NATIVE *ESCHERICHIA COLI* HU PROTEIN IS A HETEROTYPIC DIMER

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1. Introduction

Escherichia coli contains an abundant DNA binding protein, HU, [1] with a monomeric mol. wt ~10 000 which is readily isolated by affinity chromatography on DNA-cellulose. Similar protein fractions, showing immunological cross reactivity with the *E. coli* protein, have been isolated from *Salmonella typhimurium* (Prigent, J. R.-Y., unpublished), *Bacillus subtilis* (unpublished) and from cyanobacteria [2]. HU is a basic protein which bears similarities to histone H2b in its amino acid composition. Recent in vitro experiments have shown that HU can introduce negative superhelical turns in a relaxed DNA in the presence of a nicking–closing enzyme in a similar fashion to the 4 core histones [3]. A pure preparation of HU, homogeneous on SDS–polyacrylamide gel electrophoresis revealed heterogeneities at specific positions along the polypeptide chain, suggesting the existence of two closely related variants of the HU fractions [4].

Two proteins, NS1 and NS2, similar to HU, have been isolated from native 30 S ribosomal subunits of *E. coli* [5]. The complete amino acid sequences of these two proteins were determined [6] and found to be highly homologous with the N-terminal sequences of HU. This suggested that the NS proteins are identical with our HU fraction. However, we have shown [7] that only a minor fraction of HU (~10%) is bound to the ribosomes. The majority of HU protein is associated with the bacterial nucleoid isolated at low salt [7].

Here we report that the majority of native HU is a heterotypic dimer composed of two closely related proteins HUα, and HUβ. Peptide analysis indicated their homologies to NS2 and NS1, respectively.

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2. Materials and methods

Protein HU isolated from *E. coli* cells W3350 was purified as in [1] by DNA-cellulose chromatography and Sephadex G-100 filtration. After dialysis against buffer containing 10 mM Tris–HCl, (pH 7.5), 1 mM EDTA and 60 mM NaCl, the protein was adsorbed to a pre-equilibrated P11 Whatman phosphocellulose column and eluted with a linear gradient from 60–600 mM NaCl in the same buffer. Proteins were analysed either by conventional 10–25% SDS–polyacrylamide gel electrophoresis [1] or by acid–urea–Triton polyacrylamide gel electrophoresis. This later electrophoresis was performed in a 7.5% polyacrylamide gel containing 25% acetic acid (gel system no. 9 of [8]). The system was modified by the addition of urea (2.5 M) and Triton X-100 (1%) to the separation gel, the spacer gel and the sample buffer. The gel was photopolymerised with riboflavin. Electrophoresis was performed overnight at 120–150 V in the cold room. The dimethyl suberimidate cross-linking was performed according to [9]. The protein samples, dissolved in or dialysed against 0.2 M triethanolamine–HCl buffer (pH 8.2) at 200 μg/ml final conc. were treated for 3 h at room temperature with 2 mg/ml dimethyl suberimidate. After the incubation period, samples were precipitated by 50% trichloroacetic acid, dissolved in SDS–sample buffer and analysed by SDS–polyacrylamide gel electrophoresis. The N-terminal sequencing was done in a Beckman automatic protein sequencer in the labora-

tory of Dr V. Keil using a dimethyl-benzylamine program.

3. Results and discussion

While SDS–polyacrylamide gel electrophoresis of the HU reveals only a single band [1], electrophoresis in strongly acid polyacrylamide gels containing both urea and Triton X-100 shows that the HU fraction is composed of two protein species, HUα and HUβ, in about equal proportions (fig.1). Both urea and Triton X-100 are necessary to resolve the two proteins. Preparative gel electrophoresis permitted the isolation of the two HU fractions (fig.1b,c). Amino acid analysis of these proteins and of peptides isolated from their tryptic digests indicate that protein HUα corresponds to NS2 and HUβ to NS1 (table 1). Furthermore, it was found [6] that the DNA binding protein HD isolated [10] also contains proteins NS1 and NS2. Therefore, NS1 and NS2, HD and HU are identical.

