some fragments bearing this site when complexed to MC1.

MATERIALS AND METHODS

MC1 protein

Methanosarcina sp. CHTI 55 (DSM 2906) was grown as indicated in Chartier et al. [1]. The protein MC1 was prepared as previously described [1,4] with the modifications indicated in Laine et al. [11]. The protein is 99% homogeneous as ascertained by SDS/PAGE [4]. Its concentration was determined by absorption spectroscopy with an absorbance coefficient of 11000 $M^{-1} \cdot cm^{-1}$ at 280 nm.

DNA fragments

The DNA fragments containing sequences upstream of the genes coding for methyl CoM reductase were obtained by restriction digestions of a 1.2 kb fragment cloned in pUC9 [20] (Figure 1a). For convenience the positions within this fragment are numbered starting from the *Eco*RI site.

For the gel-retardation assays, the 433 bp fragment was obtained by *Sca*I and *Bst*EII digestion at positions 636 and 1070, and the 399 bp fragment by *Eco*RI and *Bgl*II digestion at positions 1 and 399. Digestion of the 399 bp fragment by *Bsp*1286 at position 190 gave two fragments of 190 bp and 209 bp respectively.

Fragments used as probes in footprinting assays were obtained by double digestion with *Hpa*II and *Alu*I at positions 256 and 422, or with *Hin*fI and *Bgl*II at positions 293 and 399 (Figure 1b).

Abbreviations used: IHF, integration host factor; TF1, transcription factor; FIS, factor for inversion stimulation; ORF, open reading frame; methyl CoM reductase, methyl coenzyme reductase.

‡ To whom correspondence should be addressed.

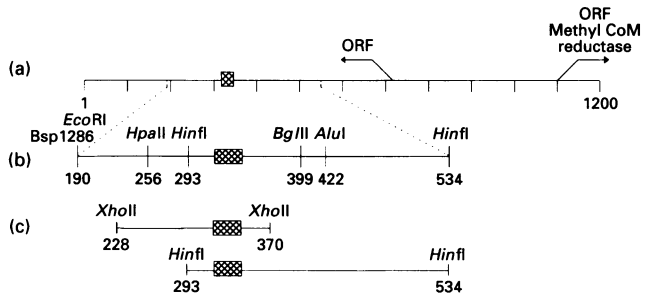

Figure 1 Schematic representation and restriction map of the 1.2 kb DNA containing the sequence upstream to the genes encoding for methyl CoM reductase

(**a**) The 1.2 kb DNA region was isolated by Allmansberger et al. [20] from the strain *Methanosarcina barkeri*. For convenience the DNA is numbered starting from the *Eco*RI site. The transcription start of the genes coding for methyl CoM reductase and the ORF in the other sense coding for an unknown protein are indicated. The preferential MC1 binding site deduced from Figure 4 is boxed. (**b**) Restriction map of the DNA region used for the footprinting experiments. (**c**) Localization of the two fragments used for the permutation assays.

For the study of the electrophoretic behaviour of the complexes, we used fragments of 133, 150, 203 and 236 bp, which have no specific binding sequence for MC1. These fragments were from *E. coli* and have been previously described [6,11].

For the circular-permutation assays, fragments of 142 bp and 241 bp, derived from digestion of the 1.2 kb DNA fragment with *Xho*II and *Hin*fI digestions respectively (Figure 1c), were labelled and circularized. The DNA ligations were performed at a DNA concentration of 0.11 μg/ml by T4 DNA ligase (BRL) according to the manufacturer's instructions. To enhance the yield of the 142 bp DNA minicircle, DNA ligation was performed in the presence of MC1 (at a protein-to-DNA molar ratio of 150), taking advantage of the fact that MC1 binding favours the ring formation of short DNA fragments [11]. After incubation, samples were deproteinized, loaded on preparative polyacrylamide gels [4% acrylamide, 0.2% bisacrylamide in TBE buffer (89 mM Tris/boric acid, 2 mM EDTA) in the presence of 0.6 μg/ml of ethidium bromide). Each DNA minicircle was extracted from the gel, and purified by phenol–chloroform treatments followed by Prepac chromatography columns (BRL). The 142 bp DNA minicircle was linearized by digestion with *Hin*fI, *Hpa*II or *Rsa*I to obtain a set of circularly permutated fragments as shown in Figure 7(b). The 241 bp DNA minicircle was linearized by digestion with *Bgl*II or *Dde*I.

DNA fragments were 5′-end labelled by T4 polynucleotide kinase with [γ^{32}P]ATP. The DNA concentrations were determined by absorption spectroscopy with an absorbance coefficient of 13000 $M^{-1} \cdot cm^{-1}$ per bp at 260 nm.

Gel-retardation assays

The binding of MC1 to defined restriction fragments was performed in 10 mM Tris/HCl, pH 7.5, containing 75 mM NaCl and 1 mM EDTA. After equilibration for 20 min at 20 °C, samples were mixed with 0.2 vol. of loading buffer (0.01% Bromophenol Blue, 50% glycerol in 10 mM Tris/HCl, pH 7.5, 1 mM EDTA), and loaded on polyacrylamide slab gels in 89 mM Tris/boric acid, 2 mM EDTA (TBE). Electrophoresis was performed at 10 $V \cdot cm^{-1}$. The gels were dried and exposed to CGR films. The autoradiographs were analysed by densitometry at 580 nm on a Camag TLC Scanner II densitometer.

