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Crystallization and preliminary X-ray diffraction analysis of the homodimeric form α_2 of the HU protein from *Escherichia coli*

Franck Coste,[a] Nadège Hervouet,[a] Jacques Oberto,[b] Charles Zelwer[a]* and Bertrand Castaing[a]

[a]Centre de Biophysique Moléculaire, Rue Charles Sadron, 45071 Orleans CEDEX 2, France, and [b]Laboratoire de Physiologie Bactérienne, UPR 9073, Institut de Biologie Physico-Chimique, 13 Rue Pierre et Marie Curie, 75005 Paris, France

Correspondence e-mail: zelwer@cnrs-orleans.fr

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The homodimeric form α_2 of the *Escherichia coli* DNA-binding protein HU was crystallized by the hanging-drop vapour-diffusion method using PEG 4000 as a precipitant. The crystals belong to space group *I*222, with unit-cell parameters $a = 31.09$, $b = 55.34$, $c = 117.63$ Å, and contain one monomer per asymmetric unit. A full diffraction data set was collected to 2.3 Å resolution on a conventional X-ray source. The molecular-replacement method, using the HU crystallographic model from *Bacillus stearothermophilus* as a starting point, gave a reliable solution for the rotation and translation functions.

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1. Introduction

HU is a small (18 kDa), basic and abundant (30000 dimers per cell) protein found in prokaryotes. This non-sequence-specific DNA-binding protein is classified among the 'histone-like' proteins because of its ability to distort DNA *in vitro* by introducing negative supercoils into relaxed circular DNA and promoting the formation of pseudo-nucleosomal structures (Rouvière-Yaniv, 1978; Rouvière-Yaniv *et al.*, 1979; Broyles & Pettijohn, 1986). In addition to this, HU acts as an accessory factor in modulating several processes such as DNA replication, gene transcription, gene recombination and DNA repair (Mensa-Wilmot *et al.*, 1989; Flashner & Gralla, 1988; Johnson *et al.*, 1986; Haykinson & Johnson, 1993; Boubrik & Rouvière-Yaniv, 1995; Nash, 1996; Fernandez *et al.*, 1997).

In enteric bacteria HU is mainly present as a heterodimer $\alpha\beta$ (Rouvière-Yaniv & Kjeldgaard, 1979), but in other eubacteria it is only present as a homodimer. In *Escherichia coli*, the α and β subunits of 90 amino-acid residues each share 70% sequence identity and are encoded by two distinct genes: *hupA* (Kano *et al.*, 1986) and *hupB* (Kano *et al.*, 1985), respectively. The relative abundance of the three possible dimeric forms (α_2, $\alpha\beta$ and β_2) varies during cell growth and in response to environmental change. Indeed, the homodimer α_2 is accumulated at the beginning of the exponential growth phase, whereas the heterodimer $\alpha\beta$ is predominant at the end of the exponential phase and during the stationary phase (Claret & Rouvière-Yaniv, 1997). These results suggest that the homodimer α_2 plays a special role during cell division, whereas in the stationary phase the heterodimer $\alpha\beta$ is required for cell metabolism.

The HU protein forms low-affinity ($K_d \simeq 10^{-7}$ M) and high-affinity ($K_d \simeq 10^{-9}$ M) complexes with DNA. In the low-affinity complexes, HU wraps DNA without sequence specificity in a regularly spaced fashion (Broyles & Pettijohn, 1986; Rouvière-Yaniv *et al.*, 1991; Groch *et al.*, 1992). Conversely, DNA containing sharp bends, kinks, branched and bulged structures or flexible structures such as single-strand breaks and gaps are high-affinity ligands for the *E. coli* HU protein (Pontiggia *et al.*, 1993; Bonnefoy *et al.*, 1994; Castaing *et al.*, 1995). Interestingly, the relative affinities of HUα_2 and HU$\alpha\beta$ for nicked or gapped DNA structures are comparable, whereas the affinity of HUβ_2 is much lower (Pinson *et al.*, 1999).

