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CHARACTERIZATION AND STRUCTURAL STUDY OF THE DNA-BINDING PROTEIN HRm FROM *Rhizobium meliloti*

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SUMMARY.- The protein HRm, a DNA-binding HU-like protein isolated from *Rhizobium meliloti*, is a single polypeptide chain (M_r 9 300) of 90 residues. Protein HRm exhibits lower affinity for double stranded DNA than *E. coli* protein HU. The sequence of the first 54 amino acid residues was established by automated Edman degradation of the protein. Many substitutions were observed by comparison with amino acid sequences of HU-like proteins from other bacteria. However a sequence of 7 residues is almost invariable and may be important in the function of the protein.

INTRODUCTION

In Prokaryotes the chromosomal DNA is packaged in a compact structure referred to as nucleoid (1) and, in electron microscopy, appears to be condensed in a beaded structure (2). The *E. coli* DNA-binding protein HU which has been shown to be associated with the nucleoid prepared at low ionic strength (3), forms *in vitro* with λ DNA a stable complex (4). Moreover protein HU can form nucleosome-like structures with circular double-stranded DNA (5). Therefore protein HU seems to be involved in the condensation of the chromosomal DNA. *E. coli* protein HU is a heterocomplex constituted of two polypeptide chains termed HU-1 and HU-2, of 90 residues each (6-8). Closely related proteins have been isolated from other bacteria (3, 9-12). Up to now, only the proteins HU-1 and HU-2 from *E. coli* (7, 8) and the protein HTa from *T. acidophilum* (13) have been completely sequenced. The partial sequences of the HU-like proteins from *P. aeruginosa* (12) and from the Cyanobacterium *Synechocystis* (14) have also been reported.

In this paper, we present the characterization and the structural study of protein HRm, a DNA-binding HU-like protein isolated from *Rhizobium meliloti*.

Abbreviations.- DFP, diisopropylfluorophosphate ; DMAA, dimethylallylamine ; EDTA, ethylene diamine tetraacetic acid ; HPLC, high pressure liquid chromatography ; M_r, relative molecular mass ; PTH, phenylthiohydantoin ; SDS, sodium dodecyl sulfate.

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MATERIALS AND METHODS

Rhizobium meliloti, strain 2011 Str 3 (streptomycin resistant mutant) was provided by J. Denarie (INRA, Versailles, France) and was grown as described in (15).

The protein was prepared by a procedure adapted from (16) and used for the preparation of *E. coli* protein HU (3). Bacteria (100 g) were suspended at 4°C in 400 ml of 20 mM Tris-HCl (pH 8.0), 1 mM Na_2 EDTA, 1.7 M NaCl, 0.1 mM DFP. Cells were disrupted in a French press with a pressure of 17 000 p.s.i. The high salt concentration is necessary to dissociate the nucleo-protein complex. The crude extract was centrifuged at 8 000 g for 1 h to remove the cell debris. An aqueous solution of polyethylene glycol 6 000 (30 %; w:v) was slowly added to the supernatant to give a final polyethylene glycol concentration of 10 % (w:v). After gentle stirring for 30 min the suspension was centrifuged at 8 000 g for 1 h to remove the precipitated nucleic acids and the supernatant was dialysed three times against 5 liters of 0.05 M NaCl in 20 mM Tris-HCl (pH 8.0), 1 mM Na_2 EDTA, 1 mM 2-mercaptoethanol. The dialysed material, cleared by centrifugation at 8 000 g for 1 h, was made 10 % glycerol (v:v) and applied to a double-stranded DNA-cellulose column (10 x 2.5 cm) at a flow rate of 40 ml/h. The DNA-cellulose column was prepared as in (17) with chicken erythrocyte DNA. The column was washed with 0.05 M NaCl in 20 mM Tris-HCl (pH 8.0), 1 mM Na_2 EDTA, 10 % (v/v) glycerol. The column was then eluted with increasing concentrations of NaCl in the Tris-EDTA-glycerol buffer : 0.1 M NaCl, then a linear gradient from 0.1 to 0.175 M NaCl, and finally 0.4 and 2.0 M NaCl.

