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Stoichiometry of the binding of chromosomal protein MC1 from the archaebacterium, *Methanosarcina* spp. CHTI55, to DNA

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We have investigated the binding stoichiometry of the chromosomal MC1 protein on DNA using the gel retardation technique. Analysis of the distribution of the complex containing 0, 1, 2, 3..... bound proteins shows that the protein MC1 interacts with the DNA as a monomer. Binding experiments with short DNA fragments of various lengths shows that the site size is 11 bp in length. These results are compared to those obtained with other chromosomal proteins including HU protein.

Chromosomal protein; Binding site; MC1; HU protein

1. INTRODUCTION

The stuctural chromatin organization of eubacteria and archaebacteria as been the subject of numerous studies in the past few years [1–4]. The protein HU (DNA-binding protein II) is encountered in a wide variety of eubacteria, whereas chromosomal proteins of different features are found in the various groups of archaebacteria [2,5]. In methanogens, proteins MC1 and HMf, respectively isolated from *Methanosarcinaceae* and *Methanothermus fervidus* [6–8] strongly differ in size and amino acid sequence. Protein MC1 isolated from *Methanosarcina* spp. CHTI 55 is a basic polypeptide of 93 amino acid residues [9]. As the protein HU from *Escherichia coli*, MC1 is unable to form stable repeating structural units reminiscent of the eukaryotic nucleosomes [10,11]. Comparison of the sequence of the protein obtained from various strains have shown that the protein contains two regions, the first one (residues 1–58) with two well-conserved amino acid sequences and a C-terminal region which is more variable and where the DNA-binding sequence is located [12]. MC1 protein preferentially binds double-stranded DNA [13] and protects DNA against thermal denaturation [14]. In a previous work [15] we have shown that MC1 protein facilitates the formation of DNA minicircles and induces bending and compaction of DNA. This effect on DNA conformation is similar to that induced by the HU protein [16].

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In vivo, the protein-to-DNA ratio is estimated to be 1 protein molecule for every 170 bp DNA. In this paper, gel retardation experiments enabled us to determine the DNA binding stoichiometry of MC1 protein. The MC1 protein interacts with DNA in a monomeric state on a binding site which is of about 11 bp long.

2. MATERIALS AND METHODS

2.1. *DNA fragments*

The different DNA fragments (236, 55, 46, 25 and 21 bp long) were obtained by electrophoresis on a 6% preparative polyacrylamide gel after restriction digestion of the appropiate plasmids, and purified on Prepac chromatography (BRL). The fragments were 5′ end labelled by T4 polynucleotide kinase with [γ^{32}P]ATP (Amersham).

2.2. *MC1 protein*

Methanosarcina spp. CHTI 55 (DSM 2902) was grown as indicated in [14]. The protein MC1 was prepared as previously described in [17] and purified as in [10] with the modifications indicated in [15].

2.3. *Formation of the complexes*

Complexes were formed by addition of MC1 protein to DNA fragment in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) containing 50 mM NaCl. After equilibration for 45 min at 20°C samples were mixed with 1/5 volume of loading buffer (0.01% Bromophenol blue, 50% glycerol in TE). The concentrations of the protein and of the DNA were determined by absorption spectroscopy with an extinction coefficient of 1,1000 $M^{-1}\cdot cm^{-1}$ per protomer at 280 nm and 1,3000 $M^{-1}\cdot cm^{-1}$ per bp at 260 nm, respectively.

2.4. *Gel electrophoresis of the complexes*

Polyacrylamide slab gels (7% acrylamide, 0.1% bisacrylamide) were cast in 45 mM Tris-boric acid, 1 mM EDTA (TBE/2). Pre-run and run electrophoreses were performed at 10 $V\cdot cm^{-1}$ for 1 and 3 h, respectively. The gels were dried and exposed to CGR films. The autoradiographs were analyzed by densitometry at 580 nm on a Camag Tlc Scanner II densitometer.