The relative amounts of α and β subunits during the cell cycle seem to be physiologically significant. However, while there is a high sequence homogeneity between both chains, the reason for the differences in DNA-binding properties between the three HU dimers remains unclear. We undertook crystallographic studies in order to characterize the different *E. coli* HU forms at the atomic level. In this paper, we report the overproduction, purification, crystallization and preliminary X-ray analysis of the *E. coli* HUα_2 protein.

2. Overproduction and purification

The T7 expression host was the *E. coli* BL21(DE3) strain, which is a lysogen of a λ phage derivative that carries the gene for the T7 polymerase under the control of an IPTG-inducible promotor (Studier & Moffatt, 1986). BL21(DE3) was transformed with the target *hupA* gene encoding the *E. coli* HUα subunit cloned into the pJES plasmid (derived from ColE1) under control of the strong *φ10*

Table 1
Data collection and reduction statistics.

Resolution limits (Å)	32–2.30	2.36–2.30
Total observations	16441	1231
Unique reflections	4755	344
Data compleness (%)	99.4	99.4
Data redundancy	3.5	3.6
Mean $I/\sigma(I)$	11.2	4.4
$R_{\rm sym}$ value (%)	5.6	16.1

† $R_{\rm sym}$ (%) = $100 \times (\sum_h \sum_i |I_{h,i} - \langle I_h \rangle| / \sum_h \sum_i I_{h,i})$, where $\langle I_h \rangle$ is the average intensity of reflection h, $\sum_h$ is the sum over all reflections and $\sum_i$ is the sum over the i measurements of reflection h.

promotor for the T7 RNA polymerase (Pinson *et al.*, 1999).

For the overproduction of HUα_2, LB broth medium (4 l) containing 100 µg ml^{-1} ampicillin was inoculated with 40 ml of an overnight culture of the BL21(DE3)/pJES-*hupA*. The culture was then supplemented with 0.5 m*M* IPTG. After 2 h induction, the cells were harvested and washed. Cell lysis was carried out as described by Castaing *et al.* (1995). After a fractionated ammonium sulfate precipitation of the lysate, the pellet produced from the 50–90% ammonium sulfate fractions, which contains the HUα_2 protein, was resuspended and loaded onto a gel-filtration column (ACA-54 IBF-LKB) and then eluted with lysis buffer containing 1 *M* NaCl. The fractions containing HUα_2 were pooled and dialyzed against lysis buffer without NaCl and then loaded onto a Sepharose SP Fast Flow column (Pharmacia) and eluted with a linear NaCl gradient (50–600 m*M*). For the last purification step, the protein was loaded onto a POROS CM column (Perseptive Biosystem) and eluted with a 50–750 m*M* linear gradient.

During the protein purification, all the collected fractions were analyzed by SDS–PAGE (Fig. 1). To estimate the HUα_2 contamination by the endogenous HU$\alpha\beta$, the fractions were also examined by acid/urea/Triton–PAGE (Fig. 2). The concentration of the homogeneous HUα_2 was assessed by the biuret method (Gornall *et al.*, 1949). After dialysis against pure water, the aliquots were dried and stored at 253 K.

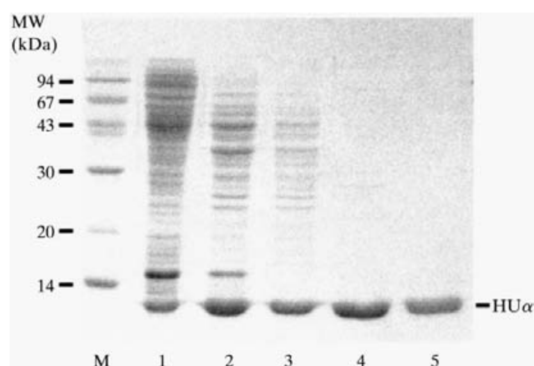

Figure 1
SDS–PAGE analysis of HUα_2 samples. Lane M, molecular-weight standards; lane 1, crude cell extract; lane 2, 50–90% ammonium sulfate fraction; lane 3, ACA54 pool; lane 4, Sepharose SP Fast Flow pool; lane 5, POROS CM pool.