The protein HRm, eluted from the DNA-cellulose column with the linear gradient of NaCl, was purified by gel filtration chromatography on a Sephadex G-100 superfine column (90 x 2.5 cm) eluted with 0.01 M HCl saturated with chloroform.

Analytical electrophoresis was performed on polyacrylamide slab gel (120 x 80 x 1 mm) (18) in three different conditions : A, in SDS using the Laemmli buffer system (19) ; B, in 0.9 M acetic acid, 2.5 M urea (20) using a 17 % acrylamide concentration ; C, in 0.9 M acetic acid, 6 M urea, and 0.38 % Triton X-100 using a 17 % acrylamide concentration (21).

Amino acid analyses were performed as described in (8).

Automated Edman degradation of the protein HRm was carried out in a Beckman 890 C Sequencer using a DMAA program (10 2974) in the presence of polybrene (22). PTH-amino acids were identified by HPLC on a column of C_{18} micro Bondapak (Waters Associates) as described in (23). Alternatively the PTH-Phe and PTH-Ile as well as PTH-Met and PTH-Val which are not separated by the previous system were identified by gas-chromatography (24) on a Hewlett-Packard 5830 A unit. The PTH-Arg was identified by HPLC on a column of micro Bondapak Phenyl (Waters Associates) using the buffer system described in (23).

RESULTS AND DISCUSSION

The elution pattern of *R. meliloti* proteins fractionated on a DNA-cellulose column is presented in fig. 1-A. Fraction I corresponds to a large amount of material which has no affinity for the DNA and therefore is not retained by the column. As assessed by polyacrylamide gel electrophoresis (Fig. 1-B) the protein HRm is mainly eluted in the fraction III by a molarity in NaCl comprised between 0.10 and 0.15 M. The protein HRm exhibits a lower affinity for the DNA than *E. coli* protein HU which is eluted from the same column at about 0.35 M NaCl. *R. meliloti* protein HRm and *B. subtilis* protein HBs (11) exhibit a similar affinity for the DNA whereas protein HTa from *T. acidophilum* tight-