3. RESULTS

3.1. *Determination of the oligomeric state of bound MC1*

In order to characterize the DNA binding properties of the MC1 protein, we performed gel retardation experiments which separate free and complexed DNA fragments [18,19]. This separation depends partly on the molecular weight and charge of the protein and partly on the DNA conformation in the complex. Fig. 1 shows the titration of a 236 bp DNA fragment by increasing protein concentrations (protein-to-DNA fragment ratios ranging from 2 to 12). Discrete and sharp bands were clearly observed, indicating that the binding is strong enough to observe stable complexes by this method. Complexes with up to 10 MC1 molecules can be seen on this gel, and probably more proteins can bind to this fragment but the higher bands are not resolved. The pattern observed is similar to those obtained for the non-specific binding of the *lac* repressor, or for the binding of HU protein to DNA fragment [19–21]. The centre of distribution of the bands regularly shifts towards larger complexes, increasing the input ratios of protein-to-DNA fragment. This is characteristic of a non-cooperative binding process.

To quantify our results we scanned the autoradiograph of the gels. Fig. 2 illustrates the results of a slot where the complexes were formed with an input ratio of 4 protein protomers per DNA fragment. We obtained the distribution of the DNA in the different species by plotting the relative area of each DNA band vs. the number of protein per DNA fragment in each band (X). The most intense bands correspond to complexes formed with 3 and 4 proteins bound per DNA fragment, and the DNA distribution is symmetrical around these two bands.

Gel retardation assay separates complexes with different stoichiometries but does allow the determination, at least directly, of whether each DNA band is retarded by the binding of a monomer or of a multimer of the protein. However, in our binding conditions, we can obtain information on the stoichiometries of the interaction by comparing the experimental distibution with theoretical ones obtained with various models. Assuming that the size of DNA fragment is large compared to the size of the binding site (as will be shown later) the distribution of the DNA in the various complexes must follow a Poisson distribution with

$$P(X) = \frac{<X>^X e^{-<X>}}{X!}$$

where $P(X)$ is the percentage of complex bearing X

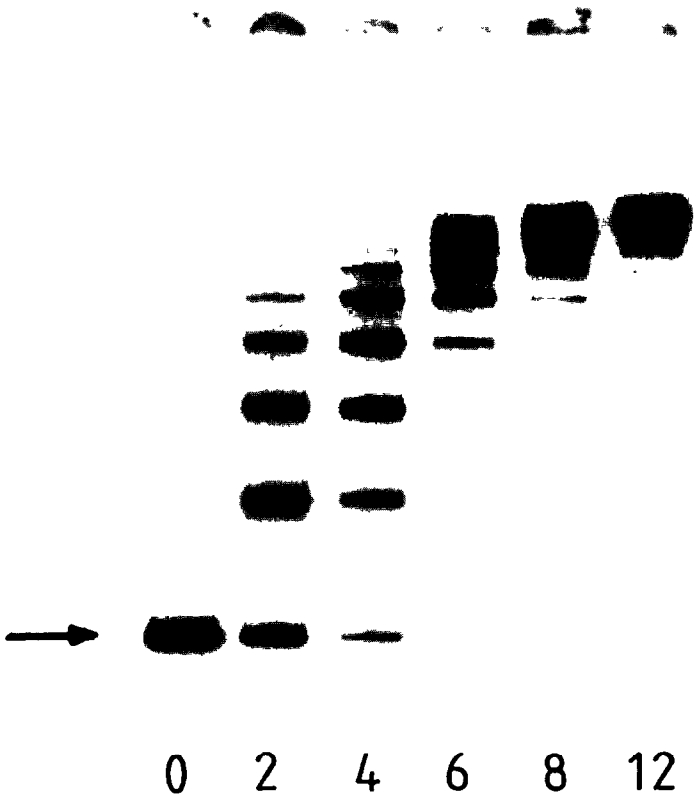

Fig. 1. Gel retardation assay of the binding of MC1 to a 236 bp DNA fragment. The binding buffer was: 10 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA pH 7.5. The gel was 7% acrylamide, 0.1% bisacrylamide. The input ratio of protein (protomer)-to-DNA fragment is indicated under each lane, the DNA fragment concentration was 2×10^{-7} M^{-1}. The arrow shows the mobility of the free DNA fragment.

