Protein Expression and Purification 110 (2015) 89–94

Contents lists available at ScienceDirect

Protein Expression and Purification

journal homepage: www.elsevier.com/locate/yprep

One-pot refolding of core histones from bacterial inclusion bodies allows rapid reconstitution of histone octamer

Young-Tae Lee [a,*], Garrett Gibbons [a], Shirley Y. Lee [a], Zaneta Nikolovska-Coleska [a], Yali Dou [a,b,*]

[a] Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109, USA
[b] Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:
Received 12 January 2015
and in revised form 7 February 2015
Available online 14 February 2015

Keywords:
Histone octamer
Nucleosome
Refolding
Histone methyltransferase
Small angle X-ray scattering

ABSTRACT

We report an optimized method to purify and reconstitute histone octamer, which utilizes high expression of histones in inclusion bodies but eliminates the time consuming steps of individual histone purification. In the newly modified protocol, *Xenopus laevis* H2A, H2B, H3, and H4 are expressed individually into inclusion bodies of bacteria, which are subsequently mixed together and denatured in 8 M guanidine hydrochloride. Histones are refolded and reconstituted into soluble octamer by dialysis against 2 M NaCl, and metal-affinity purified through an N-terminal polyhistidine-tag added on the H2A. After cleavage of the polyhistidine-tag, histone octamer is further purified by size exclusion chromatography. We show that the nucleosomes reconstituted using the purified histone octamer above are fully functional. They serve as effective substrates for the histone methyltransferases DOT1L and MLL1. Small angle X-ray scattering further confirms that the reconstituted nucleosomes have correct structural integration of histone octamer and DNA as observed in the X-ray crystal structure. Our new protocol enables rapid reconstitution of histone octamer with an optimal yield. We expect this simplified approach to facilitate research using recombinant nucleosomes *in vitro*.

Introduction

Regulation of gene expression is essential for the highly coordinated spatial and temporal patterns of gene expression, critical for cell fate determination and development. There are several distinct mechanisms that govern gene expression at transcriptional, post-transcriptional and post-translational steps. Among them, epigenetic regulation is extensively studied and includes modifications on DNA and histones. Abnormal regulation of epigenetic events leads to numerous human diseases including neurodegeneration, metabolic disorders and cancers [1]. Identification of small molecule inhibitors targeting enzymes responsible for epigenetic changes is a highly sought after therapeutics for the treatment of these diseases.

While synthesized histone tail peptides can be used as a substrate for *in vitro* assays of some histone modifying enzymes, recombinant nucleosomes are often desirable because certain histone modifying enzymes are enzymatically inactive against peptide substrates and nucleosomes provide a more physiologically relevant substrate [2]. Reconstitution of nucleosomes requires separate purifications of histone octamer and DNA template, and reconstitution of purified histones and DNA [3]. This standard protocol, however, has required multi-step column purifications under denatured conditions. A purification method with a less number of steps was published recently [4]. However, time-consuming processes of purifying each histone individually and subsequent reconstitution of octamer are still required in this revised protocol. Alternatively, soluble histone octamers or H2A/H2B dimer and H3/H4 tetramer have been purified from bacteria by coexpression [5,6]. However, overall levels of histone coexpression were low and large portions of histones were still found in inclusion bodies. In this report, we present a novel approach for nucleosome reconstitution that takes advantage of high overexpression levels of histones in inclusion bodies and bypasses all purification steps under denaturation conditions by one-pot refolding of histones into octamer.

Materials and methods

Expression plasmids

Xenopus laevis H2A was subcloned into the Ligation Independent Cloning vector pMCSG7 [7], which encodes a hexahistidine

* Corresponding authors at: Department of Pathology, University of Michigan, MSI 5215, 1301 Catherine Rd, Ann Arbor, MI 48103, USA.
E-mail addresses: lyoungta@med.umich.edu (Y.-T. Lee), yalid@med.umich.edu (Y. Dou).

http://dx.doi.org/10.1016/j.pep.2015.02.007
1046-5928/

tag (His6)[1] and a TEV protease recognition sequence followed by the SspI restriction enzyme site. PCR amplified H2A was annealed into the vector at the SspI site. After cleavage at the TEV recognition site, additional amino acids Ser-Asn-Ala remain at the N-terminus of the H2A sequence. Intact *X. laevis* H2B and H3 and H4 were subcloned into the pET3 vector.