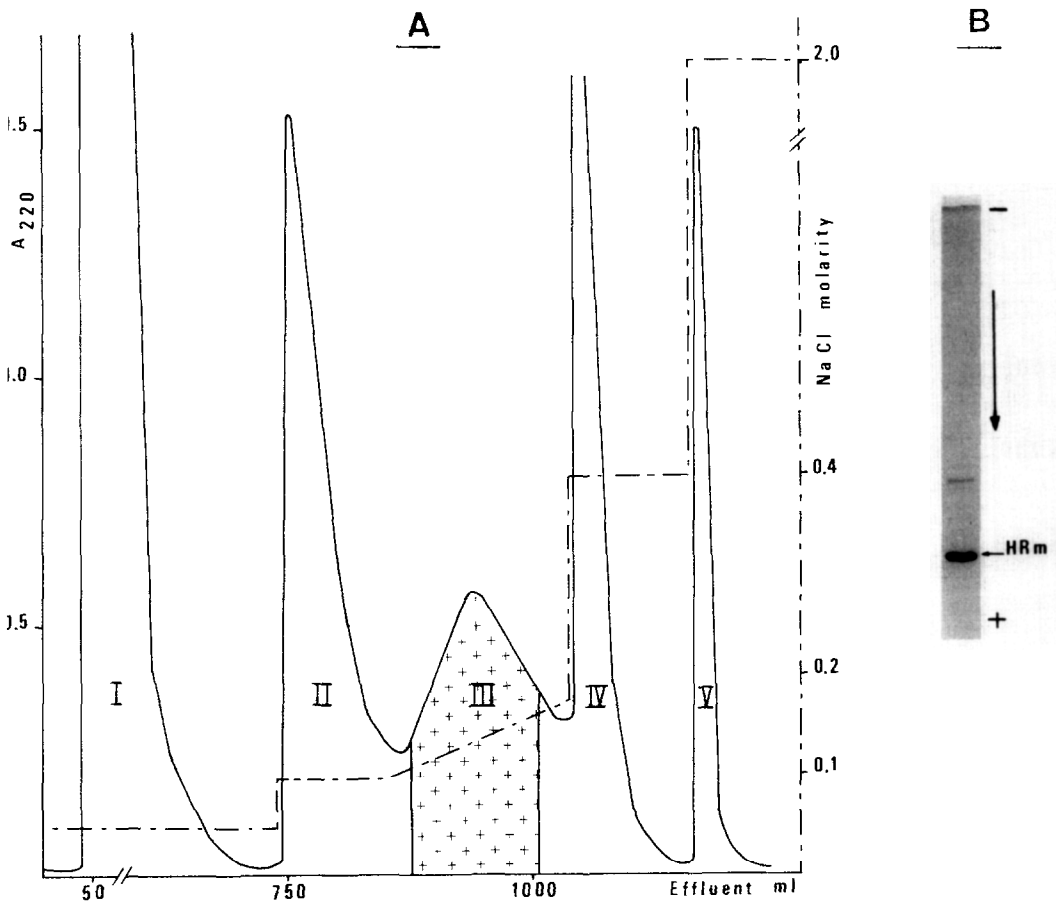

FIGURE 1.- A : Elution pattern of *R. meliloti* proteins fractionated on a DNA-cellulose column. An extract of *R. meliloti* bacteria prepared as described under "Materials and Methods" was applied to the column (10 x 2.5 cm) equilibrated with 0.05 M NaCl in 20 mM Tris-HCl (pH 8.0), 1 mM Na_2 EDTA, glycerol 10 % (v:v). The column was eluted at 40 ml/h with a gradient of NaCl in the same buffer as indicated by (– –). The linear gradient of NaCl was from 0.1 to 0.175 M (2 x 100 ml). The effluent was monitored continuously at 220 nm (——) and fractions of 5 ml were collected. B : SDS gel electrophoresis (19) of fraction III. The experimental conditions were as indicated in Fig. 2-A.

ly binds to the DNA and is eluted from the DNA-cellulose column at about 0.75 M NaCl (25).

R. meliloti protein HRm has a M_r of 10 000 as estimated by SDS gel electrophoresis (Fig. 2-A). On polyacrylamide gel electrophoresis in 0.9 M acetic acid, 2.5 M urea, the protein HRm exhibits a cathodic mobility inferior to that of protein HU (Fig. 2-B) suggesting that protein HRm is less basic than protein HU. Moreover in the presence of Triton X-100 (Fig. 2-C) the protein HRm shows a single band whereas the protein HU from *E. coli* migrates as two bands corresponding to proteins HU-1 and HU-2.

The amino acid composition of the protein HRm from *R. meliloti* is presented in Table 1. On the basis of four residues of phenylalanine present in the

