Affinity of protein HU for different nucleic acids

Askild Holck and Kjell Kleppe*

Department of Biochemistry, University of Bergen, Bergen, Norway

Received 18 March 1985

The binding of protein HU from *Escherichia coli* to nucleic acids was investigated by affinity chromatography under various conditions, by a nitrocellulose retention assay and by isopycnic centrifugations in metrizamide gradients. The results indicate that HU has a preference for binding to RNA and single-stranded DNA over double-stranded DNA. The affinity of HU for supercoiled DNA was also less than that of the corresponding relaxed DNA.

Chromatography *Affinity* *Bacterial protein* *DNA binding*

1. INTRODUCTION

The folded chromosome of *Escherichia coli* is visualized in vivo by microscopy as a dense body termed the nucleoid. Much work has been carried out to elucidate the packaging of the chromosome in this nucleoid. RNA, proteins and the cell envelope might be important factors in stabilizing and maintaining this highly organized structure [1,2]. It is therefore of interest to identify and characterize the proteins which bind to DNA [3]. One such protein is HU, a basic, low-M_r, thermostable 'histone-like' protein. This protein is relatively abundant and homologous proteins have been found in several other species of bacteria. Some reports argue that HU exists in the cell as a heterotypic dimer (ab) [4], while others suggest a tetramer $(ab)_2$ [5]. The complete amino acid sequences of both chains have been determined [6], and the 3-dimensional structure of the crystallized protein has been published [7]. HU binds to both double-stranded- (ds) and single-stranded-DNA (ssDNA), as well as to RNA [5]. It forms unstable beaded structures in vitro with circular DNA in the presence of nicking-closing enzyme [8]. The nature of the interaction between HU and DNA has been investigated by ^{1}H-NMR by Lammi et al. [9,10]. This study describes in some detail the binding characteristics of HU to different nucleic acids.

* To whom correspondence should be addressed

2. MATERIALS AND METHODS

E. coli 16 S and 23 S RNA were obtained from Boehringer Mannheim. Torula yeast RNA was from sigma, and *Ava*I restriction enzyme from New England Biolabs. Metrizamide was supplied by Nyegaard, Norway. Protein HU was purified essentially as described by Rouviere-Yaniv and Gros [11] or obtained as a gift (NS1 and NS2) from Dr C. Gualerzi, Max-Planck-Institut für Molekulare Genetik, Abt. Wittman, Berlin.

Purified DNA from *E. coli* B was covalently coupled to CNBr-activated Sepharose 4B by the method of Arndt-Jovin et al. [12]. To obtain ssDNA-Sepharose, dsDNA-Sepharose was washed with 0.1 M NaOH. Half of the amount of bound nucleic acid was eluted by this treatment. Torula yeast RNA and *E. coli* rRNA were coupled to Sepharose in the same manner.

Affinity chromatography was performed by binding HU to nucleic acid-Sepharose columns ($V = 100\ \mu l$) in a low salt buffer B, containing 20 mM Tris-HCl, 10 mM EDTA, 0.5 mM DTE, 10% glycerol and 50 mM NaCl, pH 8.0. After washing the column, the protein was eluted by increasing the NaCl concentration stepwise to 0.6 M. Various

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30 © 1985 Federation of European Biochemical Societies

modifications of the buffers are indicated in the text. The eluted protein was subjected to one-dimensional SDS-polyacrylamide gel electrophoresis as described by Laemmli and Favre [13] and the gels were stained by the silver staining technique [14]. The gels were subsequently scanned with a Zeineh soft laser scanning densitometer (Biomed Instruments, USA) and the peak areas determined by a Hewlett Packard 3390A integrator.

Supercoiled ϕX174 RFI DNA was purified by isopycnic density gradient centrifugation in CsCl twice. A fraction of the isolated DNA was linearized by digestion with *Ava*I. The ϕX174 DNA preparations migrated as single bands upon gel electrophoresis in 1% agarose with ethidium bromide. Isopycnic density gradient centrifugation in metrizamide was performed in a Beckmann L8-70 ultracentrifuge at 37 000 rpm and 4°C for 16 h 30 min using a V65Ti rotor.

3. RESULTS AND DISCUSSION

Fig.1 shows the result of a typical affinity chromatography experiment. Here HU was bound to ssDNA-Sepharose and eluted by increasing the ionic strength in the presence of 1 mM spermidine. HU eluted at 0.3 M NaCl.

The difference in the affinity of HU for dsDNA and ssDNA is clearly visualized in fig.2, where HU eluted at 0.2 M NaCl when bound to dsDNA and at 0.3 M in the case of ssDNA. The effect of binding in the presence of various counterions, known to exist in the cell in high concentrations, was also studied. Fig. 3A,B shows the effect on the binding of HU to ds and ssDNA in the presence of increasing amounts of spermidine. HU eluted at progressively lower ionic strengths as the concentration of spermidine was raised. At 5 mM spermidine the affinity of HU for dsDNA was weakened so much that it was slowly washed off the column at 50 mM NaCl. Similar experiments with 3 mM Mg^{2+} or changing the pH of the buffer to pH 7.0 gave only minimal effects (not shown).

