Analytical Biochemistry 412 (2011) 183–188

Contents lists available at ScienceDirect

Analytical Biochemistry

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

BioVyon Protein A, an alternative solid-phase affinity matrix for chromatin immunoprecipitation

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ARTICLE INFO

Article history:
Received 12 November 2010
Received in revised form 25 January 2011
Accepted 25 January 2011
Available online 1 February 2011

Keywords:
Chromatin immunoprecipitation (ChIP)
BioVyon Protein A
Protein A Sepharose
Dynabeads Protein A
CCCTC-binding factor (CTCF)
CTCF binding (target) sites (CTSs)
RNA polymerase II
TATA box
Quantitative real-time polymerase chain reaction (Q-PCR)

ABSTRACT

Chromatin immunoprecipitation (ChIP) is an important technique in the study of DNA/protein interactions. The ChIP procedure, however, has limitations in that it is lengthy, can be inconsistent, and is prone to nonspecific binding of DNA and proteins to the bead-based solid-phase matrices that are often used for the immunoprecipitation step. In this investigation, we examined the utility of a new matrix for ChIP assays, BioVyon Protein A, a solid support based on porous polyethylene. In ChIP experiments carried out using two antibodies and seven DNA loci, the performance of BioVyon Protein A was significantly better, with a greater percentage of DNA pull-down in all of the assays tested compared with bead-based matrices, Protein A Sepharose, and Dynabeads Protein A. Furthermore, the rigid porous disc format within a column made the BioVyon matrix much easier to use with fewer steps and less equipment requirements, resulting in a significant reduction in the time taken to process the ChIP samples. In summary, BioVyon Protein A provides a column-based assay method for ChIP and other immunoprecipitation-based procedures; the rigid porous structure of BioVyon enables a fast and robust protocol with higher ChIP enrichment ratios.

The chromatin immunoprecipitation (ChIP)[2] assay is an important research tool in modern molecular biology [1–5]. It allows the study and identification of DNA sequences that are specifically bound to particular proteins; they represent important regulatory elements in transcriptional machinery. The ChIP assay is a complex procedure that involves several steps: DNA/protein crosslinking, sonication, immunoprecipitation (IP) of the crosslinked DNA/protein (chromatin) complexes, capture of these complexes, DNA recovery from the precipitated product, and DNA analysis. During the IP step, antibodies specific to the protein component are employed and capture of the immunoglobulin/DNA/protein complexes is achieved by the specific binding of immunoglobulins to Protein A and/or Protein G conjugated to a solid support [6]. DNA analysis can be carried out by polymerase chain reaction (PCR), quantitative real-time PCR (Q-PCR), hybridization on microarrays (ChIP–ChIP) [7,8], or direct sequencing (ChIP–seq) [9].

ChIP assays, however, suffer from inherent problems that can often lead to misleading, or even erroneous, interpretation of the ChIP data [4]. These problems arise during IP, the most crucial part in the ChIP assay. Two main components of IP determine the quality and quantity of the ChIP DNA: the antibody and the solid support used for binding the antigen/antibody complex. The ChIP antibody may contribute to nonspecific background signal because of their possible cross-reactivity with other antigens. The ChIP antibody may also be responsible for a low yield of the recovered DNA due to a low affinity for the protein bound to the DNA. However, the bulk of nonspecific binding to the solid support, in particular to agarose-based matrices such as Protein A Sepharose beads commonly used in IP and ChIP assays, is thought to be due to the DNA/protein complexes reacting with diverse chemical groups on the surface of the Sepharose.

The Sepharose-based matrices are derived from a naturally occurring material (seaweed) and have a very chemically heterogeneous base structure with a very high surface area (see Supplemental Fig. 1A in supplementary material). The high surface area of the Protein A Sepharose variants generally favors IP. However,

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[2] *Abbreviations used:* ChIP, chromatin immunoprecipitation; IP, immunoprecipitation; PCR, polymerase chain reaction; HDPE, high-density polyethylene; Q-PCR, quantitative real-time PCR; IgG, immunoglobulin G; SPE, solid-phase extraction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LS, low-salt; MS, medium-salt; HS, high-salt; SDS, sodium dodecyl sulfate; CTS, CTCF binding (target) site.

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doi:10.1016/j.ab.2011.01.036

in ChIP assays, nonspecific binding of chromatin tends to occur as a result of ionic interactions between the differently charged surfaces of Sepharose and DNA. To minimize this problem, an additional DNA preblocking step is often recommended in ChIP protocols. During this step, the Sepharose-based solid support is preincubated with nonhomologous DNA/RNA prior to IP to block any potential active binding sites. However, this procedure might not remove all of the nonspecific binding and adds an additional step to the process.

To improve the quality of the IP step in a ChIP assay, Porvair Filtration Group has developed an alternative matrix, BioVyon Protein A, which is based on porous high-density polyethylene (HDPE). BioVyon has very different polymer chemistry from Sepharose; it is a synthetic polymer made up of repeat units of the hydrocarbon ethylene and is chemically homogeneous (Supplemental Fig. 1B), this structure is less variable and much more inert. The surface of BioVyon has been chemically etched (by a proprietary oxidation method), providing a moderate increase in surface area and allowing the covalent attachment of a linker and a Protein A molecule (Supplemental Figs. 2 and 3). The etching/oxidation process introduces a relatively low surface concentration of oxidized species, relative to a polysaccharide (Sepharose)- or methacrylate (Dynabeads)-based material, leaving an inert surface with a concentration of Protein A sufficient for the ChIP assay. The chemical structure of BioVyon Protein A, described above, is expected to exhibit reduced amounts of nonspecific binding to DNA/protein complexes.

