Production of functional chick liver HMG 2a protein in *Escherichia coli*

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Abstract **An efficient *Escherichia coli* system for the production of a variant form of high-mobility group-2a protein (HMG 2a), having the additional 5 amino acid residues (Ala-Pro-Thr-Leu-Glu) at the NH_2-terminal, has been constructed. cDNA encoding HMG 2a was ligated with the Omp A signal peptide sequence and was inserted into an inducible bacterial expression vector pSH-L. After the plasmid introduced into *E. coli* was expressed by temperature shift, the recombinant product was purified by trichloacetic acid precipitation followed by Bio-Rex 70 column chromatography. The purified product showed the expected NH_2-terminal sequence and the superhelical activity of circular DNA similar to the authentic HMG 2a isolated from chick liver.**

Key words: HMG 2a (chick liver); Expression vector; Recombinant HMG 2a; Purification; *Escherichia coli*

1. Introduction

High mobility group (HMG) proteins are a family of non-histone components in chromatin [1]. There are four major HMG proteins (HMG 1, 2, 14 and 17) in all the eukaryotic cells examined to date [2]. In chicks, HMG 2 has been further resolved by chromatography into two subfractions, called HMG 2a and 2b [3].

The interaction of HMG proteins 1 and 2 with DNA has been extensively studied. It has been shown that these proteins interact preferentially with negatively supercoiled DNA [4], AT-rich DNA [5], and bind with high selectivity to four-way junction DNA [6].

Although the function of HMG proteins are only poorly understood, these proteins has been implicated in transcription and in DNA replication [2]. A previous work in our laboratory has shown that HMG proteins 1 and 2 play an important role in nutritional modulation of chick liver RNA synthesis [7], and we have recently reported molecular cloning of chick liver HMG 2a cDNA [8]. To understand the function of the HMG protein, we expressed the HMG 2a cDNA clone in bacterial cells. The protein purified from the overproducing strain was active in inducing DNA superhelicity.

2. Materials and methods

2.1. Materials

Restriction endonuclease and nucleic acid modifying enzymes were purchased from Takara Biochemicals (Kyoto, Japan). The other reagents, except where indicated, such as chromatography resins and gels, were purchased from BioRad (Richmond, USA). The expression vector pSH-L was a generous gift from Dr. N. Habuka (The Life Science Research Laboratory, Japan Tobacco, Kanagawa, Japan). Plasmid pSP-DHFR, a recombinant plasmid containing the *E. coli* gene coding for dihydrofolate reductase, was kindly provided by Dr. A.S. Spirin (Institute of Protein Research, Moscow, Russian Federation).

2.2. General methods

Plasmid DNA was prepared by the alkaline lysis method [9] and purified by Qiagen pack-100 (Qiagen Inc., USA) [10]. Restriction enzyme digestions and ligations were carried out essentially as recommended by the purveyor. The nucleotide sequence of the insert was determined by the dideoxy chain termination procedure of Sanger et al. [11] employing Sequanase with 7-deaza-dGTP (United States Biochemicals, USA). Amino acid sequencing at N-terminal region of the recombinant protein was carried out as described previously [12].

2.3. Construction of the E. coli expression vector

The outline of the construction of pSH-HMG is shown in Fig. 1A. A plasmid pUHMG820 was constructed by inserting cDNA of HMG 2a [8] into pUC19. The expression vector, pSH-L, was constructed by ligating the linker (Fig. 1B-a) after the MAP region was removed from pSH7 [13]. Resulting expression vector, pSH-L, contained PL promoter sequence, cI857 repressor sequence and a Shine–Dalgarno sequence that are under the control of temperature. The coding sequence of HMG 2a from plasmid pUHMG820 was amplified by polymerase chain reaction (PCR). Two oligonucleotide primers containing *Xba*I site were designed for the PCR; primer 1 (30 mer) 5′–3′ CCTACTC-TAGAGGCTAAAGGTGACCCGAAG and primer 2 (30 mer) 5′–3′ TCCTCTCTAGAGAGGATCCGGGTACCATGG. PCR fragment which contained the HMG 2a coding sequence was excised by the subsequent digestion with *Xba*I. pSH-HMG was constructed by ligating this sequence with the *Xba*I-digested pSH-L. The resulting construct, pSH-HMG, encoded a fusion protein of the Omp A signal peptide and HMG 2a. Since we used the MAP-derived linker shown in Fig. 1B-a, the mature product should have additional 5 amino acids (Ala-Pro-Thr-Leu-Glu) at its NH_2-terminal when the signal peptidase cleaves a peptide bond at the correct site in the primary translation product (Fig. 1B-b).