As described [1], phosphocellulose chromatography under non-denaturing conditions resolves HU into one major and two minor fractions (fig.2). SDS gel electrophoresis of each of the three fractions resulted in single bands with identical mobilities. In addition, all three fractions react with anti-HU serum. We have now analysed these fractions on acid–urea–Triton X-100 gels and found that the major peak II contains both HUα and HUβ, whereas the minor fraction I contains only HUα and the minor fraction III only HUβ (fig.2 insert). Furthermore automatic sequence analysis of the first 20 amino acids of HUα (peak I) revealed a unique sequence identical to one of the HU variants (not shown). Since it was shown that native HU exists mainly as a dimer [1], the peaks from the phosphocellulose were analysed by Sephadex G-75 filtration. All three peaks eluted at the position of the dimer (data not shown). Crosslinking by dimethyl-suberimidate followed by SDS–polyacrylamide electrophoresis confirms that the three phosphocellulose fractions contain the proteins in predominantly dimeric form (fig.3). It should be noted that if only traces of trimers and tetramers are observed for the α2 and the αβ fractions, higher proportions of these polymer forms are found for the β2 fraction. Therefore, peak I must be

Table 1
Amino acid composition of HUα and HUβ

	HUα	NS2[a]	HUβ	NS1[a]
Asx	7.2	7	8.4	9
Thr	6.8	7	5.0	5
Ser	4.8	4	6.6	5
Glx	11.3	11	6.8	6
Pro	2.2	2	2.0	2
Gly	7.3	6	9.1	8
Ala	15.2	15	18.4	19
Val	6.7	7	7.2	6
Met	1.0	1	1.0	1
Ile	4.1	5	5.0	7
Leu	6.9	7	5.1	5
Phe	2.3	3	1.8	3
His	1.0	1	0	0
Lys	10.5	11	8.8	9
Arg	3.0	3	5.0	5

[a] The amino acid composition is calculated from complete sequence [6]

The amino acid composition was determined after 20 h hydrolysis in a Beckman 121 M amino acid analyzer. The composition is calculated as no. residues/chain of 90 amino acids

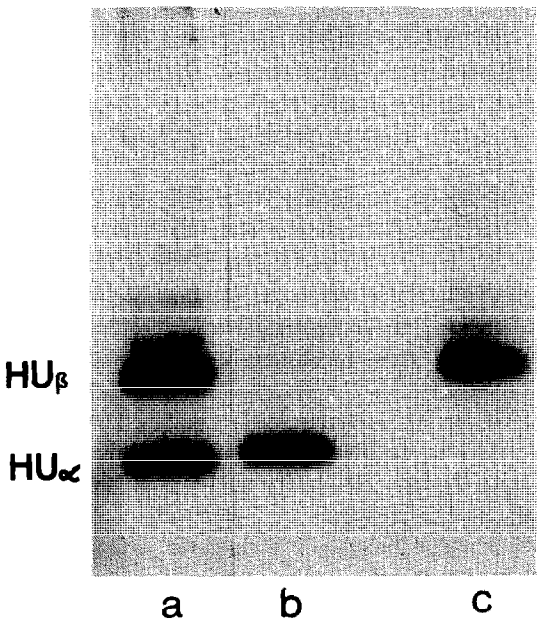

Fig.1. Electrophoretic separation of HU in acid–urea–Triton X-100 polyacrylamide gel. The samples subjected to electrophoresis were: (a) HU; (b) HUα; (c) HUβ.