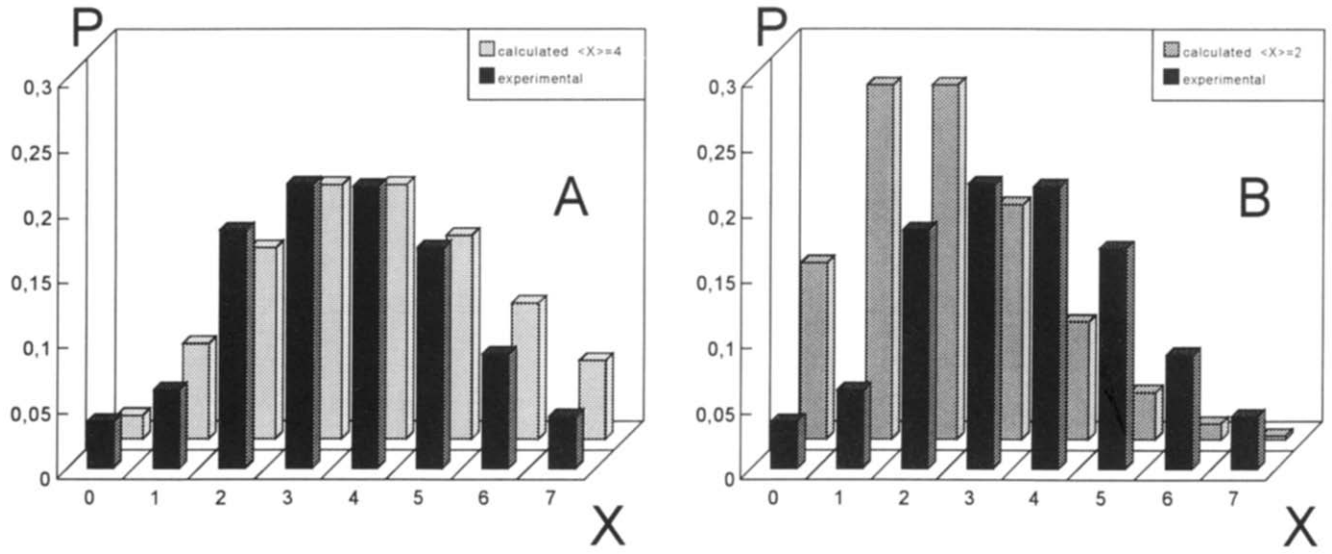

Fig. 2. Histograms of the distribution of the DNA on a retardation gel. X is the number of proteins bound per DNA fragment in each complex. Experimental conditions were as in Fig. 1, with an input ratio of protein-to-DNA fragment of 4. The experimental values were obtained by scanning the autoradiograph of the gel. P corresponds to the relative area of each DNA band. For the calculated values, P is the probability of having X MC1 molecules per DNA fragment. P is obtained by the Poisson equation with a mean average of $<X> = 4$ (A) and $<X> = 2$ (B).

bound protein and $<X>$ is the mean average of bound protein. In Fig. 2 the input protein-to-DNA ratio is 4 protomers per DNA fragment, and we have calculated the theoretical distribution for a binding in a protomeric form, which corresponds to $<X> = 4$ (Fig. 2A), and in a dimeric form, which corresponds to an effective concentration of binding species only half of that calculated in protomer, and therefore a real ratio of protein-to-DNA fragment equal to 2 ($<X> = 2$, Fig. 2B). Comparison between experimental and calculated distribution clearly demonstrates the agreement with the model in which the MC1 protein binds DNA as a monomer and not as a dimer. Of course, binding with higher oligomeric forms would have shifted the centre of distribution to even smaller X values and is also excluded. This experiments also shows that, under our experimental conditions, all the protein is bound to the DNA. The presence of free protein would have reduced the value of $<X>$ and consequently shifted the distribution to lower X values. Similar conclusions were reached when the same analysis was performed with other protein-to-DNA input ratios. Therefore it appears that the MC1 protein does not bind to the DNA fragment in a dimeric state but in a monomeric form.

3.2. *Determination of the site size of the protein on DNA*

In order to obtain the protein site size on the DNA, gel retardation experiments with different short DNA fragments were performed. Fig. 3 shows the complexes obtained with DNA fragments of 46 and 52 bp. With these short DNA fragments all the different complexes were very well separated on the gel. We observed that 5 MC1 molecules can bind to a 55 bp DNA fragment and only 4 to a 46 bp one. In the same way we observed that 3 MC1 molecules were bound to a 35 bp fragment, 2 were bound to a 25 bp fragment, whereas only 1 was bound to a 21 bp fragment (results not shown). These results suggest that the length of the site is of about 11 bp.

