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A peptide affinity reagent for isolating an intact and catalytically active multi-protein complex from mammalian cells

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ABSTRACT

We have developed an approach for directly isolating an intact multi-protein chromatin remodeling complex from mammalian cell extracts using synthetic peptide affinity reagent **4**. FOG1(1–15), a short peptide sequence known to target subunits of the nucleosome remodeling and deacetylase (NuRD) complex, was joined via a 35-atom hydrophilic linker to the StreptagII peptide. Loading this peptide onto Strep-tactin beads enabled capture of the intact NuRD complex from MEL cell nuclear extract. Gentle biotin elution yielded the desired intact complex free of significant contaminants and in a form that was catalytically competent in a nucleosome remodeling assay. The efficiency of **4** in isolating the NuRD complex was comparable to other reported methods utilising recombinantly produced GST–FOG1(1–45).

1. Introduction

To analyse the structure and function of multi-protein complexes they must first be obtained in their native form and in sufficient quantities for biophysical and biochemical analysis.[1–3] Expression of individual subunits and in vitro reconstitution of the protein complex is often undertaken but can be difficult for multi-protein complexes with many subunits.[4,5] An alternative approach is to isolate the endogenous complex directly from cell extracts using protein-derived affinity baits.[6,7] Although this typically yields smaller quantities of protein, sufficient material can be obtained for conducting preliminary analysis on the composition, structure and functional activity of the complex.[8–10]

We are interested in using synthetic molecules to isolate intact proteins and protein complexes from cell extracts. In a typical approach, peptides and small molecules are immobilized onto biocompatible solid supports through covalent attachment or using the biotin–streptavidin interaction and used in pulldown-LC–MS/MS experiments to identify their cellular targets.[11,12] In such systems, elution of captured proteins requires harsh, denaturing conditions and the structural and functional information contained within the captured protein complex is lost. We sought to develop an approach whereby short peptide or small molecule baits could be combined with affinity tags that enable gentle elution of the captured proteins. In developing such an approach we focused on isolating the multi-subunit nucleosome remodeling and deacetylase (NuRD) complex.

The NuRD complex consists of at least 10 protein components: RBBP7, RBBP4, HDAC1, HDAC2, MTA1, MTA2, GATAD2A, GATAD2B, MBD2 or 3 and CHD4[13] (Fig. 1A). The NuRD complex is unique as a chromatin remodeler because it combines two catalytic activities—ATP-dependent chromatin remodeling and lysine deacetylation. Through these two activities it is able to convert chromatin between open, poised and closed states[14,15] to regulate gene expression. In previous work, the NuRD complex was purified in a single step from murine erythroleukemia (MEL) cell nuclear extract by affinity chromatography using GST-fusions of the first 45 residues of the transcriptional co-regulator Friend of GATA 1 (GST–FOG1(1–45))[16] and later using the first 12 residues of the FOG2 (GST–FOG2(1–12)).[17] Recently a crystal structure of the NuRD component RBBP4 bound to FOG1(1–15) was reported[18] and this, along with additional biochemical experiments, revealed an Arg-Arg-Lys-Gln motif to be central to the FOG1–RBBP4 interaction (Fig. 1B). We decided to develop synthetic molecules based on this 15-residue domain from FOG1 as baits to capture the NuRD complex from MEL cell extracts.

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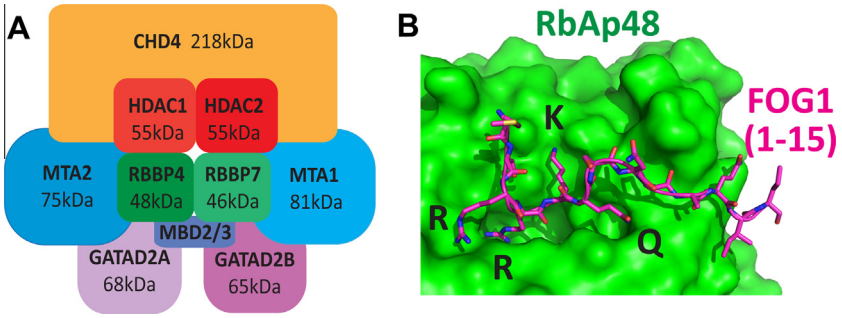

Figure 1. (A) Schematic of the nucleosome remodeling and deacetylase (NuRD) complex showing the well established subunits within the complex. (B) Crystal structure of FOG1(1–15) bound to RBBP4 (pdb 2XU7). The key RRKQ motif is shown.