2.4. Expression of HMG 2a in E. coli

Recombinant plasmid pSH-HMG was propagated in *E. coli* strain MM 294. *E. coli* transformants grew in L-broth containing 50 μg/ml ampicillin at 30°C. When the absorbance at 550 nm of the culture reached 0.8, the expression was initiated by raising the culture temperature to 42°C by the addition of the same volume of the medium at 55°C. Production of HMG 2a was achieved by continuous aerobic incubation at 42°C.

2.5. Extraction and purification of recombinant HMG 2a

Cells (from 4 l of culture) were harvested by centrifugation at $10,000 \times g$ for 10 min and the pellet was suspended in 10 volumes of water and disrupted by sonication. Purification of HMG 2a from the extract was carried out by a modification of the method described by Mathew et al. [3]. After centrifugation ($10,000 \times g$, 20 min) of the sonicate, the resultant supernatant fluid was made 0.2 M with respect to sulfuric acid by the addition of 2 M sulfuric acid and centrifuged at $10,000 \times g$ for 10 min. The supernatant was made 2% with respect to trichloroacetic acid by the addition of 100% (w/v) trichloroacetic acid and centrifuged at $10,000 \times g$ for 10 min. The supernatant was then made 25% with respect to trichloroacetic acid and centrifuged at

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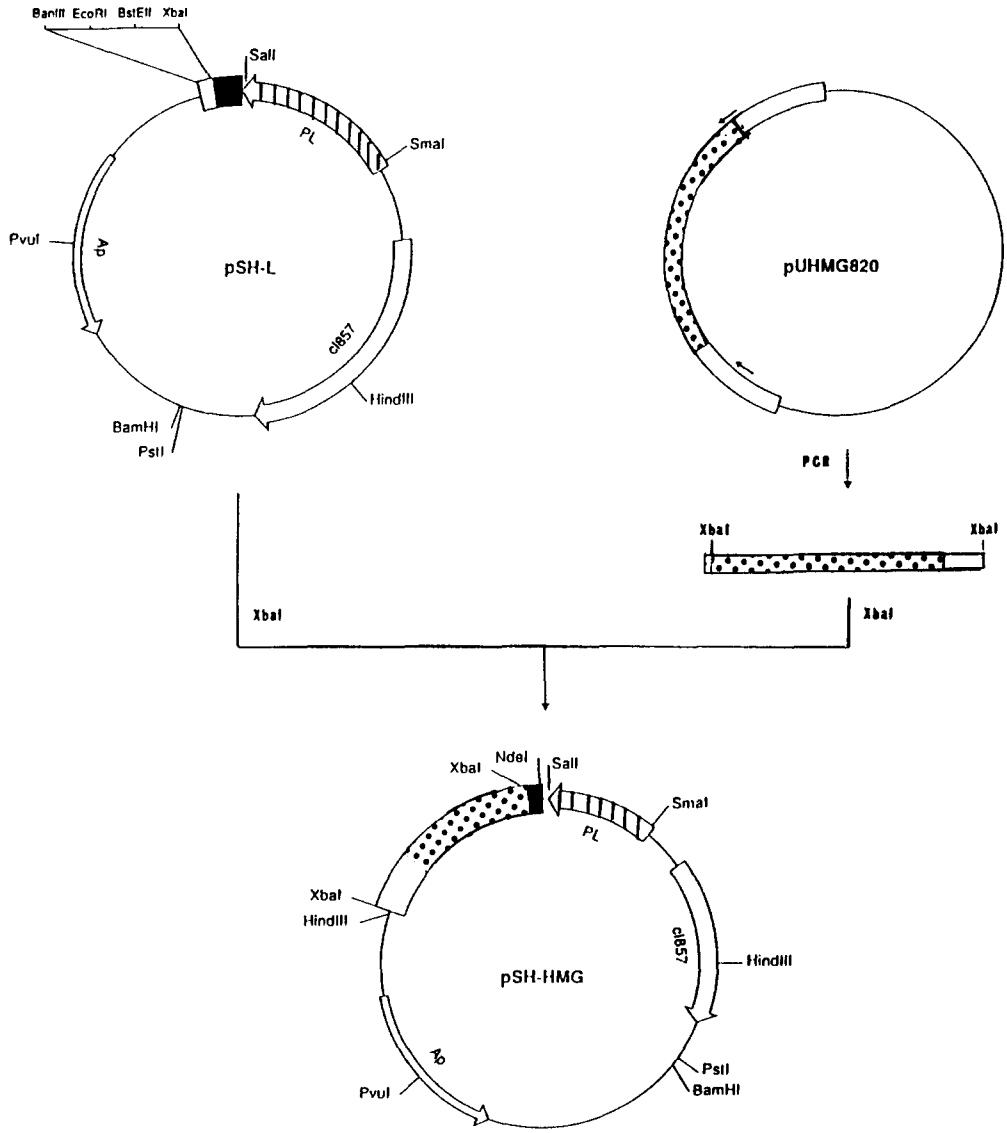
A



