Characterization of the chromosomal protein HMb isolated from *Methanosarcina barkeri*

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Received 12 December 1984; revised version received 23 January 1985

The acid soluble protein HMb is the major component of the archaebacteria *Methanosarcina barkeri* nucleoprotein complex. This protein of M_r 14 500 is rich in basic amino acids. Protein HMb differs from eubacterial HU-type chromosomal proteins by its molecular size and physico-chemical characteristics.

Archaebacteria *Methanogen* *Chromosomal protein*

1. INTRODUCTION

Prokaryotes comprise two phylogenetically distinct groups: the eubacteria and the archaebacteria which include thermoacidophiles, halophiles and methanogens [1]. Structure of macromolecules such as 5 S rRNA and elongation factor 2 proteins and properties of DNA-dependent RNA polymerase strongly suggest a closer relationship of archaebacteria with eukaryotes than with eubacteria [2–4]. In eukaryotic cells, packaging of the DNA inside the nucleus is mainly ensured by basic proteins called histones. In eubacteria, HU-type proteins ($M_r \sim 9500$) are involved in DNA condensation [5–7]. Another protein called H1 ($M_r \sim 15\,500$) which may also participate in DNA condensation has been identified in *E. coli* [8]. The change in chromosomal protein composition between eukaryotes and eubacteria explains the fundamental difference in their chromatin organization [9].

The organization of the DNA in archaebacteria has yet to be elucidated. However, chromosomal proteins have been characterized in two strains of thermoacidophiles: the protein HTa from *Thermoplasma acidophilum* [10,11] and two proteins of M_r 14 500 and 36 000, respectively, from *Sulfolobus acidocaldarius* [12]. The amino acid sequence of the former is clearly homologous to that of HU-type proteins [6,7,13]; the latter are not related to the HU-type proteins [12].

An electrophoretic study has shown the presence of a DNA-associated protein of M_r 14 300 in *Methanobacterium thermoautotrophicum* [14] but, up to now, no chromosomal protein has been isolated from methanogens and halophiles. This paper deals with the characterization of the major chromosomal protein isolated from the methanogen *Methanosarcina barkeri.*

2. MATERIALS AND METHODS

M. barkeri, strain MS, was provided by Dr J.G. Zeikus (University of Wisconsin, Madison, USA) and was grown at 35°C and pH 7 under 80% H_2/20% CO_2 in the basal medium containing carbonate, yeast extract and trypticase (BCYT) described in [15]. Cells were harvested in the post-exponential phase of growth.

The nucleoprotein complex was isolated as in [14] with the following modifications: bacteria (15 g) were suspended in 30 ml of 10 mM Tris-HCl,