In the competition experiments, due to the non-specific binding, we always used fragments of similar size to make the number of non-specific sites equivalent on both fragments. We also used low protein to DNA ratios to maximize specific versus non-specific binding.

DNAase I footprinting

Each reaction mixture (final volume 25 μl) contained 10 mM $MgCl_2$, 5 mM $CaCl_2$, 75 mM NaCl, 1 mM EDTA, 10 mM Tris/HCl, pH 7.5, the ^{32}P-end-labelled DNA fragment (10^{-9} M) and different concentrations of the protein. After incubation at room temperature for 20 min, the samples were digested with DNAase I (1 ng) for 20 s. The reaction was stopped with 12 μl of DNAase I stop solution (0.34 M EDTA, 0.9 M sodium acetate, 0.07 μg/ml carrier DNA). After ethanol precipitation the samples were loaded on a 8% sequencing gel and run at 40 W for 2 h. DNA sequencing was carried out by the Maxam–Gilbert method.

Analysis of the binding data

The analysis of the binding data was performed using the binding polynomial procedure as developed by Clore et al. [23]. As the quantitative analysis was performed at a low binding ratio (experiments were limited to protein to DNA ratios such that a maximum of three to four MC1 proteins were bound per DNA fragment), one can truncate the binding polynomial to a degree 5. We checked, by increasing this degree, that this was sufficient for accurate analysis of our binding data. Under these conditions the binding polynomial was for a fragment without a specific site

$$Z = 1 + \sum_{i=1}^{5} \frac{\prod^{j=5} NK_N^i L^i}{i!}$$

and for a fragment with a specific site

$$Z = 1 + \sum_{i=1}^{5} \frac{\prod^{j=5} N\left(\frac{K_S}{N} + K_N\right) K_N^{i-1} L^i}{i!}$$

where K_S is the binding constant to the specific site, K_N the binding constant to other sites, N the number of base pairs of the fragment and L is the free concentration of MC1 protein.

For a given free concentration of protein, L, the value of Z can be calculated for values of K_N and K_S. From the value of Z, the amount of bound MC1 is calculated as indicated by Clore et al. [23], and consequently the total amount of protein is known. One can therefore get the fraction of free DNA fragment, $(1/Z)$, as a function of the total protein concentration. K_S and K_N were deduced from comparison between this theoretical decrease of the fraction of free DNA fragment and that experimentally determined after scanning of the autoradiographs. Alternatively, we tried to use the intensities of each complex to follow the binding process but less precise results were obtained. The binding polynomial was also calculated according to the Epstein theory [24] assuming a site size of 11 base pairs as determined previously [6]. Similar results were obtained. This is due to the fact that the fragment length (about 200 bp in the case we have analysed) is large in comparison with the site size and because the binding ratio is low as previously mentioned.

RESULTS AND DISCUSSION

Localization of a preferential binding site of protein MC1

We previously studied the non-specific binding of MC1 protein to DNA by the gel-retardation method. We showed that upon MC1 binding, DNA fragments exhibit a gel electrophoretic pattern consisting of a ladder made of several discrete sharp bands with retarded mobilities when compared with the ones of the free DNA fragments. Quantitative analysis of the intensities of these bands allowed us to demonstrate that they correspond to complexes made with the DNA fragments bearing 1, 2, 3, ... MC1 molecules [6].

To look for a putative MC1 preferential binding we used the same method. The MC1–DNA binding was investigated with various fragments derived from a 1.2 kb DNA isolated from *Methanosarcina barkeri* that contains sequences preceding the methyl CoM reductase genes [20]. In order to detect even a low specific binding, and due to the strong non-specific binding of MC1 protein, we used the gel-retardation assays under particular conditions. First, in competition conditions between fragments of similar size to get an equivalent number of competing non-specific sites on both fragments. Secondly, with low protein to DNA ratios to reduce the presence of non-specific complexes. We first compared the MC1 binding to two DNA fragments, a 433 bp DNA fragment (position 636–1070) and a 399 bp DNA fragment (position 1–399). The first fragment contains the intergenic region between the methyl CoM reductase gene and an ORF of unknown function, whereas the second fragment is located in this latter ORF. Figure 2 shows the titration of the mixture of the two DNA fragments with increasing MC1 protein concentrations. To differentiate between the two species, complexes with the two separate fragments are also shown in the figure. For all the ratios of protein to DNA tested, slightly more complexes were formed with the 399 bp DNA fragment than with the 433 bp one. Upon increasing further the concentration of MC1, complexes with more than one bound protein per fragment are formed. This is apparent in Figure 2, lane F, where a band appears which can be identified as that corresponding to two MC1 proteins bound on the 399 bp fragment.