Expression and purification of histones

Colonies from a freshly transformed BL21(DE3) *Escherichia coli* strain were inoculated into starter cultures of LB media with supplement of 0.4% glucose and cultured overnight at 37 °C. Cells were 100-fold diluted into fresh LB media and cultured at 37 °C. When OD_{600} reached 0.6, IPTG was added at a final concentration of 0.4 mM and cells were grown for an additional 3 h for His6-H2A, H2B and H3 and 2 h for H4. Cells were harvested and resuspended in buffer of 50 mM Tris (pH 7.5) and 100 mM NaCl (30 mL/cells from each liter) and then stored at −80 °C until purification.

As expression levels were variable for each histone (Fig. 1), we used different amount of cells for each histone to achieve similar stoichiometry for one-pot refolding. For example, in this report we processed cells from 1 L His6-H2A, 4 L H2B, 2 L H3 and 2 L H4 cultures. After thawing from storage, cells were combined into a mixture and were lysed by sonication for 10 min per 30 mL of mixed resuspended cells (output power 9, duty cycle 20%, Branson Sonifier Model 250) and supernatant was removed by centrifugation at 32,000 g (F18-12 x 50 rotor, Fiberlite) at 4 °C. Inclusion bodies were dissolved in 10 mL of 8 M guanidine hydrochloride, 20 mM acetate (pH 5.2) and 10 mM DTT per cells from each liter and incubated for 1 h at room temperature. Then undissolved material was separated by centrifugation at 32,000 g for 20 min at 25 °C. Supernatant was transferred into a dialysis membrane of MWCO 8000 (BioDesignDialysis) and dialyzed against buffer containing 20 mM Tris (pH 8.0), 2 M NaCl and 2 mM β-mercaptoethanol in 4 L at 4 °C three times for at least 8 h. Precipitates formed heavily during the refolding were removed by centrifugation at 15,000 g for 10 min at 4 °C. Supernatant containing refolded histone octamer was loaded on a 5 mL Ni-NTA resin (Qiagen). After extensive washing with buffer containing 20 mM Tris (pH 8.0), 2 M NaCl, 10 mM imidazole and 2 mM β-mercaptoethanol, histone octamer was eluted stepwise at 30, 60, 90, 120, 150, 210 and 300 mM imidazole (5 mL each). TEV protease (Jennifer Meagher, Center for Structural Biology, University of Michigan) was added to the pooled fractions containing the histone octamer and cleavage reaction was carried out at 4 °C for overnight. The TEV protease-cleaved sample was concentrated and loaded on a HiLoad 16/600 Superdex 200 PG column (GE Healthcare). The buffer for the size exclusion chromatography contained 20 mM Tris (pH 7.5), 2 M NaCl and 1 mM DTT. Fractions were analyzed by SDS–PAGE and those containing only histone octamer were pooled and concentrated to >2 mg/mL using a MWCO 30 kDa centrifugal concentrator (EMD Millipore). The sample was dialyzed against 500 mL of 20 mM Tris (pH 7.5), 2 M NaCl, 1 mM DTT and 1 mM EDTA in 50% glycerol (v/v) for overnight at 4 °C and stored at −20 °C.

Purification of 147 bp Widom 601 DNA

Plasmid DNA containing 12 copies of the strong positioning Widom 601 DNA sequence with EcoRV sites between repeats [8] was transformed into XL1-Blue bacterial cell. Single colonies were inoculated into 0.5 L of LB media in a 2.8 L flask. A total of 2.5 L were cultured for plasmid preparation using the Qiagen Plasmid Giga Kit. Yield from the kit was about 10 mg of the DNA plasmid. The Widom 601 fragment was excised by digestion with 200 units of EcoRV per 1 mg of DNA plasmid at 37 °C for 16 h. The 601 fragment was purified by PEG fractionation as described in Dyer et al. [3].

