Similarity between the bacterial histone-like protein HU and a protein from spinach chloroplasts

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The histone-like protein HU isolated from *E. coli* is well conserved in prokaryotes. We show here that antiserum prepared against bacterial HU cross-reacts with a DNA-binding protein co-sedimenting with the nucleoid of spinach chloroplasts. Antibodies prepared against cyanobacterial HU are more reactive than those raised against *E. coli* HU. The chloroplast protein resembles HU in that both appear to be composed of two related subunits.

Histone-like protein *Chloroplast* *Phylogeny* *Higher plant* Escherichia coli Synechocystis

1. INTRODUCTION

The DNA of the bacterial chromosome is condensed and organised into discrete domains [1]. With the discovery of the HU protein of *E. coli* (HU_{EC}), it became apparent that proteins resembling the histones of eukaryotes were present in bacteria [2,3]. This observation was further supported by the fact that antiserum raised against histone H2A was found to cross-react with the H protein, a second histone-like protein isolated from *E. coli* [4].

The resemblance of HU protein to histones is based on several properties: (i) This DNA-binding protein is small, basic and abundant; (ii) it is found associated with the bacterial nucleoid [5]; (iii) in vitro the protein can introduce negative supercoiling in closed circular relaxed DNA in the presence of topoisomerase I and form nucleosome-like structures which compact the DNA [6]; (iv) the protein is well conserved in prokaryotes. Proteins of the HU family have been isolated from a variety of eubacteria such as *Salmonella typhimurium* [7], *Pseudomonas aeruginosa* [8], *Bacillus subtilis* [9], *Rhizobium meliloti* [10] and *Bacillus stearothermophilus* [11] and from two strains of cyanobacteria [12], *Synechocystis* PCC 6701 and *Anabaena* PCC 7120 (see [13] for nomenclature).

The photosynthetic cyanobacteria, previously termed blue-green algae, are rather distantly related to *E. coli* even though both are Gram-negative bacteria. Evolutionary divergence between *E. coli* and cyanobacteria occurred, according to paleontological evidence, approx. 3×10^9 years ago [14]. However, we have shown that antisera raised against HU_{EC} cross-react with the HU protein isolated from *Synechocystis* (HU_{AS}) and that partial amino acid sequence homology exists between HU_{EC} and HU_{AS} [15]. These data suggest a low rate of evolution for this protein, comparable to those found for histones H2A and H2B. For this reason it was of interest to determine whether such conserved proteins were present in eukaryotic organelles. Such evidence would be of help in establishing the evolutionary relationship [16,17] between bacteria and organelles. A protein

Abbreviations: HU_{EC}, protein HU isolated from *E. coli*; HU_{AS}, protein HU isolated from the cyanobacterium *Synechocystis*

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which is similar to HU_{EC} in structure and function but which did not show immunological cross-reactivity with antisera raised against the bacterial protein has been isolated from yeast mitochondria [18]. Here we describe the detection of immunological cross-reactivity between one of the proteins present in the DNA–protein complex isolated from spinach chloroplasts [19] and the antisera prepared against bacterial HU proteins isolated from *E. coli* and *Synechocystis*.

2. MATERIALS AND METHODS

2.1. *Chloroplast protein preparation*

Pure intact chloroplasts were obtained from spinach leaves as in [20]. A crude fraction of DNA-binding proteins was obtained by affinity chromatography of the 80000 × *g* supernatant of the osmotically lysed chloroplasts on a column of heparin–Sepharose [19]. Fractions eluted with 0.33 M ammonium sulfate were dialysed and then concentrated by precipitation with acetone [21]. The protein precipitates were recovered by centrifugation and further fractionated by polyacrylamide gel electrophoresis.

2.2. *Purification of HU proteins and preparation of antisera*

HU proteins were isolated from *E. coli* W 3150 by either one of two techniques already described, the DNase I procedure [3] or the PEG method [15]. For most preparations, the main fraction from phosphocellulose chromatography containing the $\alpha\beta$-dimer [3] was pooled, dialyzed against 1 mM HCl, and lyophilized. The protein was redissolved in saline and submitted to electrophoresis on a urea–Triton–polyacrylamide gel to establish its purity. These preparations contain only two protein bands under these conditions and possess a unique N-terminal sequence [3]. The same procedures were used to purify protein HU from *Synechocystis*.

Antisera against *E. coli* HU or *Synechocystis* HU were raised in rabbits and their specificity measured by immunodiffusion as in [7] or by immunoblotting [22]. Partially purified γ-globulins were prepared as an ammonium sulfate precipitate of whole rabbit serum [23].

2.3. *Electrophoresis*

Proteins were analyzed by electrophoresis on either an 18% SDS polyacrylamide gel usually used for histone analysis [24] with the conventional sample buffer of [25] or a urea–Triton–polyacrylamide gel as in [3]. M_r markers were purchased from Bio-Rad.