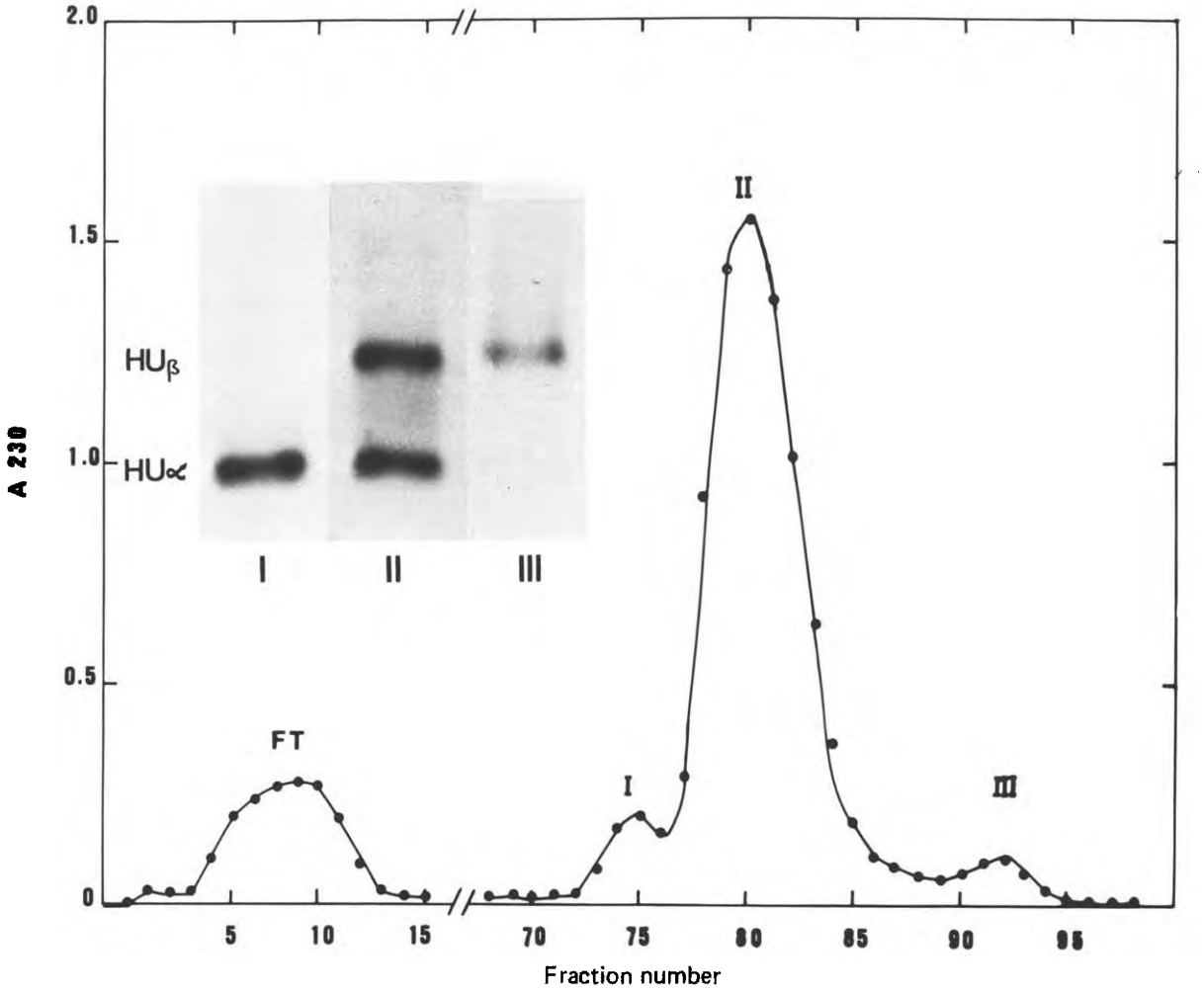

Fig.2. Fractionation of HU by phosphocellulose chromatography. FT is the flow through; the major peak is eluted at 0.4 M NaCl. Insert: fractions I, II and III were analysed on an acid–urea–Triton X-100–polyacrylamide gel as in section 2.

composed of $\alpha 2$, peak II of $\alpha\beta$ and peak III of a majority of $\beta 2$.