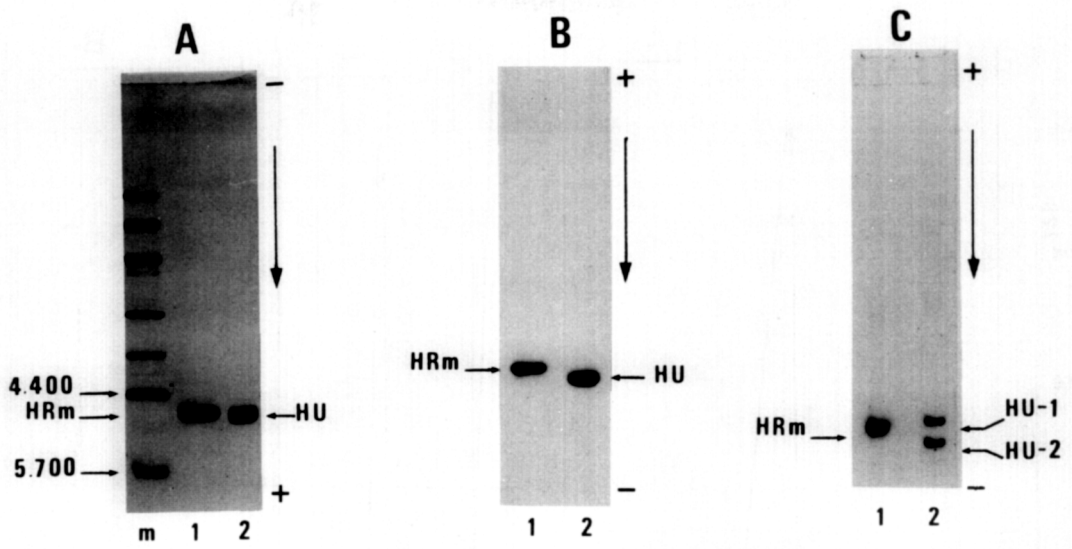

FIGURE 2.- Polyacrylamide gel electrophoresis of purified *R. meliloti* protein HRm (slot 1) and *E. coli* protein HU (slot 2). 2-A, in the presence of SDS on a 5-30 % polyacrylamide gradient gel (19). 2-B, in 0.9 M acetic acid, 2.5 M urea (20) on a gel containing 17 % acrylamide. 2-C, in 0.9 M acetic acid, 6 M urea and 0.38 % Triton X-100 (21) on a gel containing 17 % acrylamide. Electrophoresis were performed on a slab gel (18) at room temperature. In the case of Fig. 2-A samples (7 µg) were treated with 5 % SDS, 2 % 2-mercaptoethanol for 2 min at 100°C and run at 40 mA for 150 min. Electrode buffer (Tris-glycine, pH 8.3) and gel buffer (Tris-HCl, pH 8.9) were made 0.1 % in SDS. m, molecular mass markers : Phosphorylase b (94,000), Bovine serum albumin (67,000), Ovalbumin (43,000), Carbonic anhydrase (30,000), Soybean trypsin inhibitor (20,100), α-Lactalbumin (14,400) and Insulin (5,700). In the case of Fig. 2-B and 2-C, 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 3 h. 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 in the case of Fig. 2-A and for 3 h in the case of Fig. 2-B and 2-C. The gels were destained by diffusion in acetic acid/ethanol/water (7:20:73, v/v/v).

protein, the total number of amino acid residues is 90 which corresponds to a calculated M_r of 9 311. This value is in good agreement with that estimated by SDS gel electrophoresis. The protein is characterized by a high content of hydrophobic residues which represent 42 % of the total amino acids and is devoid of cysteine, tyrosine, histidine and tryptophan. Similar features were observed for proteins HU-1 and HU-2 from *E. coli* but protein HU-2 contains one histidine residue.

Automated Edman degradation of the *R. meliloti* protein HRm was performed in two different runs, with samples of 260 and 420 nM, respectively. The repetitive yield calculated from the alanine residues at positions 8 and 24 was 94 % in both experiments. Positive identifications were achieved through step 54. The sequence of the first 54 residues of the protein HRm thus determined by automated Edman degradation was confirmed by structural studies of the peptides isolated from a tryptic hydrolysate of the protein (Fig. 3). The identification of only one PTH amino acid at each step of the Edman degradation indi-

Table 1

Amino acid composition of *R. meliloti* protein HRm.

	R. meliloti		*E. coli*	
	Mol %	Residues[a]	Residues[a]	
			HU-1	HU-2
Aspartic acid	14.17	12.66 (13)[b]	9	7
Threonine[c]	3.34	2.99 (3)	5	7
Serine[c]	7.68	6.86 (7)	5	4
Glutamic acid	7.07	6.32 (6)	6	11
Proline	3.30	2.94 (3)	2	2
Glycine	10.93	9.77 (10)	8	6
Alanine	14.11	12.61 (13)	19	15
Cysteine	0	0	0	0
Valine	10.14	9.06 (9)	6	7
Methionine	0.79	0.70 (1)	1	1
Isoleucine[d]	3.32	2.97 (3)	7	5
Leucine	5.80	5.18 (5)	5	7
Tyrosine	0	0	0	0
Phenylalanine	4.48	4	3	3
Histidine	0	0	0	1
Lysine	9.25	8.26 (8)	9	11
Arginine	5.62	5.01 (5)	5	3
Tryptophan[e]	0	0	0	0
TOTAL		90	90	90

[a]The results are expressed in number of residues per mole of protein.

[b]Number in parentheses is the nearest integer.

[c]Values for threonine and serine were obtained by linear extrapolation to zero hydrolysis time.

[d]72 h hydrolysis values.

