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QUARTERLY

Purification of IHF-like protein from gram-negative bacteria in one chromatographic step*

Beata Krawczyk and Józef Kur**

Department of Microbiology, Technical University of Gdańsk, G. Narutowicza 11/12, 80-952 Gdańsk, Poland

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We describe a fast and very efficient method of purification which yields highly purified integration host factor-like proteins in one chromatografic step. IHF-like proteins from *Acinetobacter junii* or *Proteus vulgaris* are each an αβ heterodimer (subunits of 10 and 11 kDa) similar to the IHF of *Escherichia coli* when analyzed by polyacrylamide gel electrophoresis. The purified IHF are able to bind to the same *ihf* sites as IHF of *E. coli*. The results presented confirm that IHF is conserved during evolution in gram-negative bacteria.

The IHF protein of *Escherichia coli* is a histone-like, heterodimeric protein consisting of the products of the *him*A gene (encoding the α subunit of IHF) and the *him*D/*hip* gene (encoding the β subunit of IHF). The IHF protein is involved in several regulatory processes, including modulation of transcription, phage packaging, plasmid replication and transfer, bacterial phase variation (for a review see [1]). Several related consensus binding sequences have been proposed [2–7]. According to Kur *et al.* [5], IHF specifically binds to the A+T-rich *ihf* consensus sequence:

$W_6N_7W_4C$-AR-NWN_2TTR.

It has recently been shown that an IHF homolog is present in other gram-negative bacteria like *Salmonella typhimurium* [8], *Haemophilus influenzae* [9], *Rhodobacter capsulatus* [10], *Pseudomonas aeruginosa* [11], *Aeromonas proteolytica* [12] and *Serratia marcescens* [12].

MATERIALS AND METHODS

Purification of the IHF-like protein. The bacteria were grown in 6 l of LB medium at 30°C (*Acinetobacter junii*) or 37°C (*E. coli* and *Proteus vulgaris*). The cells were harvested at A_{650} = 1.0 by centrifugation and the cell paste was frozen and stored at –70°C. About 30 g of the cell paste obtained was resuspended in 100 ml of lysis buffer (100 mM Tris/HCl pH = 7.5, 20 mM EDTA) containing 0.2 mg of egg white lysozyme per ml and the mixture was incubated for 30 min at 37°C. The cooled (4°C) suspension (130 ml) was sonicated in 65 ml batches. Each batch was subjected to 4 bursts of 45 s each at 15 s intervals. The homogenate was centrifuged for 90 min at 120000 × *g* in a Beckman model XL-70 centrifuge. The supernatant (100 ml) was

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**Correspondence to: Dr J. Kur, Technical University of Gdańsk, G. Narutowicza Str. 11/12, 80-952 Gdańsk, Poland. Tel. (48-58) 47-23-02; Fax (48-58) 47-26-94; E-mail: kur@altis.chem.pg.gda.pl

Abbreviations: IHF, integration host factor; *ihf*, IHF-binding site on DNA; *att*, phage λ attachment site; H', one of the *ihf* in the *att*P site; *him*A and *hip*/*him*D genes encoding the α and β subunit of IHF, respectively; LB, Luria-Bertani medium; PAGE, polyacrylamide gel electrophoresis; R, A or G; W, A or T.

directly loaded, at a flow rate of 30 ml per hour, onto a 10-ml column of Macro-Prep Q anion-exchange equilibrated with buffer A (25 mM Tris/HCl, pH = 7.4, 1 mM EDTA, 10% glycerol). This column was connected with a 10-ml heparin-Sepharose column equilibrated with buffer A. Next, the columns were washed with buffer A for 6 h at a flow rate of 30 ml/h, then the anion exchange column was disconnected and the heparin column was eluted by 100 ml of 0.1–1.0 M NaCl gradient in buffer A. The A_{280} was monitored and 2 ml fractions were collected. NaCl concentration in selected fractions was determined by measuring conductivity using a conductivity meter. The IHF protein present in the collected fractions was detected by gel retardation assays using the 346-bp DNA fragment containing the H′ *ihf* site from *att*P region of bacteriophage λ (*Hin*dIII restriction fragment of plasmid) [13]. The IHF containing fractions were concentrated by dialysis against buffer A and then were supplemented with glycerol to a final concentration of 50%, aliquoted and stored at –20°C.

RESULTS

The purification scheme presented here is an one-step procedure that allows fast recovery of microgram quantities of highly purified IHF. It does not include the polymin P fractionation steps of the procedure used earlier to purify IHF from *E. coli* [14], it makes use of commercial Macro-Prep Q anion-exchange column (Bio-Rad) and heparin-Sepharose CL-6B column (Pharmacia) without prior removal of DNA to avoid the loss of IHF, and it allows direct isolation of the protein from cells containing IHF. The protein fractions collected at 0.8–1.0 M NaCl (fractions 37 to 40; 200 μg of protein), analyzed by SDS/PAGE (Fig. 1), contained mainly two polypeptides of 11000 and 10000. The IHF protein from the tested bacteria *A. junii*, *P. vulgaris* or *E. coli* was obtained with an enrichment factor of about 450. SDS/urea/PAGE analysis of the protein fractions at each purification step showed that most of the contaminants present in the extracts were removed by the first anion exchange column (not shown).