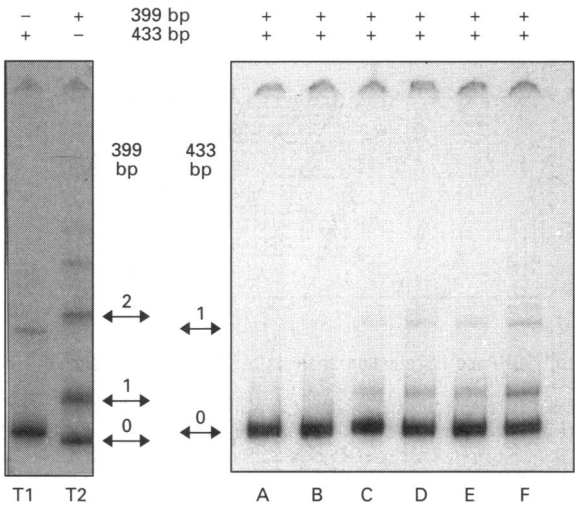

Figure 2 Competition between two DNA fragments derived from the 1.2 kb DNA upstream of the genes coding for methyl CoM reductase for MC1 binding

Autoradiograph of gel-retardation assay under competition conditions between a 433 bp (position 636–1070) and a 399 bp (position 1–399) DNA fragment. The protein concentrations were in lanes A–F: 0, 0.5 nM, 1 nM, 1.6 nM, 2.1 nM and 3.2 nM respectively. The DNA fragment concentration was 0.21 nM for the 399 bp and 0.19 nM for the 433 bp fragments. The arrows indicate the number of bound protein molecules per DNA fragment. The lanes T1 and T2 display the positions of complexes constituted of MC1 and either the 399 bp or the 433 bp DNA fragments. The samples were separated on a 7% acrylamide/0.1% bisacrylamide gel, in TBE buffer.

The 399 bp DNA fragment was cleaved with the endonuclease *Bsp*1286 to obtain two fragments of nearly the same length, one corresponding to the region 1–190 (190 bp) and the other to the region 191–299 (209 bp) Their relative affinities for MC1 protein were compared by a competitive gel-retardation assay using conditions mentioned above (low protein to DNA ratios). Upon increasing amounts of protein, we observed that the free 209 bp fragment disappears more rapidly than the 190 bp one (Figure 3). This clearly demonstrates that the binding is stronger for the 209 bp DNA fragment than for the 190 bp one. Simultaneously with the disappearing of the free DNA fragments, higher bands regularly appear. As expected, they are more abundant with the 209 bp fragment. So, for the higher protein to DNA ratio (lane D) essentially only a complex bearing one MC1 molecule is visible with the 190 bp fragment, whereas complexes containing one, two, and three MC1 molecules (complexes 1, 2 and 3 respectively) are visible with the 209 bp fragment. As shown later, with the 209 bp fragment, the complex 1 results from the specific binding of one MC1 molecule, and the complexes 2 and 3 are due to the non-specific binding of one and two MC1 molecules in addition to one specific binding.

Furthermore, we have to point out that complex 1 with the 209 bp DNA migrates slightly faster than the one formed with the 190 bp DNA fragment, although the two free DNAs and the two complexes containing two MC1 molecules per fragment migrate as expected according to the length of the DNA fragments used.

To go further into the location of the preferential binding of protein MC1, we subjected the DNA–protein complexes to DNAase I footprinting. Figure 4(a) shows the results for the top strand. A region of partial protection is clearly observed between positions 318 and 338. The amounts of protein necessary to detect the footprint and the specific complex by gel retardation under the same binding conditions are similar. The footprint is interrupted at two positions: a lack of protection adjacent to a highly enhanced cleavage site. Protection of the bottom strand is extended over about the same DNA region (Figure 4b). As for the top strand an enhanced cleavage site is observed. Relative to that of the top strand, its position is located with an offset of 3 bp on the 3′ side of the sequence (Figure 4c).

The precise boundaries of the protection are difficult to locate, because on one hand the protection is not total, and on the other hand several positions are not cleaved by DNAase I; this is particularly evident for the stretch of six adenines at the 5′ end on the top strand (Figure 4a). Nevertheless, we estimate that the extent of protection by MC1 ranges from 20 to 30 bp. This sequence exhibits no sequence similarity with other sequences of the DNA banks. Its base composition (about 60% AT) is close to that of the whole 1.2 kb DNA fragment.

When reported on an 'unwrapped' cylindrical projection, the protected area draws a large surface. It spreads on one 'side' of the DNA over about two double-helix turns and surrounds a minor groove region which is DNAase-hypersensitive (Figure 4c).

Experiments with larger amounts of protein did not reveal other areas of protection before the saturation of the fragment. This step was accompanied by several enhanced cleavages that probably reflect some important DNA conformational changes

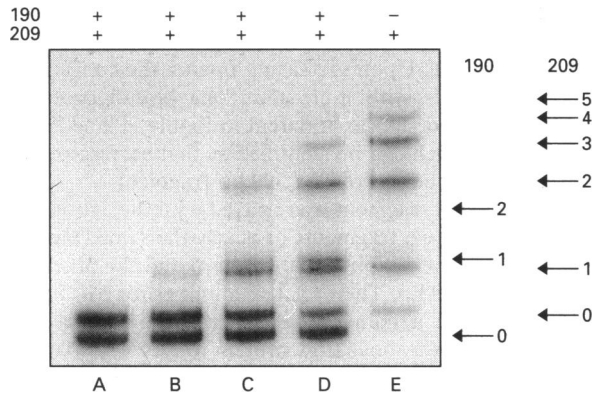

Figure 3 Competition between two DNA fragments isolated from an ORF sequence for MC1 binding

The 209 bp (position 191–399) and the 190 bp (position 1–190) DNA fragments derived from a digestion of the 399 bp DNA fragment with *Bsp*1286. Equimolar amounts (0.45 nM) of the two fragments were titrated with the following total concentrations of MC1 in lanes A–D: 0, 4 nM, 8 nM and 20 nM. Lane E shows the position of the complexes formed with the 209 bp DNA fragment. The samples were separated on a 6% acrylamide/0.3% bisacrylamide gel, in TBE buffer.