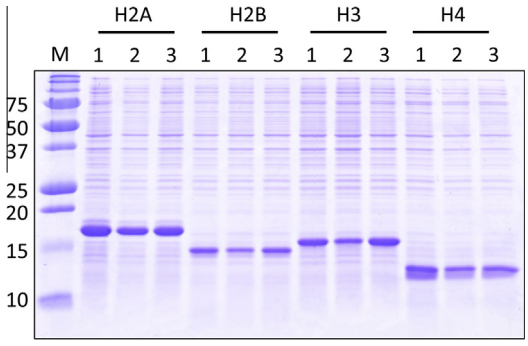

Fig. 1. Expression levels of histones in the host bacteria BL21(DE3) are variable. Histones were expressed using the starter cultures grown overnight at 37 °C with a supplement of 0.4% glucose (lanes 1) or its omission (lanes 2). Lanes 3; cells were kept below the log phase until protein induction. M; size markers (kDa).

Reconstitution of mononucleosome

Histone octamer in 50% glycerol was dialyzed against 20 mM Tris (pH 7.5), 2 M NaCl, 1 mM EDTA and 1 mM DTT for overnight at 4 °C. Octamer concentration was measured based on UV absorption at 280 nm with the extinction coefficient of 39,020 cm^{-1} M^{-1} or 2.78 mg/mL for OD_{280} = 1. Next, DNA and histone octamer were combined in a 1:1 molar ratio for reconstitution with concentrations of 0.70 mg/mL of the 601 DNA and 0.85 mg/mL of histone octamer. Nucleosome reconstitution was carried out by salt dialysis either by stepwise gradient for a small scale or linear gradient for a large scale as described by Dyer et al. [3]. Briefly, for salt dialysis by stepwise gradient, samples were dialyzed against 500 mL of 20 mM Tris (pH 7.5), 1 mM DTT, 1 mM EDTA with 2, 0.85, 0.65, 0.2 and 0.05 M NaCl for at least 1.5 h each at 4 °C. For long-term storage, reconstituted nucleosomes were further dialyzed against 20 mM cacodylate (pH 6) and 1 mM EDTA and stored at 4 °C as recommended [3].

Histone methyltransferase (HMTase) assay

GST-DOT1L catalytic domain (amino acids 1–420), with N-terminal His6 tag, was recombinantly expressed in *E. coli* and purified by nickel affinity chromatography and subsequent size exclusion chromatography using a Superdex-75 column with 20 mM Tris (pH 8.0), 200 mM NaCl, 1 mM DTT, 1 mM EDTA buffer. Human MLL1 SET domain, WDR5, RbBP5, and ASH2L were cloned on a pET28a-based vector encoding a polyhistidine-SUMO tag, and purified as previously described [9]. HMT assays for human MLL1 were carried out as described previously [10]. The HMTase reaction for DOT1L was carried out by incubation of 125 nM GST-DOT1L with 0.7 μg of either recombinant nucleosomes or HeLa extracted nucleosomes (52015; BPS Bioscience) and 125 nM (0.28 μCi) ^{3}H-S-adenosyl methionine (NET155250UC; Perkin Elmer). S-adenosyl homocysteine (SAH) was added to the reaction at varying concentrations before the addition of ^{3}H-S-adenosyl methionine to inhibit DOT1 HMTase activity. The reaction was carried out in 20 mM Tris (pH 7.9), 4 mM EDTA, 1 mM DTT, 0.01% Triton X-100 at a final volume of 26.5 μL. After 1 h of reaction, 5 μL of reaction mixture was transferred to P81 filter paper. Filter papers were dried and washed three times with 50 mM $NaHCO_3$ (pH 9.0). Filter papers were dried

[1] *Abbreviations used:* His6, hexahistidine tag; HMTase, histone methyltransferase; SAH, S-adenosyl homocysteine.