When fraction II is rerun on a column of phosphocellulose no minor fraction is generated. This indicates that the homologous dimers ($\alpha 2, \beta 2$) are not, under our conditions, in equilibrium with the mixed dimer ($\alpha\beta$). If the dimeric HU protein were formed in the cell by a random association of the subunits, one would expect to find 25% of each of the homologous dimers. Our results (fig.2) show that the mixed dimer accounts for ~90% of the HU protein. It is therefore most likely that this heterotypic dimer represents the natural form of the HU complex, which however also might exist in tetrameric and (or) higher polymeric forms. The strong sequence homologies between the two subunits allow for a pseudo-symmetrical structure of the polymeric forms of the protein. An ancestral gene of HU which was probably duplicated during evolution gave rise after diversion to two closely related polypeptides chains. The heterotypic dimer of native HU, or higher polymers formed in the presence of DNA, may play a similar role to that of the H3–H4 tetramer in the condensation of DNA [3].

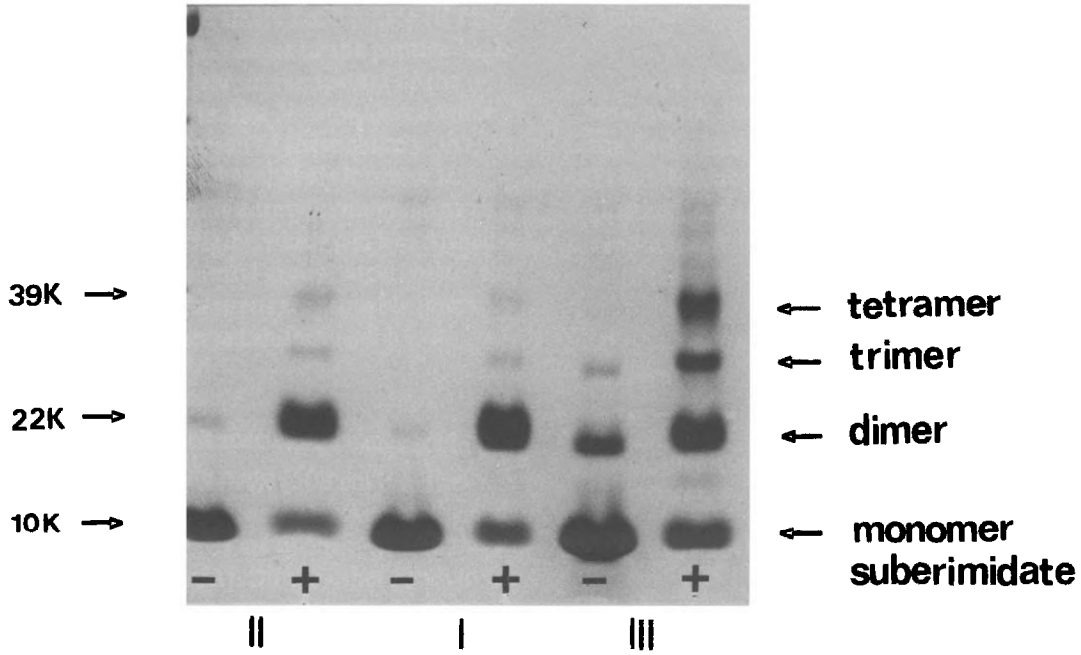

Fig.3. SDS–polyacrylamide gel electrophoresis of HU fractions crosslinked by dimethyl suberimidate. The three fractions I, II and III eluted from the phosphocellulose column were treated with dimethyl-subermidate as in section 2. Samples processed in the same way but without crosslinking agent were run in parallel. Fraction II contains α and β; fraction I contains α; fraction III contains β. Molecular weight markers are: *E. coli* HU (10K); *E. coli* helix destabilizing protein (22 000) and *E. coli* RNA polymerase (α subunit 39 000).

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