Assays of nucleosome assembly and the inhibition of histone acetyltransferase activity: 1. Assay of the inhibition of histone acetyltransferase (HAT) activity

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Method Article

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Abstract

Introduction

We describe the protocol here using transcription factor JDP2 as a model protein with HAT-inhibitory and nucleosome-assembly activity. Filter-binding assays are performed as described elsewhere¹ with minor modifications (see Figure 1a). For a detailed introduction to assays of nucleosome assembly and the inhibition of histone acetyltransferase activity, please go here:

"http://www.natureprotocols.com/2007/07/30/assays_of_nucleosome_assembly.php":http://www.natureprotocols.com/2007/07/30/assays_of_nucleosome_

Reagents

500 mM Tris-HCl (pH 8.0) (Sigma-Aldrich Corp., St. Louis, MO, USA; cat. no. 93349) 0.5 mM Ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich Japan, Tokyo, Japan; cat. no. E9884) 5.0 mM Dithiothreitol (DDT; Sigma-Aldrich Japan; cat. no. D0632) 50% Glycerol (Sigma-Aldrich Japan; cat. no. G5516) Core histones (Upstates Biotech. Co.; Charlottesville, VA, USA; cat. no. 13-107) [³H]Acetyl-CoA (4.3 Ci/mmol; GE Healthcare Bioscience Co., Tokyo, Japan) 0.5 mM Acetyl-CoA trilithium salt (Wako Pure Chemical Industries Ltd., Osaka, Japan; cat. no. 018-10811) P-81 Phosphocellulose filter paper (Upstate Biotech. Co.; cat. no. 20-134) 50 mM Na₂HPO₄·12H₂O (pH 9.0) (Wako Pure Chemical Industries Ltd.; cat. no. 194-02835) Acetone (Wako Pure Chemical Industries Ltd.; cat. no. D18-01146) 5x Reaction buffer, 500 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 5.0 mM DDT, 50% glycerol [³H]Acetyl-CoA mix; [³H]acetyl-CoA (4.3 Ci/mmol), non-radiolabeled 0.5 mM acetyl-CoA trilithium salt and distilled water (ddH₂O) combined at 1:1:3 (v/v) Jun dimerization protein 2 (JDP2) purified as glutathione S-transferase-JDP2 (GST-JDP2), as described elsewhere^{2,3} p300 purified as (His)₆-tagged-p300, as described elsewhere⁴

Procedure

1. Incubate the sample at 30 °C for 60 min in 25 ml of assay buffer [which contains 1x reaction buffer, namely, 50 mM Tris-HCl (pH 8.0), 10% glycerol, 0.1 mM EDTA, 1.0 mM DTT] with 6 pmol of [³H]acetyl-CoA (4.3 mCi/mmol; Amersham Biosciences Co., Tokyo, Japan) and core histones as indicated: [See figure in Figures section](#). p300 protein is used as the HAT for acetylation of core histones in vitro. In the absence of JDP2, the HAT is taken as 100%. 2. In some cases, in our model experiments, reconstituted nucleosomes that contain the cis-element CRE, DRE, mDRE or mCRE^{2,5}, prepared as described elsewhere^{4,6}, and JDP2, as well as bZIP/ATF-2, are incubated first at 30 °C for 15 min. Then p300 and [³H]acetyl-CoA are added and incubation is continued for a further 45 min at 30 °C. 3. After incubation, spot each reaction mixture onto P-81 phosphocellulose filter paper (Upstate Biotechnology Co.). 4. Wash filters five times for 5 min each in 50 ml of 50 mM Na₂HPO₄ buffer and once in 50 ml of acetone for 5 min at room temperature. 5. Measure the radioactivity on air-dried filters in a liquid scintillation counter (Figure 1b). 6. Analyze reaction mixtures by SDS-PAGE (18% polyacrylamide) after reactions have been performed as described above. In experiments for which results are shown in Figures 1c and 1d, we used 100 pmol of [¹⁴C]acetyl-CoA (55 mCi/mmol; Amersham Life Science Inc.) instead of [³H]acetyl-CoA (Figures 1c and 1d). 7. The same protocol can be applied using reconstituted nucleosomes (Figure 1e).

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Figures

Figure 1

Inhibition of the HAT activity of GST-JDP2. a) Schematic representation of the design of the experiment for measuring HAT-inhibitory activity. AcCoA, acetyl CoA. (b) Dose-dependent inhibition of the acetylation of histones by JDP2. Filter-binding assays were performed with 2 µg of core histones and increasing concentrations (10 pmole and 30 pmole) of GST-JDP2 (columns 6 and 7), GST (columns 8 and 9) and BSA (columns 10 and 11). Lane 1, B buffer; lanes 5-11, 4 pmole of p300; lane 3, 30 pmole of BSA; and lane 4, 30 pmole of GST-JDP2. (c) Analysis by SDS-PAGE of HAT activity. Assays of HAT activity were performed and [¹⁴C]-acetylated histones were detected by autoradiography after separation by SDS-PAGE. Lanes 1-4, 500 ng of core histones; lanes 2-4, 4 pmole of p300; lane 3, 10 pmole of GST-JDP2; and lane 4, 5 pmole of GST. (d) Analysis by SDS-PAGE of HAT activity. Lanes 1-8, 4 pmole of p300; lane 2, with 10 pmole of GST; lanes 3-7, with 10 pmole of mutant GST-JDP2 (mutations at amino acid positions 39, 47, 49, 50 and 51 of JDP2, respectively) and lane 8, with 10 pmole of wild-type GST-JDP2. (e) JDP2 did not inhibit the HAT activity of p300 in nucleosomes. [¹⁴C]-Acetylated histones were detected by autoradiography after separation by SDS-PAGE. Lanes 1-4, 500 ng of mononucleosomes; lanes 2-4, 3 pmole of p300; lane 3, 5 pmole of GST-JDP2; and lane 4, 5 pmole of GST.

Component	Amount
5x Reaction buffer	5.0 µl
Core histones	5.0 µl
JDP2 (sample)	1.0 µl
6 pmol of [³ H]acetyl-CoA	2.5 µl
Preparation of p300	1.0 µl
ddH ₂ O	to 25.0 µl

Figure 2