BioVyon is manufactured in the form of a solid but porous disc that is rigid and incompressible and is inserted into a column (Supplemental Fig. 4). This makes the BioVyon matrix easier to handle than bead-based matrices and allows a column to be prepared with a precise amount of Protein A fixed at the base.

In this study, we carried out ChIP assays with two different antibodies and several DNA loci using BioVyon Protein A in microcolumns. These results were compared with the results of ChIP assays obtained using other matrices under similar conditions (Protein A Sepharose and Dynabeads Protein A). Dynabeads are superparamagnetic particles coated with a polyacrylate polymer layer. From these experiments, we concluded that the BioVyon Protein A matrix presents an attractive alternative to the existing matrices in ChIP and, potentially, in other IP-based assays.

Materials and methods

Cells

NIH 3T3 mouse fibroblasts were maintained in Dulbecco's modified Eagle's medium (DMEM, Lonza, Basel, Switzerland) supplemented with 10% donor serum and 50 μg/ml gentamicin (both from Invitrogen, Carlsbad, CA, USA). MCF7 human breast cancer cells were cultured in RPMI-1640 with Ultraglutamine 1 (both from Lonza) supplemented with 10% fetal bovine serum (FBS, Biosera, East Sussex, UK) and 50 μg/ml gentamicin.

Antibodies

Antibodies for this study were as follows: anti-RNA polymerase II (8WG16) mouse monoclonal (Covance Research Products, Princeton, NJ, USA), previously used in ChIP assays [10]; anti-CTCF (CCCTC-binding factor) rabbit polyclonal (Upstate (Millipore), Billerica, MA, USA), employed for genome-wide ChIP analyses (e.g., Refs. [11,12]); anti-His-tag rabbit polyclonal (Abcam, Cambridge, UK); anti-β-actin mouse monoclonal (Sigma–Aldrich, St. Louis, MO, USA); mouse and rabbit immunoglobulin G (IgG, Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Q-PCR

The primers and conditions for Q-PCR are described in Supplemental Table 1 in the supplementary material. Q-PCRs were performed as reported previously with modifications [13]. In brief, the reaction components were assembled in a 25-μl mixture containing 3 μl of sample, 200 nM of each primer, and 12.5 μl of SensiMix Plus SYBR Green PCR Kit (Quantace, London, UK). Amplification, data acquisition, and analysis were conducted using the Chromo4 Real Time PCR (Bio-Rad Laboratories, Hercules, CA, USA). Dissociation curve analysis was performed for each sample after PCR to verify that a single amplicon of the expected melt curve characteristics was obtained. The amount of precipitated DNA was calculated relative to the total input chromatin and expressed as a percentage of the total according to the formula% input = $2^{\Delta Ct} \times 100\%$, where $\Delta Ct = Ct\,(\text{input}) - Ct\,(\text{IP})$ and Ct is the mean threshold cycle of the corresponding PCR reaction [14]; this method has been widely used in the scientific community (for examples, see Refs. [13,15]). These experiments were carried out in triplicate, and the average was obtained from the percentage input.

BioVyon Protein A columns

BioVyon Protein A columns were developed and manufactured by Porvair Filtration Group and provided by the company for this study. The columns have the same dimensions as a standard 1-ml solid-phase extraction (SPE) tube and contain a rigid porous HDPE BioVyon Protein A frit approximately 6 mm in diameter and 2 mm long (Supplemental Fig. 4). The frits have been chemically treated to increase surface area using a proprietary selective oxidation method, which preferentially etched the surface and provided carboxylic acid anchor groups, for further covalent attachment. The pits caused by the etching process can be clearly seen in Supplemental Fig. 3, which compares the microstructure of the surface before and after etching. The pitting was responsible for the increased surface area, and further details regarding this are available in Supplemental Fig. 2. The surface has been tailored by this process to provide sufficient functionality for the IP stage of the ChIP assay. The anchor groups formed on the surface of the HDPE were then covalently coupled to the Protein A via a linker to form the BioVyon Protein A solid phase.

ChIP assays

ChIP assays were conducted according to the manufacturer's manual for the ChIP Kit (Upstate (Millipore)) for Protein A Sepharose (Sigma–Aldrich) with some modifications. In ChIP experiments involving BioVyon Protein A, the above protocol with some modifications was applied; the Protein A Sepharose slurry there was replaced by the BioVyon Protein A columns. In the ChIP assays with Dynabeads Protein A (Invitrogen), we followed the manufacturer's protocol. The detailed protocols of the ChIP experiments with the three different matrices are provided in the Supplemental Materials and Methods Section of the supplementary material.

Statistical analysis

Statistical analysis was carried out using unpaired Student's t test. A significant value was detected when the probability was below the 5% confidence level ($P < 0.05$).

Results and discussion

In the ChIP protocol developed for BioVyon Protein A, the IP step required optimization for use with this solid support. As a model