Abbreviations: DFP, diisopropylfluorophosphate; PTH, phenylthiohydantoin

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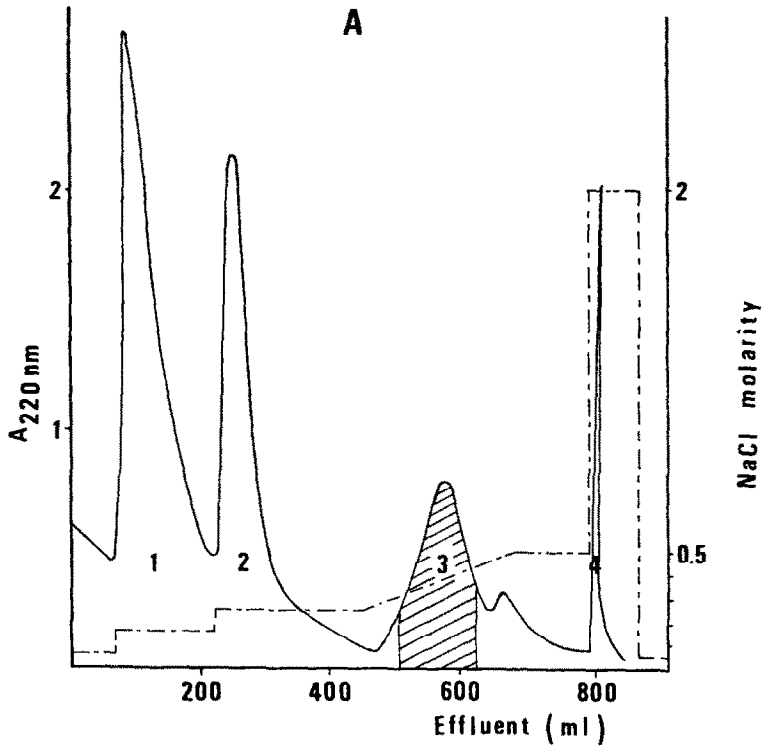
Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30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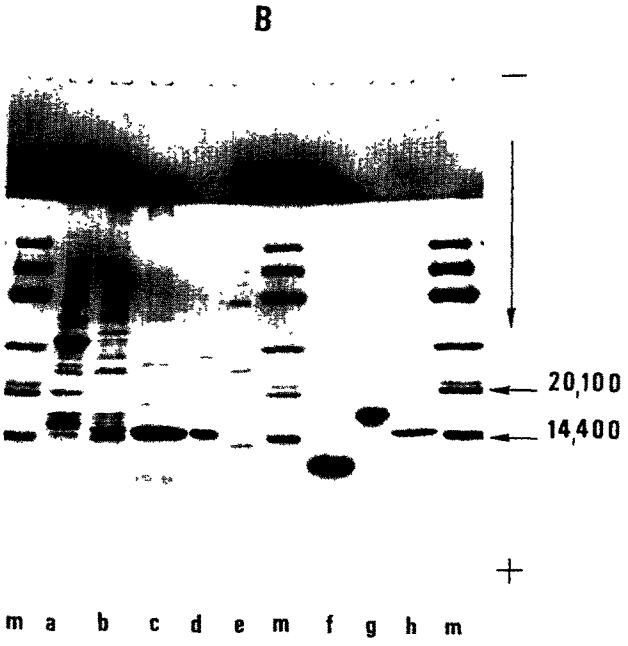
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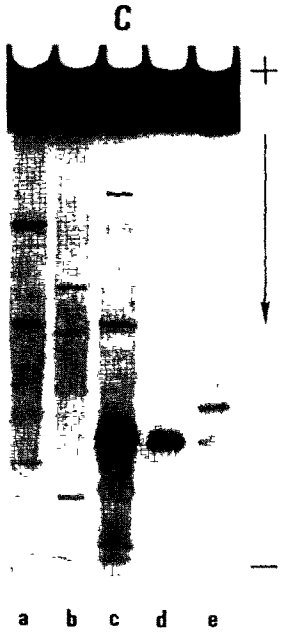
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200 mM NaCl, 1 mM Na_2EDTA at pH 7.4 in the presence of 0.1 mM DFP, and isopycnic centrifugation was performed for 68 h at $120000 \times g$. The acid-soluble proteins were extracted from the nucleoprotein complex overnight at 4°C with 0.25 M HCl under gentle agitation. Proteins were precipitated by 6 volumes of acetone at −20°C and redissolved in 0.01 M HCl.

Protein HMb was prepared by affinity chromatography on a DNA-cellulose column as described in [16] with the following modifications: bacteria were suspended in 10 mM sodium phosphate, 1 mM Na_2EDTA buffer adjusted to pH 6.8 (buffer A) containing 2 M NaCl, 0.1 mM DFP and 1 mM 2-mercaptoethanol, and the DNA-cellulose made with *Rhizobium meliloti* DNA was eluted with increasing concentrations of NaCl in buffer A as indicated in fig.1A. Protein HMb was purified by gel filtration chromatography on a Sephadex G100 column (90×1.6 cm) equilibrated and eluted with 0.01 M HCl saturated with chloroform.

Analytical gel electrophoreses and amino acid analyses were performed as indicated in [16,17], respectively.