(results not shown). The presence of a unique preferential binding site along the studied DNA fragment allowed us to quantitatively analyse the binding competition shown in Figure 3. This is not straightforward since the very large number of potential non-specific sites on the fragments has to be taken into account. On a random DNA fragment each base is the beginning of a non-specific site except the n terminal bases, where n represents the number of bases covered by the protein. For the mixture of the two fragments of about 200 bp, one can calculate that there are about 700 potential non-specific sites in competition with the preferential site for the MC1 binding. The binding features of MC1 were quantitatively analysed as indicated in the Materials and methods section. The decrease of the quantity of the free DNA has been calculated for several values of ratio of the specific to non-specific binding constants and compared with the experimentally observed decreases of the intensity of the free DNA bands. We find that this ratio is about 2000. This indicates a difference of binding energy of about 4 kcal which is far smaller than that calculated between the specific and non-specific binding of prokaryotic repressors. For example, in the *Lac* operator–repressor system $K_S/K_N \sim 10^6$–10^7 [25]. However, the concentration of MC1 in the cell is markedly larger than that of a prokaryotic repressor: it is estimated to be one MC1 molecule for every 150 bp, corresponding to a concentration of 10^{-4}–10^{-5} M [26]. With such a concentration we can assume that this preferential site is permanently more occupied by MC1 than its neighbouring sequences *in vivo*.

Behaviour of the complexes in polyacrylamide gels

The mobility of complexes made with various DNA fragments of different lengths containing the preferential binding sequence were compared with the ones of complexes made with fragments lacking this sequence. An example of such an experiment is shown in Figure 5. The DNA–protein complexes are progressively and regularly retarded with increasing amounts of bound protein, except with the fragment containing the preferential binding sequence (lane E). The relative mobilities of the different

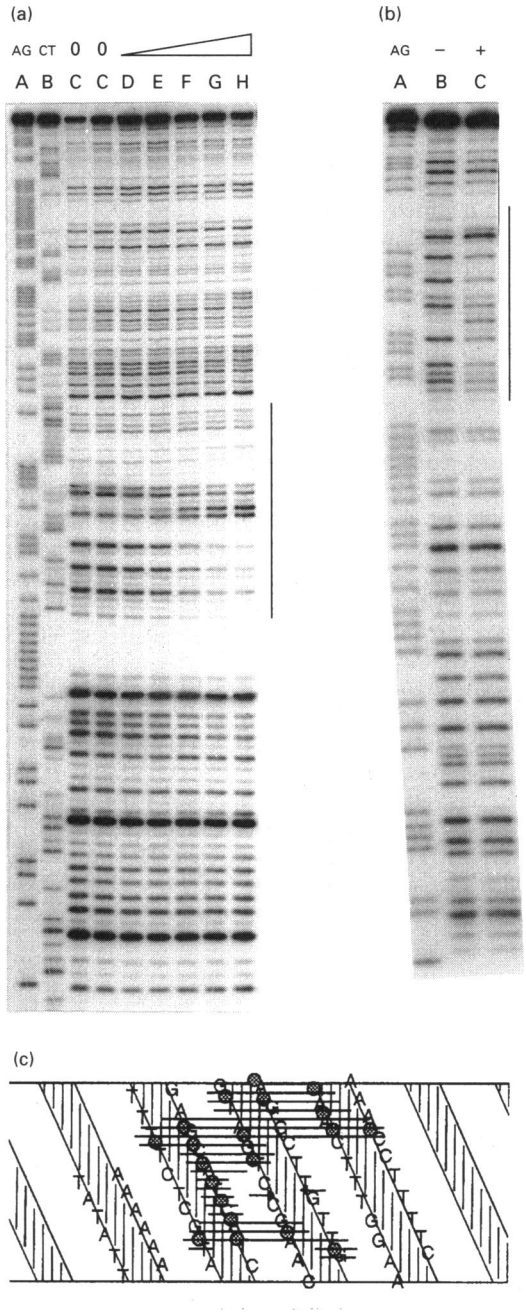

Figure 4 DNAase I protection analysis of MC1–DNA complexes

(**a**) Top strand labelled (position 257–422). Lanes A and B correspond to Maxam and Gilbert sequencing reactions. Lane C is the DNAase I digest in the absence of protein. Lanes D to H are the DNAase I digest in the presence of increasing MC1 concentrations (26 nm, 52 nM, 87 nM, 140 nM, 170 nM). The DNA fragment concentration was 9 nM in each lane. (**b**) Bottom strand labelled (position 191–399). Lane A is the A+G sequencing reaction. Lane B is the DNAase I digest in the absence of protein. Lane C is the DNAase I digest in the presence of protein (50 nM). The DNA fragment concentration was 5 nM. (**c**) Planar representation of the cylindrical projection of the DNA molecule (10.5 bp per turn) in the region of the preferential binding site of MC1. The internucleotidic bonds protected from DNAase I cleavage are symbolized by the full circles. The two arrows indicate the bonds with enhanced cleavage in the presence of MC1 protein.