2.4. *Protein transfer and immunoreaction*

Proteins fractionated on polyacrylamide gels were blotted on nitrocellulose filters (Schleicher and Schüll BA 85) using the Western blot technique described in [26] with the following modifications: after electrophoresis the gels were treated to allow partial protein renaturation except that the urea step was shortened by a factor of 3 for the urea–Triton gel. Renatured proteins were blotted on two identical nitrocellulose filters by a modification of the diffusion technique described in [27,28]. Transfer was for 3 days at room temperature. Filters were incubated for 2 h at 37°C with our partially purified IgG diluted 1/300. The specific immunoreaction complex was revealed by incubation of the filter for 1 h at 37°C with 10^6 cpm/ml protein A from *Staphylococcus aureus* labeled with ^{125}I (30 mCi/mg, Amersham). Excess radioactive material was removed by extensive washing with 0.1% Triton X-100 (Koch-Light) in Tris–HCl 10 mM (pH 7.4), NaCl 9‰. Autoradiography of the blot revealed the protein bands that interact specifically with the immunoglobulins.

3. RESULTS

The DNA-binding proteins which co-sediment with the nucleoid of spinach chloroplasts were partially purified by affinity chromatography on a column of heparin–Sepharose [19]. Fractions containing a multipeptide complex which includes the chloroplast RNA polymerase [29] were further fractionated by polyacrylamide gel electrophoresis (PAGE). Two electrophoretic systems were used in parallel, an SDS system in which the closely related α- and β-chains of HU_{EC} migrate as a single band with an apparent M_r of 9500 (fig.1) and a urea–Triton system which permits the resolution of the two different HU chains which differ in hydrophobicity [3] (fig.2). Following electrophoresis, half of each gel was stained with

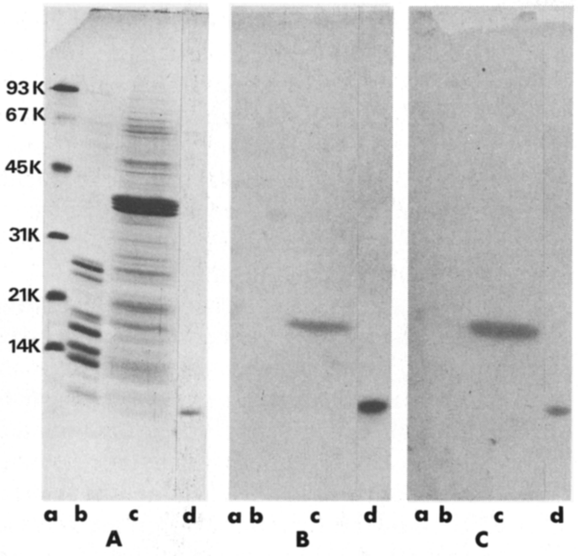

Fig.1. Separation of chloroplast DNA-binding proteins by SDS-polyacrylamide gel electrophoresis and immunochemical reaction with antisera raised against bacterial HU proteins. (A) Coomassie blue staining of the left half of the gel. (B,C) Autoradiograms of the blots of the right half of the gel after immunoreaction with serum prepared against the HU protein isolated from *E. coli* (B) or from *Synechocystis* (C). The gel lanes contained the following different samples: (a) M_r markers; (b) DNA-binding proteins from *E. coli*, depleted of HU; (c) DNA-binding proteins from spinach chloroplasts; (d) purified HU from *E. coli*.

Coomassie blue to reveal total protein. The identical second half was treated with urea-containing buffer [26] to allow partial protein renaturation and the proteins were then transferred to filters.

The total stained proteins observed in the SDS system are shown in fig.1A. As previously mentioned, proteins isolated from the chloroplast nucleoid are quite heterogeneous. Approx. 30 protein bands are visible under these denaturing conditions; the apparent M_r values range from 12000 to 70000. The major stained component, a complex of 3 bands, displayed an M_r of approx. 34000. The identical second half of the gel was transferred to two nitrocellulose filters, one of which was treated with antiserum prepared against $\mathrm{HU}_{\mathrm{EC}}$ (fig.1B) and the other with antiserum prepared against $\mathrm{HU}_{\mathrm{AS}}$ (fig.1C). It is clear that serum against $\mathrm{HU}_{\mathrm{EC}}$ recognizes, in addition to the $\mathrm{HU}_{\mathrm{EC}}$ protein (fig.1B, lane d), a polypeptide of approx. 17 kDa present in the chloroplast nucleoid (fig.1B, lane c). It should be noted that anti-$\mathrm{HU}_{\mathrm{EC}}$ and anti-$\mathrm{HU}_{\mathrm{AS}}$ have not reacted with a mixture of *E. coli* DNA-binding proteins depleted of HU protein (fig.1B, lane b). In fig.1C, it can be seen that antibodies prepared against $\mathrm{HU}_{\mathrm{AS}}$ recognize not only $\mathrm{HU}_{\mathrm{EC}}$ (lane d) as expected, but also the 17-kDa protein of the chloroplast preparation. Since blotting of the gel by diffusion permitted us to obtain