3. RESULTS

A large number of protein species are retained on DNA bound to cellulose, but all of them cannot be considered as chromosomal proteins [12]. Therefore the preparation of the nucleoprotein complex is essential for identifying the proteins associated with the DNA in vivo. The *M. barkeri* nucleoprotein complex was prepared in the presence of 0.2 M NaCl to avoid non-specific binding of cytoplasmic or membranous proteins [14]. A protein of apparent M_r 14500 is the major component extracted from the nucleoprotein by 0.25 M HCl (fig.1B, lane c). By analogy with the *T. acidophilum* protein HTa and *S. acidocaldarius* protein HSa, this protein was named HMb.

Since protein HMb was recovered with a low yield from the nucleoprotein, it was prepared simultaneously by affinity chromatography on a DNA-cellulose column. The *M. barkeri* protein fractions eluted from the DNA-cellulose column (fig.1A) were analysed by polyacrylamide gel electrophoresis in the presence of SDS (fig.1B) and in 0.9 M acetic acid, 2.5 M urea (fig.1C). The identity of the major protein eluted in fraction 3 at a molarity of NaCl between 0.32 and 0.40 M with protein HMb was ascertained by the following criteria: first, it is the only protein recovered by affinity chromatography showing an electrophoretic mobility identical to that of protein HMb in two different electrophoretic systems; secondly, both proteins have the same retention times on reverse-phase high-pressure liquid chromatography (not shown) and identical amino acid compositions.

Protein HMb was purified to homogeneity by gel filtration chromatography on Sephadex G100 as assessed by polyacrylamide gel electrophoresis (Fig.1B, lane h). Moreover, the purity of the protein was confirmed by the identification of only one amino acid derivative (PTH-Ser) at its N-terminus.

The amino acid composition of protein HMb is presented in table 1. Protein HMb is devoid of cys-

←

Fig.1. (A) Elution pattern of *M. barkeri* proteins fractionated on a DNA-cellulose column. The column (15×2.5 cm) was eluted at a flow rate of 40 ml/h with a gradient of NaCl (– – –) in buffer A (see section 2). The effluent was monitored at 220 nm (——) and fractions of 6.7 ml were collected. Protein HMb is eluted in the hatched peak (peak 3). (B) SDS-polyacrylamide gel electrophoresis of *M. barkeri* and *E. coli* DNA binding proteins. lanes a, b, d, e correspond respectively to fractions 1, 2, 3, 4 eluted from the DNA-cellulose in (A); c, acid extract of the nucleoprotein complex; f, *E. coli* protein HU; g, *E. coli* protein HI; h, protein HMb; m, M_r markers: phosphorylase *b* (M_r 94000), bovine serum albumin (M_r 67000), ovalbumin (M_r 43000), carbonic anhydrase (M_r 30000) soybean trypsin inhibitor (M_r 20100), α-lactalbumin (M_r 14400). Samples (7 μg) were treated with 5% SDS, 2% 2-mercaptoethanol for 3 min at 100°C and run at 40 mA for 150 min on a 5–30% polyacrylamide gradient gel [22]. Electrode buffer (Tris-glycine, pH 8.3) and gel buffer (Tris-HCl, pH 8.9) were made 0.1% in SDS. The gels were stained with 0.5% Coomassie blue R-250 in acetic acid/ethanol/water (1:2:7, v/v/v) for 15 h. The gels were destained by diffusion in acetic acid/ethanol/water (4:5:31, v/v/v). (C) Polyacrylamide gel electrophoresis of *M. barkeri* DNA binding proteins in 0.9 M acetic acid, 2.5 M urea. Lanes a–e are as in (B). Samples (3 μg) were dissolved in 0.01 M HCl, 8 M urea, 0.5 M 2-mercaptoethanol and were run at 22 mA for 4 h on a gel containing 17% acrylamide. Gel was stained and destained as in (B).