DNA fragments (the quotient of the migration distances of free DNA and complexed DNA) were calculated, and plotted as a function of the number of bound protein molecules per fragment. For the non-specific fragments, a linear relationship is observed

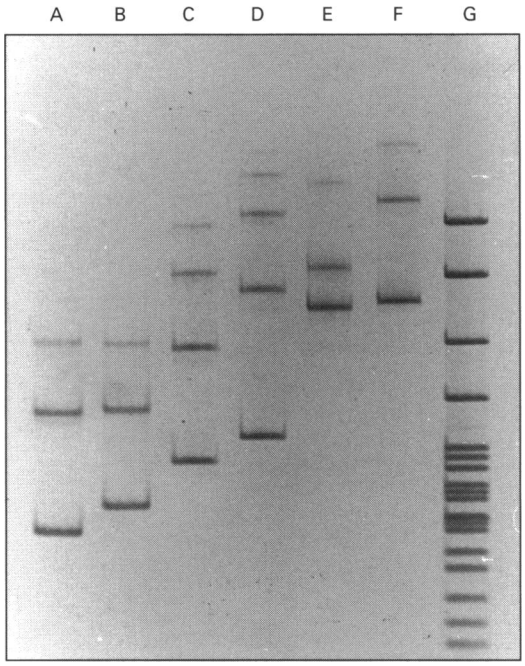

Figure 5 Comparison of mobilities of complexes formed between MC1 and various DNA fragments

Autoradiograph of gel-retardation assay with DNA fragments of, respectively, from lanes A to F, 133, 150, 203, 236, 399 and 433 bp. Only the 399 bp fragment (position 1–399) contains the preferential binding site of MC1 protein. The 399 and 433 bp fragments were derived from the 1.2 kb DNA from *Methanosarcina barkeri* whereas the other fragments were from *E. coli*. Lane G: size markers obtained from a pBR 322-MSP I digest. The samples were separated on a 7% acrylamide/0.1% bisacrylamide gel, in TBE buffer.

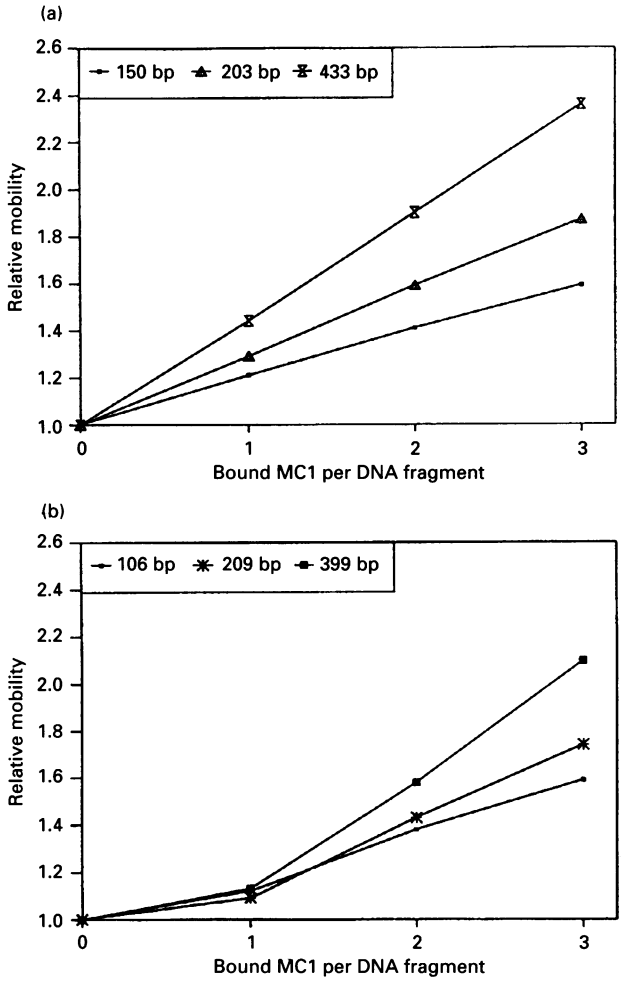

Figure 6 Analysis of mobilities of complexes formed between MC1 and fragments containing and not containing the preferential binding site

The relative mobilities of the complexes (free/complexed DNA) are plotted as a function of the amount of bound MC1 molecules per DNA fragment. (**a**) Non-specific binding DNA fragments. The 150 bp and the 203 bp are *E. coli* DNA fragments [11], and the 433 bp DNA encompasses sequence 636–1070 within the 1.2 kb DNA from *Methanosarcina barkeri*. (**b**) Fragments containing the preferential MC1 binding site. The fragments of 106, 209 and 399 bp encompass sequences 294–399, 191–399 and 1–399 respectively.

(Figure 6a). In this figure only the data obtained with protein to DNA ratios up to three are shown, but the graphs were still linear with higher ratios when observed. This is in accordance with the behaviour observed by Bading [27] on the basis of experiments performed with the *lac* repressor binding to a 203 bp DNA fragment. On the contrary, the complexes formed with three DNA fragments containing the preferential binding site have mobilities which do not follow the same rule. The curves are not linear and the specific complexes migrate faster than expected (Figure 6b).