The DNA binding activity of *A. junii* and *P. vulgaris* IHF was first demonstrated with the 346-bp DNA fragment containing the H′ *ihf* site from *att*P region of bacteriophage λ. By splitting that fragment into two smaller subfragments (*Ava*I digestion), we could observe that IHF-like proteins bound only to this fragment (236 bp) containing H′ *ihf* site, whereas the other fragment (110 bp) is not affected at all (Fig. 2). It is evident (from that experiment) that the binding of IHF-like proteins is specific, because, when a mixture of two DNA fragments is present, IHF preferentially binds to and retards only the DNA fragment containing the H′ site. We also performed gel retardation assays

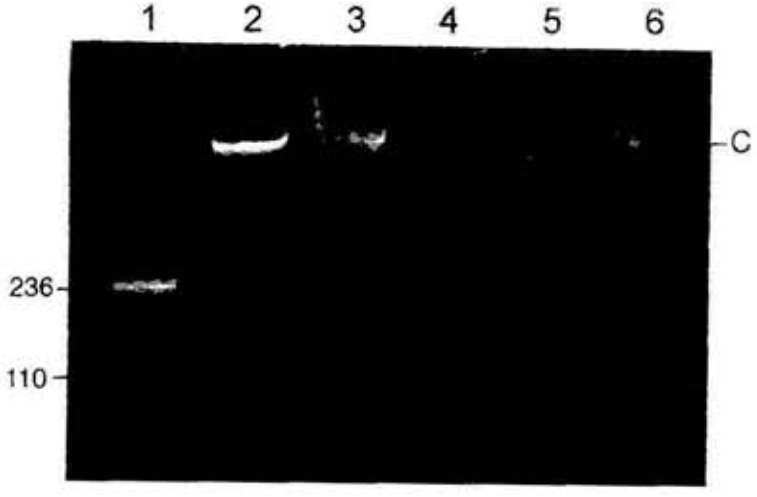

Fig. 2. Binding of A. junii IHF to the mixture of two fragments created by AvaI digestion of the 346-bp DNA fragment containing the H′ ihf site from the attP region of bacteriophage λ.

The amounts of proteins (micrograms) indicated in parentheses were added to the DNA fragments: Lane 1, free DNA; lane 2, *E. coli* IHF (0.1); lane 3, *E. coli* IHF (0.2); lane 4, *A. junii* IHF (0.025); lane 5, *A. junii* IHF (0.1); lane 6, *A. junii* IHF (0.2). The IHF binding was carried out in the presence of unlabeled DNA fragment (100 ng) prepared (by standard procedure) according to Maniatis *et al.* [16]. The DNA-protein complexes were studied by the gel retardation technique of Fried & Crothers [17], as modified by Kur *et al.* [5]. The DNA fragments and their complexes with IHF were separated on 6% polyacrylamide gel at 5 V/cm. Note that only fragment 236 bp is retarded. Fragment sizes are indicated on the left margin and their complexes on the right margin.

using the *Hind*III fragment of the λ *b*2 region (λ coordinates 23 130-25 157) to compare the IHF-like proteins of *A. junii* or *P. vulgaris* with the *E. coli* IHF. After digestion with *Hin*fI restriction enzyme 6 subfragments were obtained. Four out of the six fragments were found to interact