Table 1

Amino acid compositions of protein HMb and of other chromosomal proteins

	Archaebacteria				Eubacteria	Eukaryote
	HMb		HSa	HTa	HU	HCc
	M. barkeri		*S. acidocaldarius* [12]	*T. acidophilum* [11]	*E. coli* [16]	*Crypthecodinium cohnii* [19]
Amino acids	(mol%)	(Residues)	(mol%)	(mol%)	(mol%)	(mol%)
Aspartic acid	10.3	12.7 (13)[e]	9.1	5.6	8.9	5.5
Threonine	4.3[a]	5.3 (5)	3.7	5.6	6.8	7.2
Serine	3.4[a]	4.2 (4)	8.2	7.8	4.5	4.5
Glutamic acid	13.0	16.0 (16)	10.8	13.3	9.5	9.0
Proline	6.4	7.9 (8)	3	3.3	2.6	4.3
Glycine	6.8	8.3 (8)	9.5	6.7	7.2	7.6
Alanine	6.4	7.9 (8)	7.1	7.8	18.5	15.8
Cysteine	0[b]	0	1.8	0	0	trace
Valine	7.0[c]	8.6 (9)	4.5	8.9	7.4	7.3
Methionine	2.3	2.9 (3)	1.1	1.1	1.2	0.9
Isoleucine	4.1[c]	5.1 (5)	4.1	7.8	6.6	3.1
Leucine	3.5[c]	4.3 (4)	14.7	3.3	6.9	7.5
Tyrosine	0	0	2.8	1.1	0	3.6
Phenylalanine	4.1	5.1 (5)	1.8	5.6	3.3	2.6
Histidine	2.2	2.7 (3)	0.2	0	0.9	2.1
Lysine	15.7	19.3 (19)	10.2	15.6	11.1	17.8
Arginine	8.9	11.0 (11)	5.3	6.7	4.7	3.6
Tryptophan	1.5[d]	1.9 (2)	nd	0	0	nd
Total	99.9	123				
M_r		14360	14500	9934	9500	12000
% basic amino acids	26.8		15.7	22.3	16.7	23.5

[a]Values for threonine and serine were obtained by linear extrapolation to zero hydrolysis time
[b]Cysteine was determined as cysteic acid after performic acid oxidation [20]
[c]72 h hydrolysis values
[d]Determined after hydrolysis in 3 N mercaptoethanesulfonic acid [21]
[e]Numbers in parentheses are the nearest integers
nd = not determined

teine and tyrosine, it contains two residues of tryptophan. This protein is characterized by a high content of basic amino acids (~27%) and dicarboxylic amino acids (~23%). With 123 amino acid residues, the calculated M_r of protein HMb is 14360.

4. DISCUSSION

The acid-soluble protein HMb of M_r 14360 constitutes the major protein of the nucleoprotein complex of *M. barkeri* (strain MS). Protein HMb is not homologous to any of the eubacterial or archaebacterial DNA binding proteins. It differs in size and in amino acid composition from HU-type proteins [11,16] and from the *Sulfolobus solfataricus* DNA binding protein [18]. Chromosomal proteins from *M. thermoautotrophicum* [14] and from *S. acidocaldarius* (protein HSa) [12] have a molecular size similar to that of protein HMb but exhibit different physico-chemical characteristics. Thus, the protein HMb is acid soluble whereas the

M. thermoautotrophicum protein is not. On the other hand, protein HSa is distinct from protein HMb particularly with respect to the amounts of lysine, arginine and leucine.

Elucidation of protein HMb amino acid sequence will allow us to determine if this protein shares some structural homology with HU-type proteins or with histones. Since phylogenetic data indicate that archaebacteria are more closely related to eukaryotes than to eubacteria, it is of interest to investigate whether protein HMb may represent an ancestral histone. It is also of interest to know if this protein can be related to the basic protein HCc isolated from a lower eukaryote, the dinoflagellate *Crypthecodinium cohnii* which lacks histones [19].

Knowledge of the properties of protein HMb and of archaebacterial nucleoid structure will serve as a model for better understanding the radical difference in chromatin organization between eubacteria and eukaryotes.

ACKNOWLEDGEMENTS

The authors are grateful to M.J. Dupire, T. Ernout and A. Hemez for their skillful technical assistance. The work was supported by a grant from the Centre National de la Recherche Scientifique.

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