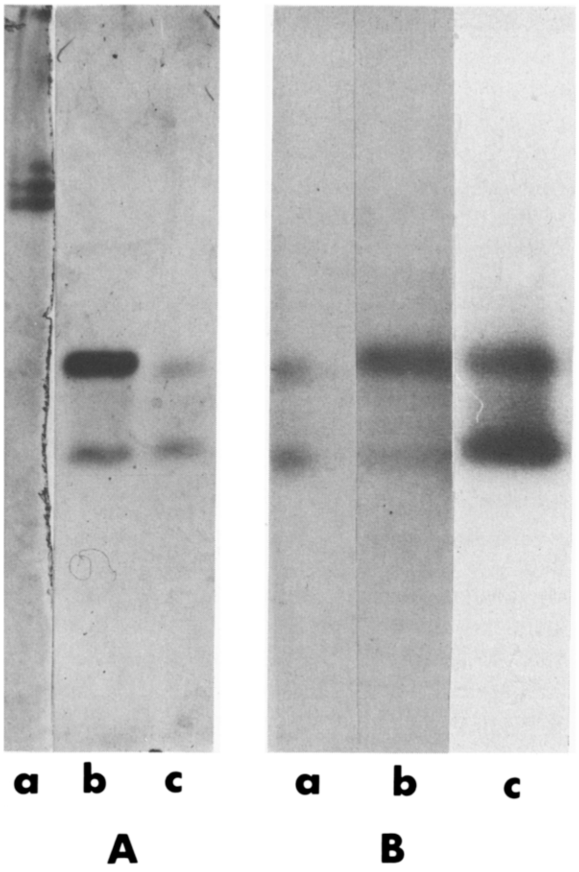

Fig.2. Separation of chloroplast DNA-binding proteins by urea–Triton polyacrylamide gel electrophoresis and immunochemical reaction with antisera raised against *E. coli* HU proteins. (A) Coomassie blue staining of the first 3 lanes of the left half of the gel. (B) Autoradiogram of the blot of the corresponding lanes from the right half of the gel after treatment with serum prepared against HU isolated from *E. coli*. The lanes contained: (a) DNA-binding proteins from spinach chloroplasts; (b) purified HU from *Synechocystis*; (c) purified HU from *E. coli*. Lane c of the autoradiograms corresponds to a shorter exposure time to compensate for the strong homologous immunoreaction.

two identical filters with equal amounts of protein [30], it is clear by visual inspection of these two blots (fig.1B,C) that antibodies against HU_{AS} react more avidly with the 17-kDa chloroplast protein than do antibodies against HU_{EC}. As expected, on these blots HU_{EC} (lane d) reacted less strongly with serum raised against HU_{AS} than with the homologous serum. If the nitrocellulose filters were treated with control non-immune serum, no trace of any band could be found on the autoradiographs.

A variety of different sera prepared against HU_{EC} (10 different preparations) and against HU_{AS} (3 different preparations) were used in this work. All gave similar results, although we occasionally found the chloroplast specific protein migrating with an apparent M_r of approx. 34000 (not shown). This result was found independently of the serum used. The possibility exists that this band represents a dimer of the 17-kDa polypeptides which were either cross-linked, aggregated or incompletely denatured under our electrophoresis conditions. In fact, in certain cases, the pure *E. coli* HU protein also migrates in the SDS system as a mixture of monomers, dimers, and tetramers, even in the absence of a cross-linking agent [3]. Strong interactions between molecules of HU due to long stretches of hydrophobic residues could favour the formation of such homopolymers. To test this hypothesis the proteins were run in parallel on SDS and on urea–Triton gels. Both were transferred and developed using the same conditions. The chloroplast protein gave identical results in the urea–Triton system, independently of whether the 17- or the 34-kDa proteins were found in the SDS system. The single protein band found in the SDS system was resolved in the urea–Triton system into two bands (fig.2B, lane a) as are HU_{AS} (lane b) and HU_{EC} (lane c). It has been shown that the two bands observed in the urea–Triton system correspond to the α- and β-subunits of HU_{EC} [3] and HU_{AS} [15]. The present results suggest that the chloroplast protein could similarly be composed of two different subunits, not separated on SDS gels, which show equal affinity for the HU_{EC} antiserum.

4. DISCUSSION

The results presented here show an immunological cross-reactivity between antisera raised against the histone-like protein HU of bacteria and a protein bound to the chloroplast nucleoid. Like HU_{EC} and HU_{AS}, this protein seems to be composed of two different subunits α and β. The monomeric forms appear to correspond to two peptides, both of which display an M_r of 17000 on the SDS gel. Such cross-reactivity was not observed with the histone-like protein (HM) isolated from yeast mitochondria [18].

The problem of the origin of eukaryotic organelles is still unsolved. By criteria such as size, structure and function, the genetic material of mitochondria and chloroplast is similar to that of prokaryotes. Like bacteria, mitochondria and chloroplasts do not contain histones [18] but, as we have shown, they do possess a histone-like protein. Our result showing that the chloroplast histone-like protein has a much stronger affinity for antiserum prepared against HU isolated from cyanobacteria than HU isolated from *E. coli* is in accordance with the endosymbiotic theory: chloroplasts could result from the symbiotic invasion of a photosynthetic bacterium into a plant cell. It will be of interest to determine whether the chloroplast HU-like protein is encoded by the chloroplast genome or, as for the mitochondrial HM proteins [18], by the nuclear genome.

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