DNA conformation can greatly affect DNA migration through polyacrylamide gel matrix. So, intrinsically curved DNA and protein-induced bent DNA are strongly retarded [28,29]. The degree of the retard induced by the protein greatly depends on the position of the binding site on the DNA fragment [29]. To determine whether DNA bending accounts for the abnormal mobility observed, we used the circular-permutation assay. We therefore circularized a 142 bp *Xho*II fragment containing the preferential binding site, then we cut it by different single site restriction enzymes yielding a set of four permuted fragments. The result of this circular permutation is shown in Figure 7. The mobilities of the free DNA fragments are nearly identical, indicating that there is little or no sequence-directed DNA bending in the free DNA fragment investigated. On the other hand the mobilities of the specific complex bearing one MC1 protein vary considerably, depending on the location of the preferential binding site within the fragment. The lowest mobility is observed with the fragment with a centrally located binding site, as deduced from the footprint experiments. Clearly this permutation assay strongly indicates that MC1 binding to the preferential binding site induces DNA bending. It is likely that, due to the moderate specificity of the binding, the observed effect is underestimated. Indeed as suggested in similar cases with the histone-like proteins IHF and TF1 [30–32], the DNA mobilities are certainly averaged by the different positions occupied by the protein on the DNA fragments during the time of the electrophoresis. We therefore think that the permutation curve is flattened and that MC1 binding to the preferential binding site induces quite a large DNA bending.

DNA bending requires multiple protein–DNA contacts. This is consistent with the result showing that the site size protected against DNAase I is quite large (20–30 bp). It is likely that the DNA is wrapped around the protein, as has been shown for other complexes between DNA and histone-like proteins [30,33]. Furthermore, as enhanced sensitivity to DNAase I can be generated by expanding the minor groove [34], the two strongly enhanced cleavage sites are probably located in an outward

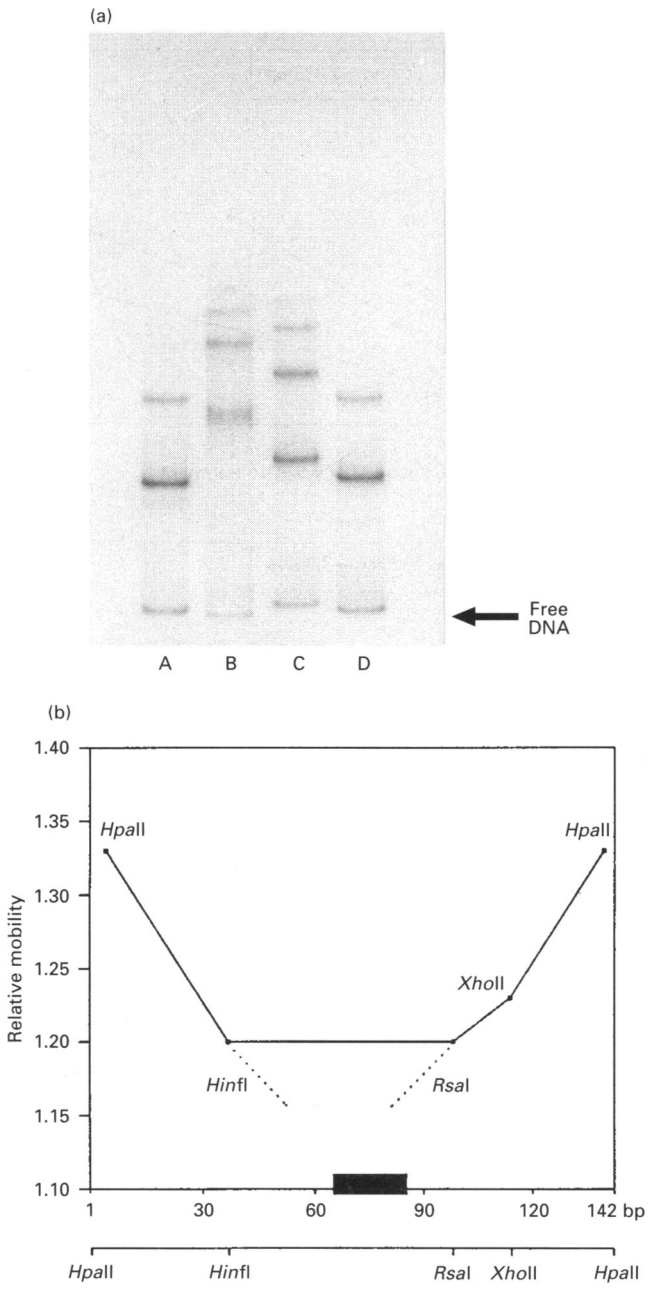

Figure 7 Bending of DNA by MC1

(**a**) A 142 bp *Xho*I fragment was circularized and cut by single-site restriction enzymes to obtain a set of four circularly permuted fragments containing the preferential binding site as indicated in the Materials and methods section. DNA fragments were: lane A, *Hin*fI; lane B, *Hpa*II; lane C, *Xho*II; lane D, *Rsa*I. The samples were separated on a 6% acrylamide/0.3% bisacrylamide gel, in TBE buffer. (**b**) The relative mobilities of the complexes were plotted as a function of the position of the site relative to the fragment ends. The closed box denotes the MC1-binding site.