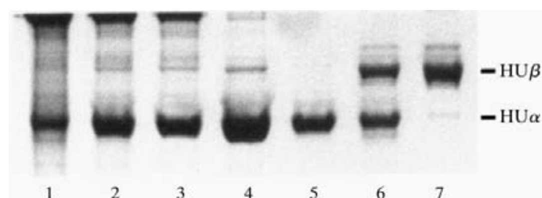

Figure 2
Acid/urea/Triton–PAGE analysis of HUα_2 samples. Lane 1, crude cell extract; lane 2, 50–90% ammonium sulfate fraction; lane 3, ACA54 pool; lane 4, Sepharose SP Fast Flow pool; lane 5, POROS CM pool with purified HUα_2; lane 6, purified HU$\alpha\beta$; lane 7, purified HUβ_2.

3. Crystallization

HUα_2 was crystallized by the hanging-drop vapour-diffusion technique using the Crystal Screen I solutions (Hampton Research). Drops of 2 µl containing a 1:1(*v*:*v*) mixture of the Crystal Screen and the protein (15 mg ml^{-1}) solutions were equilibrated against 500 µl of Crystal Screen solutions at 293 K. Numerous needle-shaped crystals appeared after one month in solution number 41 (20% PEG 4000, 10% 2-propanol, 0.1 *M* HEPES pH 7.5). Crystals (Fig. 3) suitable for X-ray diffraction can be obtained at 277 or 293 K with either a 1:1 or 1:2(*v*:*v*) protein to reservoir ratio and with protein concentrations in the range 10–30 mg ml^{-1}.

4. X-ray analysis

A needle-shaped crystal of dimensions 0.4 × 0.1 × 0.1 mm was cryoprotected by soaking in a solution which included 20% glycerol in the mother liquor and was flash-frozen at 100 K in a nitrogen-gas stream (Oxford Cryosystems Cryostream). X-ray data were collected using Cu $K\alpha$ radiation (λ = 1.5418 Å) from a Rigaku RU200 X-ray generator (40 kV, 100 mA) coupled with a 30 cm MAR Research imaging-plate detector. The crystal-to-detector distance was set at 175 mm and 0.5° oscillation images were collected with a 1200 s exposure time. The diffraction patterns were processed with *MOSFLM* (Leslie *et al.*, 1996) and scaled with *SCALA* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). This data set was 99.4% complete at a maximum resolution of 2.3 Å. The crystals are orthorhombic, with unit-cell parameters a = 31.09, b = 55.34, c = 117.63 Å and with $I222$ or $I2_12_12_1$ as possible space groups. Assuming one monomer (9500 Da) per asymmetric unit, the specific crystal volume is V_m = 2.64 Å^3 Da^{-1}, corresponding to a solvent content of 53%, which falls within a reasonable range observed in most protein crystals (Matthews, 1968). Detailed data-collection statistics are given in Table 1.

The molecular-replacement method, using the X-ray structure of HU from *Bacillus stearothermophilus* (PDB code 1huu; White *et al.*, 1999) as a search model and a data set limited to the 20–3 Å resolution range, was carried out using *AMoRe* (Navaza, 1994). The space-group ambiguity was solved on the basis of the translation-function results. With space group $I222$, a well defined molecular-replacement solution was found. Its correlation coefficient was 1.5 times higher than the best solution obtained with the alternative space group $I2_12_12_1$. Rigid-body refinement in *AMoRe* resulted in a correlation coefficient of 0.57 and an R factor of 0.46. Visual inspection of the crystal packing using *TURBO-FRODO* (Roussel & Cambillau, 1991) revealed no bad contacts between neighbouring molecules. The biologically relevant homodimer is generated by a crystallographic twofold rotation axis parallel to c. Structure refinement is in progress.

Figure 3
Photograph of an orthorhombic crystal of HUα_2. Its approximate dimensions are 0.5 × 0.06 × 0.06 mm.

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