Note

Overproduction and Rapid Purification of the *Escherichia coli* Histone-like Protein, H-NS

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E. coli cells produce a set of small and usually basic DNA-binding proteins, which were collectively termed histone-like proteins because their biochemical properties were thought to resemble those of eukaryotic histone.¹⁾ Of the five such histone-like proteins so far characterized, H-NS (or H1a) is an abundant and neutral one, with an apparent molecular weight of 16,000. The gene for H-NS has recently been cloned, sequenced, and mapped at 27 min on the *E. coli* genetic map.^{2,3)} Results of previous extensive genetic studies on H-NS suggested that the product of the *hns* locus is an important DNA-binding protein for both the function and the architecture of

nucleoprotein complexes concerned in crucial biological processes.⁴⁾ Another intriguing finding on a possible function of H-NS was obtained with a different biochemical approach.^{5,6)} We searched for *E. coli* proteins that preferentially bind *in vitro* to a synthetic curved DNA sequence, and succeeded in purifying such a protein. The purified protein was identified as H-HS.⁵⁾ In this study, as a first step to gain a new insight into the complex functions of the curved DNA binding protein, H-NS, we developed a procedure for the purification of a large quantity of H-NS at one time.

We previously purified H-NS from an *E. coli* strain carrying the *hns* gene on its chromosome⁵⁾; less than one mg of purified H-NS being obtained from one liter of culture. Here we constructed a recombinant plasmid (pHOP-11) that carries the entire *hns*-coding sequence connected downstream to the *tac* promoter-operator,⁷⁾ (Fig. 1). *E. coli* JM83, transformed with pHOP-11, was grown in a rich medium, isopropyl- β -D-galactopyranoside being added at logarithmic phase to induce the synthesis of H-NS. After incubation for 4 hr, the cells were harvested. When total cellular proteins were analyzed by SDS-polyacrylamide gel electrophoresis, a thick protein band was detected (Fig. 2, lane b). The apparent molecular weight estimated for this protein band (16,000) agreed with that calculated from the amino acid sequence of H-NS. After precipitating proteins from the soluble cell-lysate with ammonium sulfate (40% to 60% saturation) (Fig. 2, lane c), the sample was chromatographed on a phosphocellulose column. When proteins were eluted from the column, a large protein peak appeared on the chro-

Fig. 1. Schematic Representation of the Plasmid Constructed for Overproduction of H-NS, and Purification of H-NS Phosphocellulose Column Chromatography.

Plasmid pHOP-11 carries the entire *hns*-coding sequence (bold arrow) including its own ribosome binding site (RBS) connected downstream to the *tac* promoter-operator (*tac^{P/O}*). A total cell-lysate prepared from the cells transformed with pHOP-11 was fractionated on a phosphocellulose column with linear gradient elution (10–700 mM K_2HPO_4 – KH_2PO_4 , pH 6.9). A large protein peak containing H-NS is shaded.

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Fig. 2. Purification of H-NS.

The samples of each step of H-NS purification were analyzed by SDS polyacrylamide gel electrophoresis: lane a, total cell lysate from the uninduced cells (100 μ g); lane b, total cell lysate from the induced cells (100 μ g); lane c, after ammonium sulfate fractionation (120 μ g); lane d, after phosphocellulose chromatography (15 μ g). Molecular mass markers were run in lane M (numbers represent kDa).

matogram (Fig. 1). This protein peak was found to contain H-NS, which was predominant and nearly homogeneous in the SDS-polyacrylamide gel (Fig. 2, lane d). Thus a large amount of H-NS was purified by the single-step-chromatography. This purification step, if necessary, was followed by Heparin-Sepharose chromatography and Mono-S chromatography (data not shown). The N-terminal amino acid sequence of the purified protein was the same as that deduced from the nucleotide sequence of the *hns* gene. Thus the plasmid we constructed enabled us to easily purify a large quantity of H-NS as a soluble form (10 mg of protein from one liter of culture). This large amount of H-NS will certainly allow us to analyze the structure of H-NS by nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography. Experiments along these lines are currently under way in our laboratory.

We then raised a polyclonal rabbit antiserum against the purified H-NS protein and used it for the following experiment to gain insight into the features of H-NS. It has been reported that the absolute amount of H-NS in cells varies with the growth phase, e.g., 5800 copies per cell at the early logarithmic phase and 26,000 copies per cell at the stationary phase.⁸⁾ Taking advantage of the antiserum prepared in this study, immunoblot analysis was

Fig. 3. Immunoblot Analysis.

A: *E. coli* MC4100 was grown in L-broth until appropriate growth phases: a, early-logarithmic phase; b, mid-logarithmic phase; c, late-logarithmic phase; d, stationary phase. Total soluble cellular proteins were run on an SDS polyacrylamide gel (30 μ g each). Molecular mass markers were run in lane M (number represent kDa). B: The same samples as in panel A were analyzed by immunoblotting analysis with mixed antisera raised against H-NS and OmpR. The latter was used as an internal control.

done for cellular proteins of *E. coli* MC4100 grown until appropriate growth phases (Fig. 3). We did not, however, observe such accumulation of the protein, which was crossreactive with a polyclonal anti-H-NS-antiserum, during the stationary phase. Therefore, our results clearly suggest that the intriguing previous notion that there is marked accumulation of H-NS during the stationary phase should be reassessed carefully.

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