B

(a) Xba I Bst EII Eco RI Ban III

5' -CTAGA GGTGACC GAATTC AT- 3'

3' -T CCACTGG GTTAAG TAGC- 5'

(b)

Val Ala Gln Ala Ala Pro Thr Leu Glu Ala Lys Gly Asp

5' -GTA GCG CAG GCC GCG CCT ACT CTA G AG GCT AAA GGT GAC- 3'

3' -CAT CGC GTC CGG CGC GGA TGA GAT CTC CGA TTT CCA CTG- 5'

Xba I

Omp A signal ◄ ► HMG 2a

amino acid originated from MAP

10,000 × g for 10 min. The resultant pellet was dissolved in 20 ml of 100 mM sodium phosphate buffer (pH 6.5) and dialyzed against the same buffer. The dialyzed sample was applied to a Bio-Rex 70 column (26 × 40 mm) and eluted with a linear gradient of 4% to 8% guanidium chloride in 100 mM sodium phosphate buffer (pH 6.5). The fractions reactive against anti-HMG 2a antibodies were pooled and concentrated. The yield of protein was 1.0 mg from 4 l of culture.

2.6. Identification of HMG 2a

Proteins in the samples were separated on 15% polyacrylamide gels containing 0.1% sodium dodecyl sulfate (SDS) according to Laemmli [14]. Gels were either stained with Coomassie brilliant blue R-250 or proteins were transferred to nitrocellulose membrane. Immunodetection was carried out by first incubating the membrane with rabbit polyclonal antibody raised against HMG 2 protein from chick liver [15] and then with horse radish peroxidase-conjugated goat anti-rabbit IgG (Cappel Products, USA) [16]. The quantity of the recombinant HMG 2a expressed in the transformant was estimated by an enzyme-linked immunosorbant assay (ELISA) as described previously [12,15].

2.7. Functional analysis of the recombinant HMG 2a

The assay for the binding of the recombinant HMG 2a with DNA was performed by a modification of the procedure described by Waga et al. [17]. Reaction mixtures (100 μl each) contained 35 mM Tris-HCl (pH 8.0), 75 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 5 mM spermidine, 0.01% bovine serum albumin, 30 mM NaCl, 3 units of DNA topoisomerase I and 1 μg of pSP-DHFR DNA (Form 1). The reaction mixtures were incubated at 37°C for 30 min. Varying amounts of HMG 2a proteins were added to the reaction mixtures and incubated for additional 2 h. The reaction was stopped by the addition of sodium dodecyl sulfate, followed by the ethanol precipitation. The DNA samples were analyzed on a 1% agarose gel at 2.3 V/cm for 12 h. The gel was stained with 1 μg/ml of ethidium bromide for 1 h and photographed under the ultraviolet light.

3. Results

3.1. Expression of recombinant HMG 2a

Fig. 2 shows the time-course of HMG 2a production in the *E. coli* transformant MM 294/pSH-HMG. The extracts obtained by a brief sonication of the cell were analyzed by SDS-PAGE. The intensity of a band, slightly slower moving than the authentic HMG 2a, gradually increased during 5 h after the induction by temperature shift. As immunostaining experiment showed that the increase of the band intensity was due to an expression of recombinant HMG 2a (data not shown). The analysis of samples obtained by cell fractionation indicated that the mature type of recombinant HMG 2a was accumulated in the periplasmic space (data not shown). Using ELISA, the amount of recombinant HMG 2a produced in the cells 5 h after the induction was estimated to be 0.5 mg/l of culture.

3.2. Characterization of recombinant HMG 2a

HMG 2a was extracted and purified as described in section 2. An analysis of the purified protein by SDS-PAGE revealed a single band, having a slightly lower mobility than the authentic HMG 2a (Fig. 3A), which was immunostained by anti-HMG 2 antibody (Fig. 3B). The first eight amino acid residues from the NH_2-terminal of the purified protein were found to be Ala-Pro-Thr-Leu-Glu-Ala-Lys-Gly. The first five residues

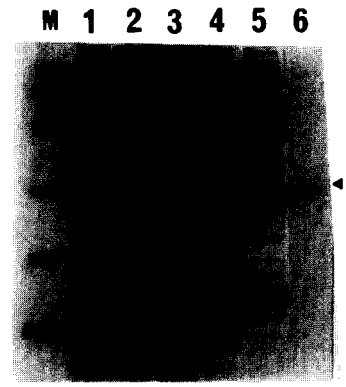


Fig. 2. Time course of HMG 2a production after induction by temperature shift. Bacteria were precultured in L-broth and expression was initiated by raising the culture temperature to 42°C as described in section 2. Each extract, containing 30 μg protein, was separated on 15% SDS-PAGE and the protein bands were visualized by Coomassie brilliant blue. Lane 1, start of the induction; lanes 2 to 5, 0.5, 1, 3, 5 h after the induction, respectively; lane 6, authentic HMG 2a. Arrowhead indicates the position of expressed recombinant HMG 2a. Molecular weight markers (M) are phosphorylase b, 94,000; bovine serum albumin, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; trypsin inhibitor, 20,000; and α-lactoalbumin, 14,000 (from top to bottom).

correspond to the amino acid residues originated from the linker sequence of pSH-L. This result indicates that the cleavage of a peptide bond in the fusion protein by signal peptidase between the Omp A signal peptide and the designed HMG 2a took place at the expected site in the bacterial cells.