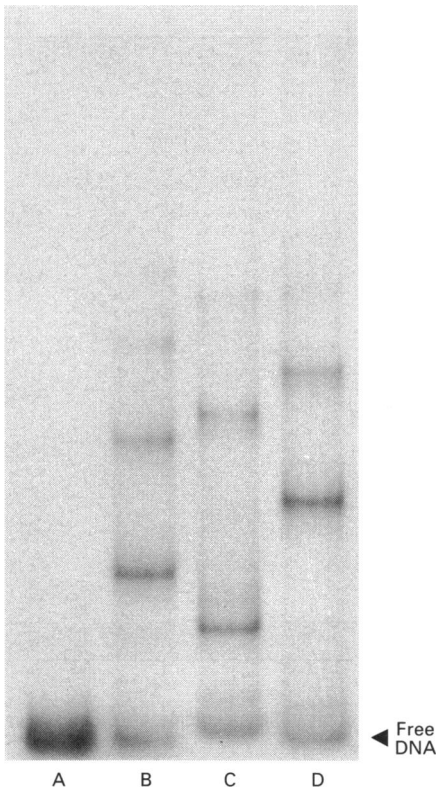

Figure 8 Unusual mobility of the complexes formed with *Bgl*II fragments

A 241 bp *Hin*fI fragment was circularized and cut by *Bgl*II or *Dde*I to obtain three circularly permuted fragments as explained in the Materials and methods section. Lanes B–D are respectively the *Hin*fI, the *Bgl*II, and the *Dde*I DNA fragments in the presence of MC1. Lane A is the free *Hin*fI DNA fragment. The samples were separated on a 5% acrylamide/0.25% bisacrylamide gel, in TBE. The positions of the sites relative to the left end of the fragment are: *Hin*fI (0.12), *Bgl*II (0.68) and *Dde*I (0.78).

facing position on the bent DNA, with the minor groove expanded.

In this permutation assay, there is no abnormally high mobility of the specific complexes, and particularly the behaviour described in Figure 6(b) is no longer observed whatever the position of the specific site on the fragments. We then suspected that a sequence outside the binding site and lacking the permutated fragment could be necessary to observe this particular mobility of the specific complexes. In fact the sequence *Xho*II–*Bgl*II (position 370–399) is absent in the permutated fragment, whereas the three fragments used in the experiment shown in Figure 6(b) span up to the position 399 (*Bgl*II cut). A second circular-permutation experiment with a fragment encompassing the *Bgl*II restriction site (*Hin*fI fragment, position 293–534) was then performed. The result of this permutation is shown in Figure 8. When the DNA is linearized with *Bgl*II, and only in that case, the abnormal behaviour of the specific complex is again observed (lane C): it migrates abnormally fast in regard to the complex containing two MC1 proteins on the same fragment, furthermore it has the highest mobility although the preferential binding site is located nearest to the centre of the fragment (68% from the left end). The two other specific complexes migrate as expected (Figure 8, lanes B and D).

To our knowledge that is the first time that such behaviour has been reported. Only the mobility of the complexes is affected, and neither their order of appearance nor their respective proportion is modified. We did not find any clear-cut explanation of this phenomenon, but we can formulate some of its characteristics. (1) A sequence with a maximal length of 30 bp located between 30 and 60 bp from the preferential binding site is required (*Xho*II–*Bgl*II). (2) The position of this sequence in the

DNA fragment is crucial: it must be at the end of the fragment, as demonstrated by the circular-permutation experiment. On the other hand the phenomenon occurs independently of the length of the *Bgl*II fragments (at least between 100 and 399 bp). As a consequence it does not depend on the relative position of the preferential binding site on the DNA fragment.

MC1 is a small protein (93 amino acids) which binds DNA in a monomeric form [6]. it is therefore unlikely that the effect observed is due to a differential frictional drag effect. On the other hand, it is probably related to a DNA conformational change we cannot define at present. This conformational change probably occurs in addition to the DNA bending of the preferential binding site, but does not influence this latter as the DNAase I enhanced cleavages, which reflect the structural modification of the DNA, are similar on fragments with or without the *Xho*II–*Bgl*II sequence (results not shown). One may speculate, for example, that this type of sequence favours some DNA condensation in the neighbourhood of the binding site. However, we do not detect any peculiarity of the corresponding sequences and in particular it does not induce intrinsic DNA curvature (see the circular permutation Figure 8).

The search for sequence-dependent binding is one of the approaches used to elucidate the biological functions of histone-like proteins such as MC1. Specific bindings of MC1 could organize crucial regions of the *Methanosarcina* genome for biological processes. In addition, even weak specific binding sites could contribute to phase the position of MC1 on the bacterial chromosome. Our results indicate that no specific binding occurs in a region controlling the transcription of the strongly expressed genes encoding methyl CoM reductase. It will be of interest to check whether this is of general character. The results presented in this paper establish additional features of the MC1 binding to DNA. First, we demonstrate that MC1 exhibits a specific binding sequence. The recognized sequence is located inside an ORF about 360 bp from its start. Secondly, the protein induces a bending of the specific site. These features are shared by proteins such as IHF, FIS and TF1, which have been shown to be involved in site-specific recombination, DNA replication or transcription [35]. Furthermore, the result of DNA bending is likely to facilitate direct protein–protein contact or protein–DNA interaction by bringing distal regions of the genome into functional contact. Even if the function of protein MC1 remains elusive, it appears possible that MC1 belongs to a family of prokaryotic proteins which both compact DNA and organize specific regions.