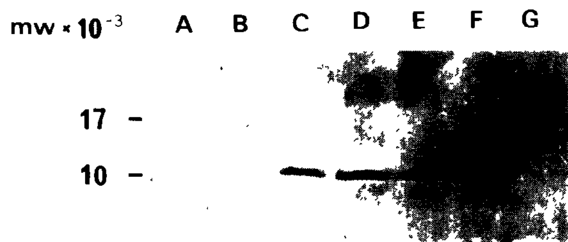

Fig.1. SDS-polyacrylamide gel electrophoresis of eluates from a typical affinity chromatography experiment: The affinity of HU for ssDNA-Sepharose in the presence of 1 mM spermidine. Protein HU was bound to ssDNA-Sepharose in buffer B and eluted by increasing the ionic strength stepwise. Spermidine (1 mM), was added to all buffers. Lane A, buffer B (see section 2); 50 mM NaCl; B–G, eluates of increasing ionic strength, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 M NaCl, respectively.

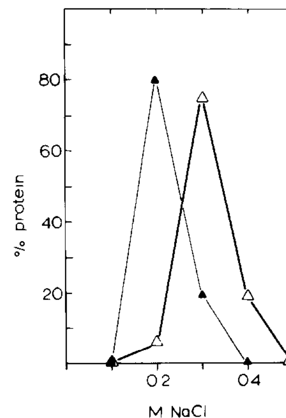

Fig.2. Binding of HU to ds and ssDNA-Sepharose. Eluates from affinity columns were subjected to SDS gel electrophoresis (as in fig.1). The gels were scanned and total eluted protein set to 100%. (▲) Elution profile from dsDNA-Sepharose. (△) Elution profile from ssDNA-Sepharose.

Likewise, the affinity of HU for RNA was investigated (fig.4). The results showed that HU had a significantly higher affinity for *E. coli* ribosomal RNA than unspecific foreign RNA, and was equal to the affinity for ssDNA.

The interaction of HU with supercoiled ϕX174 RFI DNA was very weak as evident from fig.5A. In this experiment HU was complexed to linearized and supercoiled replicative forms of ϕX174 DNA and the DNA-protein complexes were retained on nitrocellulose filters. In the case of supercoiled ϕX174 RFI less than 30% of the DNA was retained on the filters at 50 mM KCl. Similar results were obtained when HU-DNA complexes were focused by isopycnic gradient centrifugation in metrizamide as shown in fig.5B. A DNA-protein complex having a density of 1.22 $g \cdot cm^{-3}$ indicates a protein-DNA ratio of 1.8:1 [15]. At low ionic

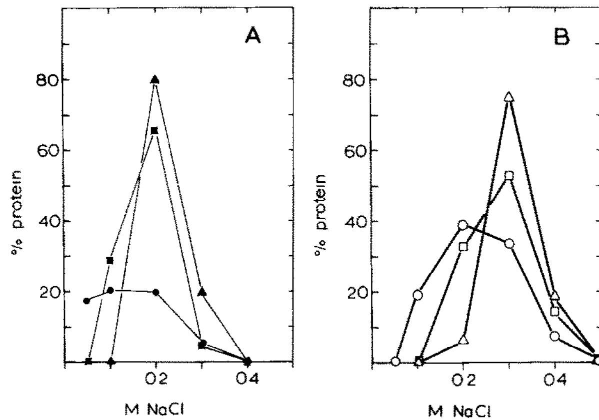

Fig.3. Binding of HU to DNA in the presence of spermidine. The data were obtained as described in fig.2. (A) dsDNA-Sepharose chromatography, (▲) 0 mM, (■) 1 mM, (●) 5 mM spermidine added. (B) ssDNA-Sepharose chromatography, (△) 0 mM, (□) 1 mM, (○) 5 mM spermidine added.

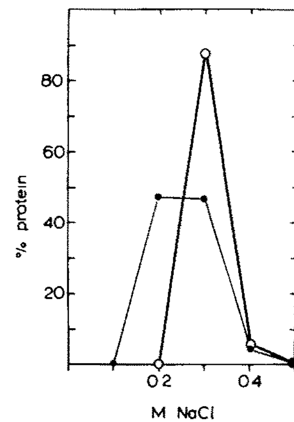

Fig.4. Binding of HU to RNA. Experimental details were similar to those described in fig.2. (●) Elution profile from torula yeast RNA-Sepharose. (○) Elution profile from *E. coli* 16 S, 23 S rRNA-Sepharose.

strength, 15 mM NaCl, HU seemed to bind nearly quantitatively to linearized DNA, whereas only 30% of HU was found associated with the supercoiled DNA under the same conditions.

This study indicates that HU has a preference for binding to RNA and ssDNA over dsDNA. The HU-dsDNA complexes dissociate at relative low salt concentrations and are also affected by low concentrations of spermidine. In procaryotic organisms the chromosome exists in a highly supercoiled form. The affinity of HU for supercoiled DNA is very low. Together these findings raise some doubts as to whether HU functions as a bacterial analogue to eucaryotic histones.