4. DISCUSSION

We have used the gel retardation technique to study the interaction of the chromosomal protein, MC1, with DNA. We focused this work on the binding stoichiometry of the protein (oligomeric state of the protein in the binding process) and protein site size on the DNA, in order to obtain a comprehensive view of the interaction. The quantitative analysis of the gel electrophoresis experiments allows the determination of the oligomeric state of MC1 protein bound to DNA. The data demonstrate that MC1 protein interacts with DNA as a monomer under our conditions. The stoichiometry of the interaction of a protein–DNA complex is often determined by the use of a labelled protein [22,23]. The method we have used is easier to perform. It requires that the binding is non-cooperative and a knowledge of the amount of bound protein. The absence of cooperativity can be assessed by retardation gel experiments. The amount of bound protein is more difficult to obtain and generally requires an independent method such as, for example, the fluorescence. However, in the case of a binding in a monomeric state, the result is unambiguous, even if the amount of bound protein is unknown. This is the case for MC1 binding.

The protein, MC1, is present in the deoxyribonucleo-

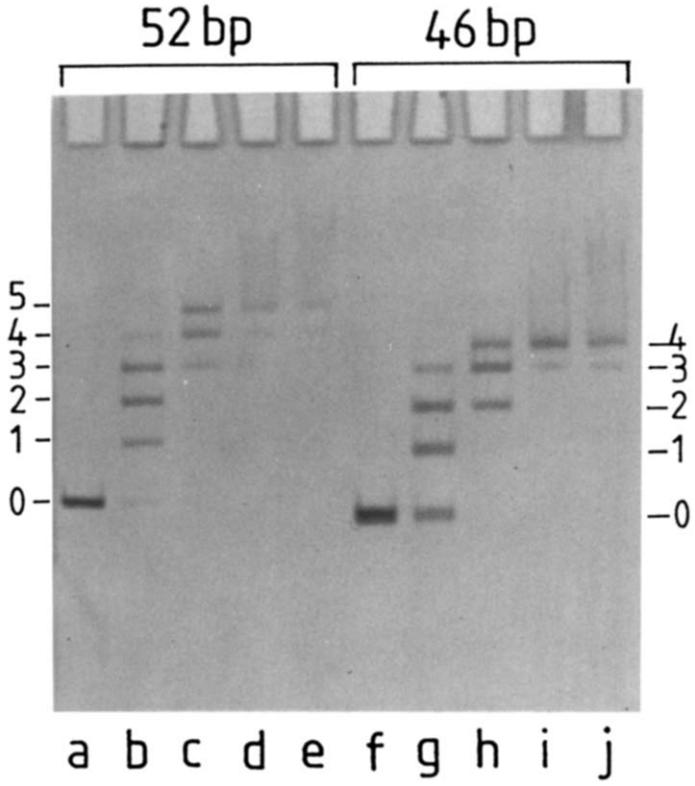

Fig. 3. Gel retardation assay with a 52 bp (left part) and a 46 bp DNA fragment (right part). The input protein-to-DNA fragment ratio was, respectively: lanes a–e, 0, 2, 4, 5.5, 7; and lanes f–j, 0, 1.5, 4, 5.5, 7. The amount of protein bound per DNA fragment is indicated. The gel was stained by ethidium bromide.

protein complex of the archaebacterium at a protein-to-DNA mass ratio of 0.11 [24], i.e. 1 protein molecule for every 170 bp, and so, in vivo, less than 10% of the genome can be complexed with MC1 even if we consider that all proteins are bound.

This property of the binding is similar to that of the protein, HU, of the eubacteria [11,21]. On the other hand, HU is bound to DNA as a dimeric structure [24–26]. This binding behaviour of MC1 let us to think that in vivo MC1–DNA interactions are different from those observed with HU.

The site size of the MC1 protein on the DNA (11 bp) is large in connection with the protein size (93 amino acids). It is slightly larger than that observed using the same method for the HU protein, 9 bp [21], but in this case the site was obtained for an HU dimer whereas here it is obtained for a MC1 monomer. Two other chromosomal proteins, IHF and TF1, have a large site size (about 30 bp per dimer); in these two cases it was suggested that the DNA must be wrapped around the proteins [23,27]. We have previously shown that MC1 bends and compacts DNA [15]. The large site size observed in these experiments agrees with an important DNA conformational change and supports our belief that one of the biological functions of the MC1 protein is to participate in in vivo DNA packaging.

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