2. Results and discussion

We first sought to confirm that FOG1(1–15) could indeed recruit the NuRD complex from MEL nuclear extract. We obtained FOG1(1–15) biotinylated at the C-terminus via the side chain of an additional lysine residue (bFOG1(1–15), **1**). In three parallel experiments, peptide **1** was loaded onto streptavidin beads and then incubated with a nuclear extract prepared from cultured MEL cells. Each set of beads was then washed with a different salt concentration, then the bound proteins were eluted by heat denaturation and the eluates for each experiment were analysed by SDS PAGE. Figure 2 shows the presence of many protein bands in the eluate from beads washed with 150 mM NaCl. However, when the beads were washed with higher salt concentrations (300 mM NaCl and 500 mM NaCl), fewer protein bands were observed in the eluates by SDS–PAGE. To confirm the presence of NuRD proteins, the gel lane corresponding to the eluate of beads washed with 300 mM NaCl was cut it into 10 pieces, each was individually digested with trypsin and the extracted peptides were analysed by LC–MS/MS. The NuRD components RBBP4, RBBP7, HDAC1, HDAC2, MTA1, MTA2, GATAD2A, GATAD2B, MBD3, MBD2 and CHD4 were all identified with 1–50 significant peptides and Mascot scores >500 (see Supporting information), demonstrating that **1** can recruit the entire NuRD complex. The data also show that a significant amount of non-specific binding takes place under these conditions.

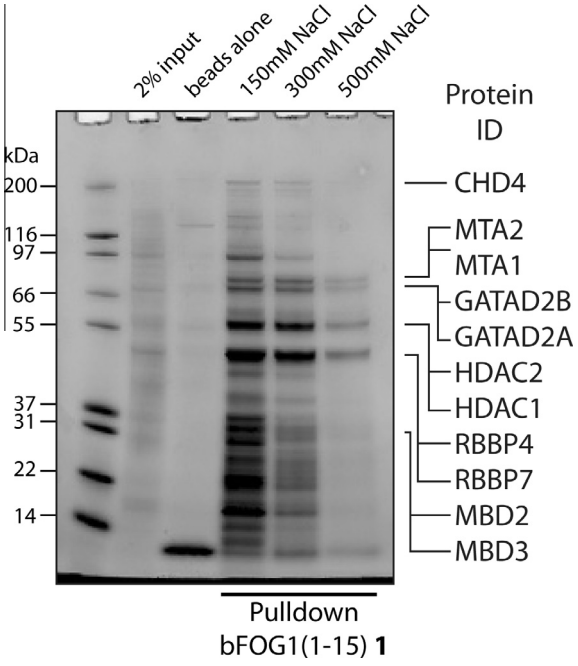

Figure 2. SDS PAGE analysis of eluates from protein pulldowns from MEL cell nuclear extract using **1** (bFOG1(1–15)) and different concentrations of salt during bead washes. Gel stained with Sypro-Ruby.

To facilitate gentle elution of the captured NuRD complex we next chose to investigate FOG1(1–15) joined to the StreptagII peptide affinity tag. This peptide tag can be loaded onto commercially available Streptactin beads and gently eluted using biotin or desthiobiotin.[19] A relative comparison of peptide tags for affinity purification indicated that the StreptagII–Streptactin system gives the best compromise between highly specific capture (e.g., relative to 6xHis-NiNTA), and cost of the affinity resin (e.g., relative to FLAG and HA antibody resins).[20] Therefore we decided to pursue this approach.

Initially we synthesised FOG1(1–15) with the eight-residue StreptagII peptide at the C-terminus (**2**, Fig. 3), loaded it onto Streptactin beads and attempted to pulldown the NuRD complex from MEL cell nuclear extracts. We attempted to elute **2**, along with any captured proteins, by three treatments with 10 mM biotin. However, no NuRD subunits were observed by SDS–PAGE in the elutions (Fig. 4A). We reasoned that upon binding to the Streptactin beads, peptide **2** might position the NuRD recruiting FOG1 domain in an awkward orientation that renders it unable to bind to RBBP4 or other NuRD components. We therefore prepared a second peptide in which the FOG1 domain and StreptagII peptide were separated by an aminohexanoic acid linker (**3**, Fig. 3) and repeated the pulldown from MEL cell nuclear extract (Fig. 4A). Once again, no NuRD proteins were observed by SDS–PAGE. Next, we decided to introduce a much longer linker between the StreptagII and FOG1(1–15). We also decided to incorporate fluorescein into the molecule so we could visually monitor peptide attachment to, and elution from, the beads.

The new peptide (**4**) was assembled entirely on solid phase according to Figure 3. After preparing the 8-residue StreptagII peptide, the bifunctional amine 4,7,10-trioxa-1,13-tridecanediamine was incorporated at the N-terminus via a urea linkage. Introduction of this linker was conducted at −20 °C to minimise hydantoin formation[21,22] which could prematurely cap the growing peptide chain. A lysine residue modified at its side chain Nε with carboxyfluorescein was then incorporated, followed by attachment of a second molecule of 4,7,10-trioxa-1,13-tridecanediamine through a urea linkage. The FOG1 peptide was then assembled to produce peptide **4** which had a 35-atom spacer between the StreptagII peptide and FOG1(1–15). This synthetic strategy gave the desired peptide in 13% purified yield and could quickly provide hundreds of milligrams that could conveniently be stored as a solid or in frozen aliquots.

Peptide **4** was then immobilized on Streptactin beads and incubated with MEL cell nuclear extract. The beads were washed in 500 mM NaCl and elution with 10 mM biotin was performed.