We thank D. A. Klein for the gift of the 1.2 kb DNA fragment containing the region preceding the methyl CoM reductase genes, Dr. P. Sautière for stimulating discussions, and Dr. J. P. Touzel for bacteria cultures. C.T. was supported by a grant from the Région Centre. We acknowledge a grant from the Comité du loiret de la ligne contre le cancer.

Received 24 December 1993/28 April 1994; accepted 4 May 1994

REFERENCES

1 Chartier, F., Laine, B., Sautière, P., Touzel, J. P. and Albagnac, G. (1985) FEBS Lett. **183**, 119–123
2 Chartier, F., Laine, B. and Sautière, P. (1988) Biochim. Biophys. Acta **951**, 149–156
3 Chartier, F., Laine, B., Belaiche, D. and Sautière, P. (1989) J. Biol. Chem. **264**, 17006–17015
4 Imbert, M., Laine, B., Helbecque, N., Mornon, J. P., Hernichart, J. P. and Sautière, P. (1990) Biochim. Biophys. Acta **1038**, 346–354
5 Laine, B., Chartier, F., Imbert, M. and Sautière, P. (1990) in Microbiology and Biochemistry of Strict Anaerobes Involved in Interspecies Hydrogen Transfer (Belaiche, J. P., Bruschi, M. and Garcia, J. L., eds.), pp. 291–301, Plenum Press, New York
6 Culard, F., Laine, B., Sautière, P. and Maurizot, J. C. (1993) FEBS Lett. **315**, 335–339
7 Chartier, F., Laine, B., Bélaïche, D., Touzel, J. P. and Sautière, P. (1989) Biochim. Biophys. Acta **1008**, 309–314
8 Isabelle, V., Franchet-Beuzit, J., Sabattier, R., Laine, B., Spotheim-Maurizot, M. and Charlier, M. (1993) Int. J. Radiat. Biol. **63**, 749–758
9 Katouzian-Safadi, M., Laine, B., Chartier, F., Cremet, J. Y., Belaiche, D., Sautière, P. and Charlier, M. (1991) Nucleic Acids Res. **19**, 3041–3045
10 Hodges-Garcia, Y., Hagermann, P. J. and Pettijohn, D. E. (1989) J. Biol. Chem. **264**, 14621–14623
11 Laine, B., Culard, F., Maurizot, J. C. and Sautière, P. (1991) Nucleic Acids Res. **19**, 3041–3045
12 Drlica, K. and Rouvière-Yaniv, J. (1987) Microbiol. Rev. **51**, 301–319
13 Pettijohn, D. E. (1990) in Nucleic Acids and Molecular Biology (Eckstein, F. and Lilley, D. M. J., eds.), vol. 4, pp. 152–162, Springer-Verlag, Heidelberg
14 Greene, J. and Geiduschek, P. (1985) EMBO J. **4**, 1345–1349
15 Craig, N. L. and Nash, H. A. (1984) Cell **39**, 707–716
16 Hübner, P., Haffter, P., Lida, S. and Arber, W. (1989) J. Mol. Biol. **205**, 493–500
17 Dersch, P., Schmidt, K. and Bremer, E. (1993) Mol. Biol. **5**, 875–879
18 Yoshida, T., Ueguchi, C., Yamada, H. and Mizuno, T. (1993) Mol. Gen. Genet. **237**, 113–122
19 Ellefson, W. and Wolfe, R. (1981) J. Biol. Chem. **256**, 4259–4262
20 Allmansberger, R., Knaub, S. and Klein, A. (1988) Nucleic Acids Res. **16**, 7419–7436
21 Bollschweiller, C., Kuhn, R. and Klein, A. (1985) EMBO J. **4**, 805–809
22 Thomm, M. and Wich, G. (1988) Nucleic Acids Res. **16**, 161–163
23 Clore, G. M., Gronenborn, A. M. and Davies, R. W. (1982) J. Mol. Biol. **155**, 447–466
24 Epstein, I. R. (1978) Biophys Biophys. Chem. **8**, 327–339
25 Pfahl, M. (1976) J. Mol. Biol. **106**, 857–869
26 Imbert, M., Laine, B., Prensier, G., Touzel, J. P. and Sautière, P. (1988) Can. J. Microbiol. **34**, 931–937
27 Bading, H. (1988) Nucleic Acids Res. **16**, 5241–5248
28 Wu, H. M. and Crothers, D. M. (1989) Nature (London) **308**, 509–513
29 Gartenberg, M. R. and Crothers, D. M. (1988) Nature (London) **333**, 824–829
30 Schneider, G. and Geiduschek, E. P. (1991) J. Biol. Chem. **265**, 10198–10200
31 Prentki, P., Chandler, M. and Galas, D. M. (1990) EMBO J. **6**, 2479–2487
32 Sayre, M. and Geiduschek, P. (1990) J. Mol. Biol. **216**, 819–833
33 Yang, C. and Nash, H. (1989) Cell **57**, 869–880
34 Suck, D., Lahm, I. A. and Oefner, C. (1988) Nature (London) **332**, 464–468
35 Schmid, M. (1990) Cell **63**, 451–454