3.3. Superhelical activity of recombinant HMG 2a

The ability of recombinant HMG 2a to induce superhelical conformation in pSP-DHFR form I DNA was examined by electrophoresis on an agarose gel. As shown in Fig. 4, both authentic (lanes 3–5) and recombinant (lane 6 and 7) HMG 2a proteins induced DNA superhelicity, depending on the HMG-to-DNA ratios.

4. Discussion

The HMG 1/2 proteins have a highly conserved tripartite structure. Two folded domains A and B (HMG boxes) are homologous to each other and are followed by a highly charged COOH-terminal tail [18]. The NH_2-terminal A-domain and the central B-domain are positively charged and bind to DNA while the COOH-terminal domain is acidic and has been shown to interact with histones [18,19]. Recent studies have suggested that the COOH-terminal domain modulates the specific interaction of HMG 1/2 with negatively supercoiled DNA [4,20]. Transcription can lead to localized supercoiling of DNA because the topology of the elongation complex requires a relative rotation between RNA polymerase and DNA [21,22]. Involvement of HMG proteins in the regulation of transcription can

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Fig. 1. Construction of expression vector, pSH-HMG. (A) The plasmid pSH-HMG contains the following genetic loci: dotted box, HMG 2a coding sequence; open box, HMG 2a non-coding sequence; black box, Omp A signal sequence; hatched arrows, ribosome binding site and PL promoter. (B) a. The nculeotide sequence of the linker from pSH-L. b. The nucleotide sequence at around the ligation site between Omp A signal sequence and HMG 2a cDNA.

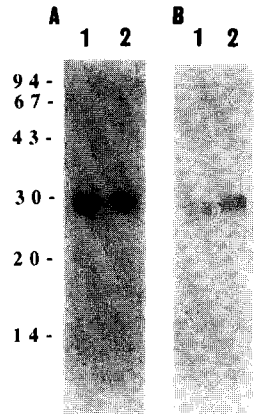

Fig. 3. SDS-PAGE analysis of purified HMG 2a. Authentic (lane 1) and purified recombinant (lane 2) HMG 2a proteins were separated, and stained (A) or immunoblotted (B) as described in section 2. Molecular weight markers are the same as in Fig. 2.

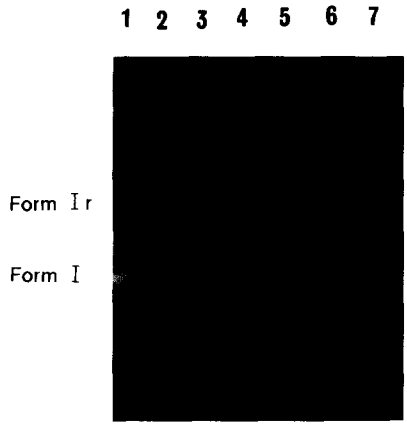

Fig. 4. Superhelical activity of recombinant HMG 2a. The assay was conducted as described in section 2. Lane 1, markers of superhelical DNA I and relaxed circular DNA Ir; lane 2, incubation without HGM 2a; lanes 3–5, incubation with authentic HMG 2a; lanes 6 and 7, incubation with recombinant HMG 2a. Lane 3, HMG/DNA 1; lanes 4 and 6, HMG/DNA 2; lanes 5 and 7, HMG/DNA 4.

thus be implicated. Waga et al. [23] have shown that the cruciform structure, formed under negative supercoiling, blocks transcription and that HMG 1 can remove the block by altering the DNA conformations.

Recently, Bianchi [24] reported the production of HMG 1 in *E. coli* under the control of the T7 promotor/T7 RNA polymerase system. However, the yield of HMG 1 in this method was 200 μg HMG 1 from 10 l of culture. We have now developed an efficient *E. coli* system for the production of HMG 2a (1 mg from 4 l of culture) and a convenient procedure for its purification. The recombinant HMG 2a, presently produced, had additional 5 amino acids at its NH_2-terminal but was shown to have essentially the same superhelical activity as the authentic HMG 2a isolated from the animal source. We believe that our system will provide a useful tool for further study on the mechanism of action of HMG proteins.

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