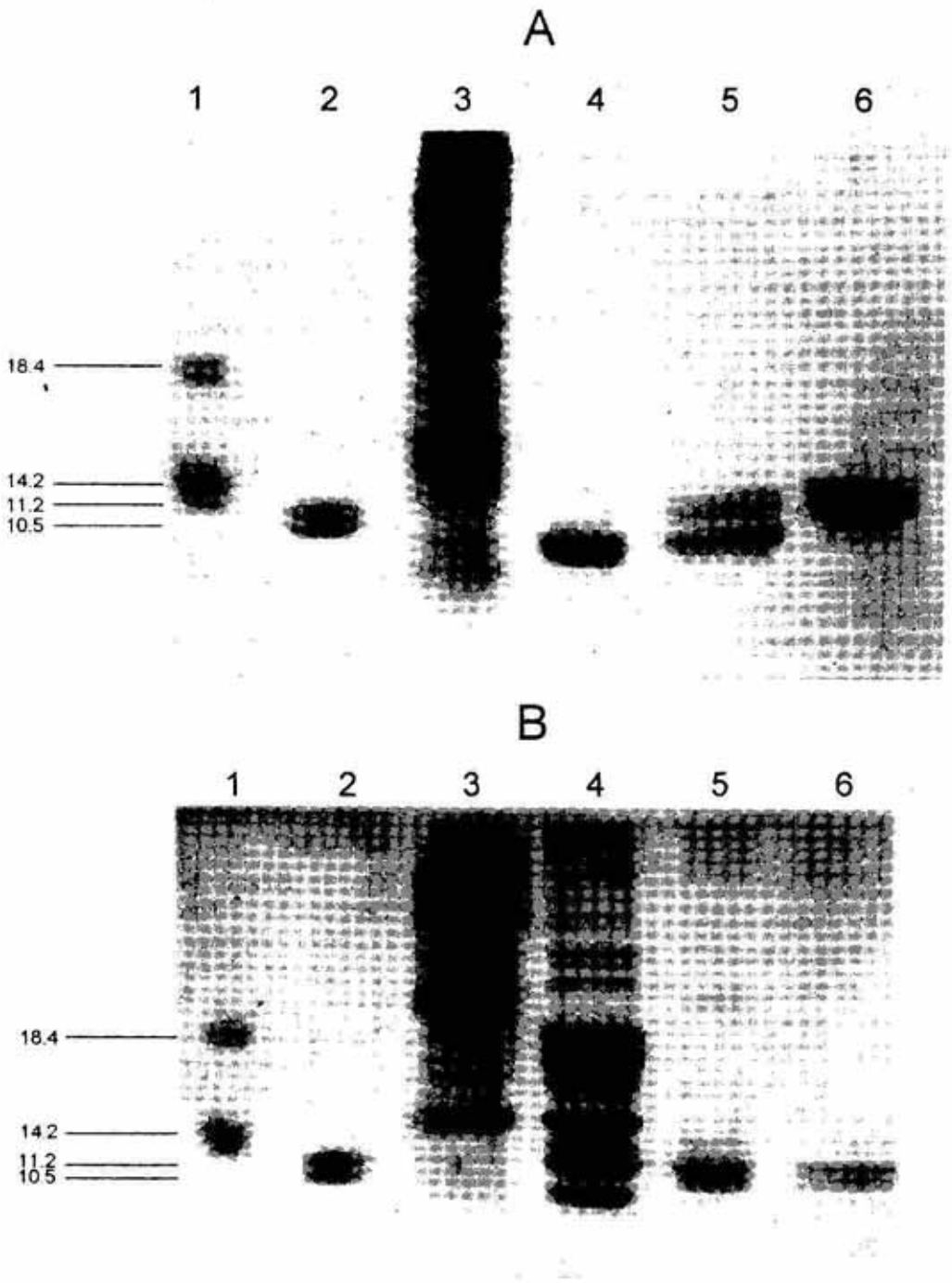

Fig. 1. SDS/urea/18% PAGE analysis of the protein fractions obtained by one-step purification of the A. junii (A) and P. vulgaris IHF (B).

(A) Lane 1, molecular mass markers (18.4, 14.2, and 6.5 kDa); lane 2, *E. coli* IHF standard (1 μg; *E. coli* IHF was purified by the Biotechnology Center, University of Wisconsin, according to the protocol of Nash *et al.* [15]; lanes 3, 4, 5 and 6, fractions 33–34 (10 μl at protein concentration of 0.1 μg/μl), 35–36 (10 μl at 0.15 μg/μl), 37–38 (10 μl at 0.15 μg/μl) and 39–40 (10 μl at 0.2 μg/μl), respectively. (B) Lane 1, molecular mass marker as in panel A; lane 2, *E. coli* IHF standard (1 μg); lanes 3, 4, 5 and 6, fractions 33–34 (10 μl at protein concentration 0.1 μg/μl), 35–36 (10 μl at 0.15 μg/μl), 37–38 (10 μl at 0.15 μg/μl) and 39–40 (10 μl at 0.2 μg/μl), respectively.

specifically with both *A. junii* and *E. coli* IHF. The same results were obtained with *P. vulgaris* IHF (not shown).

DISCUSSION

It is evident from this study that the method presented here can be applied for purification of IHF homologous proteins from other organisms. The purified IHF was highly active in DNA-binding. The IHF activity in extracts was stable when storage took place at –20°C. It should also be noted that the most highly purified fractions of IHF were virtually free of the HU protein homolog, a troubling contaminant in IHF preparations. The recovery of more than 2 mg of apparently homogeneous material from 6 liters of cells indicates that sufficient amounts of IHF can be readily prepared to carry out crystallization, spectrophotometric, and chemical studies.

The results presented here suggest also that IHF proteins are probably widespread and are evolutionarily conserved in gram-negative bacteria.

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