[e]Determined spectrophotometrically.

The amino acid composition of *E. coli* protein HU (8) is given for comparison.

cates that *R. meliloti* protein HRm consists indeed of a single polypeptide chain as already evidenced by gel electrophoresis performed in the presence of Triton X-100 (Fig. 2-C). The sequence of the amino terminal half of the molecule contains 24 hydrophobic residues. The hydrophobic character is outstandingly important in the regions comprising residues 6-11, 24-29 and 42-52.

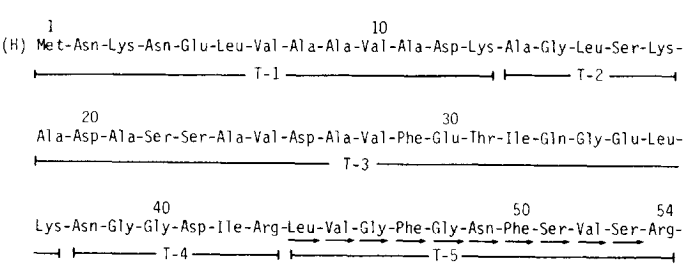

FIGURE 3.- Amino-terminal amino acid sequence of *R. meliloti* protein HRm. The tryptic peptides, lettered T, are numbered according to their position in the polypeptide chain. (→) indicates manual Edman degradation of peptide T-5.

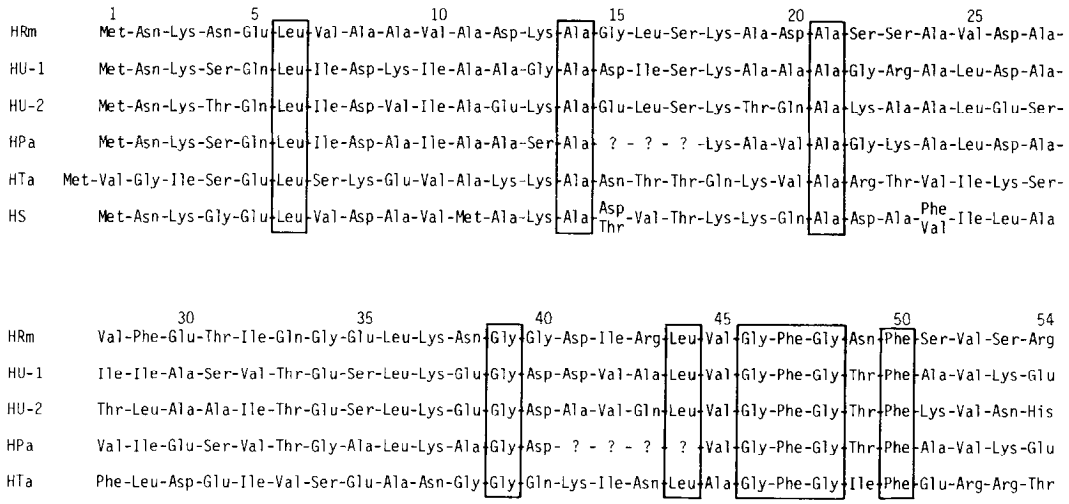

FIGURE 4.- Comparison of the amino-terminal amino acid sequences of DNA-binding HU-like proteins from different bacteria : HRm from *R. meliloti*, HU-1 and HU-2 from *E. coli* (8), HPa from *P. aeruginosa* (12), HTa from *T. acidophilum* (13), HS from the Cyanobacterium *Synechocystis* (14). Boxes indicate highly conserved residues.(?), not identified amino acid residues in *P. aeruginosa*.

A comparison of the sequence of protein HRm with that of other HU-like DNA binding proteins shows many substitutions (Fig. 4) (8, 12-14). However 9 residues indicated by boxes appear highly conserved during the evolution. Particularly the sequence between residues 44 and 50 is almost invariable and may be important in the function of the protein. The two phenylalanine residues at positions 47 and 50 can be made more accessible for interaction since the adjacent glycine residues give to this sequence a high potential for β-turn conformation. Indeed a β-turn conformation is predicted for this region when a statistical predictive method (26) is applied to proteins HU-1 and HU-2 from *E. coli* (27).

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