In bacterial chromatin isolated under mild conditions, HU has been found to be associated with the RNA rather than with DNA [16]. It has been suggested that HU might have a regulatory function in the cell. In vitro trancription of DNA is stimulated by HU [11] and it has been reported

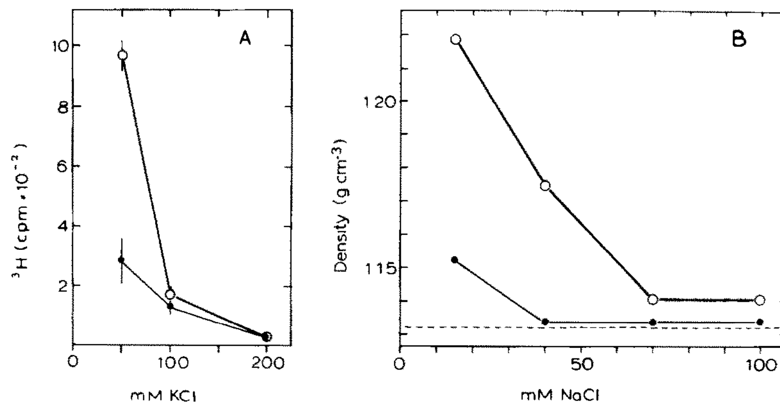

Fig.5. The binding of HU to supercoiled ϕX174 DNA RFI and linearized ϕX174 DNA RFIII. (A) Retention of HU-DNA complexes on nitrocellulose filters. 50 ng [^{3}H]DNA (1100 cpm) was incubated with 100 ng HU in 10 mM Tris-HCl, pH 8.0, 10 mM β-mercaptoethanol with different salt concentrations as indicated. (○) Linearized ϕX174 DNA RFIII, (●) supercoiled ϕX174 DNA RFI. Each point is the average of 3 measurements, σ is indicated by the vertical lines. (B) HU-DNA complexes in metrizamide. HU was mixed with DNA in a 2:1 ratio in 1.5 mM Na-citrate, pH 7.0, prior to centrifugation at different salt concentrations. (○) HU-linearized ϕX174 DNA RFIII. (●) HU-supercoiled ϕX174 DNA RFI. Both ϕX174 DNA RFI and RFIII alone banded at a density of 1.32 g$\cdot$cm^{-3}.

that it also stimulates replication and suppresses the initiation of replication at sites other than the *oriC* [17]. Moreover, HU has been isolated as a protein tightly associated with the 30 S subunit of ribosomes [18]. Thus, the in vivo function of HU still remains uncertain.

ACKNOWLEDGEMENTS

This study was supported by grants from Norwegian Research Council for Science and Humanities.

REFERENCES

[1] Pettijohn, D.E. (1982) Cell 30, 667-669.
[2] Kleppe, K., Øvrebø, S. and Lossius, I. (1979) J. Gen. Microbiol. 112, 1-13.
[3] Geider, K. and Hoffmann-Berling, H. (1981) Annu. Rev. Biochem. 50, 233-260.
[4] Rouviere-Yaniv, J. and Kjeldgaard, N.O. (1979) FEBS Lett. 106, 297-300.
[5] Berthold, V. and Geider, K. (1976) Eur. J. Biochem. 71, 443-449.
[6] Mende, L., Timm, B. and Subramanian, A.R. (1978) FEBS Lett. 96, 395-398.
[7] Tanaka, I., Krysztof, A., Dijk, J., White, S.W. and Wilson, K.S. (1984) Nature 310, 376-381.
[8] Rouviere-Yaniv, J., Yaniv, M. and Germond, J.-E. (1979) Cell, 17, 265-274.
[9] Lammi, M., Paci, M., Pon, C.L., Losso, M.A., Miano, A., Pawlik, R.T., Gianfranceschi, G.L. and Gualerzi, C.O. (1983) in: Proteins Involved in DNA Replication (Hübscher, H. and Spadari, S. eds) pp. 467-477, Plenum, New York.
[10] Lammi, M., Paci, M. and Gualerzi, C.O. (1984) FEBS Lett. 170, 99-104.
[11] Rouviere-Yaniv, J. and Gros, F. (1975) Proc. Natl. Acad. Sci. USA 72, 3428-3432.
[12] Arndt-Jovin, D.J., Jovin, T.M., Bähr, W., Frischauf, A.-M. and Marquardt, M. (1975) Eur. J. Biochem. 54, 411-418.
[13] Laemmli, U.K. and Favre, M. (1973) J. Mol. Biol. 80, 575-599.
[14] Marshall, T. and Latner, A.L. (1981) Electrophoresis 2, 228-234.
[15] Rickwood, D., Birnie, G.D., MacGillivray, A.J. (1975) Nucleic Acids Res. 2, 723-733.
[16] Aasland, R., Holck, A., Lossius, I., Haarr, L. and Kleppe, K. (1985) Eur. J. Biochem. submitted.
[17] Kaguni, J.M. and Kornberg, A. (1984) Cell 38, 183-190.
[18] Suryanarayana, T. and Subramanian, A.-R. (1978) Biochim. Biophys. Acta 520, 342-357.