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Concerted action of the PHD, chromo and motor domains regulates the human chromatin remodelling ATPase CHD4

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ABSTRACT

CHD4, the core subunit of the Nucleosome Remodelling and Deacetylase (NuRD) complex, is a chromatin remodelling ATPase that, in addition to a helicase domain, harbors tandem plant homeo finger and chromo domains. By using a panel of domain constructs we dissect their roles and demonstrate that DNA binding, histone binding and ATPase activities are allosterically regulated. Molecular shape reconstruction from small-angle X-ray scattering reveals extensive domain-domain interactions, which provide a structural explanation for the regulation of CHD4 activities by intramolecular domain communication. Our results demonstrate functional interdependency between domains within a chromatin remodeller.

1. Introduction

Chromodomain helicase DNA binding protein 4 (CHD4), also known as Mi2β, belongs to the SNF2 family of helicases [1] and was first identified as a dermatomyositis-specific autoantigen [2]. CHD4 is the main subunit of the Nucleosome Remodelling and Deacetylase (Mi-2/NuRD) complex, a chromatin remodelling complex which is involved in many fundamental biological processes [3,4]. Mi-2/NuRD is thought to act as transcriptional repressor working in opposition to other chromatin remodellers such as SWI/SNF [5]. Like other chromatin remodelling complexes, Mi-2/NuRD achieves diversity in regulatory function through combinatorial assortment of its motor protein, CHD4, with other subunits including histone deacetylases HDAC1 and HDAC2 [6]. Despite the wealth of information available for CHD4, little is known on how this ATPase is targeted to specific sites within a chromatin environment and about the molecular mechanism of its chromatin remodelling activity.

In addition to its SNF2-type ATPase domain, the 218 kDa CHD4 protein harbours tandem plant zinc finger homeodomains (tPHD), which are found in a number of chromatin remodelling factors involved in nucleosome/histone binding [7–9], and tandem chromodomains (tCHD) which have been shown to mediate chromatin interaction by binding directly to either DNA, RNA or methylated histone H3 [10–13]. While the combination of tPHD and tCHD is a characteristic specific to CHD4 and two other members of the CHD family (CHD3 and CHD5) [14], the simultaneous presence of several histone-binding modules is a characteristic of many chromatin remodelling ATPases. So far, the mechanism by which these domains cooperate, and their role in the context of regulation of the ATPase motor and nucleosome remodelling remains unclear.

Here we dissect the roles of the individual domains of CHD4 and investigate their contribution to its enzymatic activity and targeting specificity. Low-resolution shape reconstructions, obtained from small angle X-ray scattering, suggest that the tight cooperation between the domains is mediated by intramolecular interactions due to their spatial proximity within CHD4. We present a plausible regulatory mechanism necessary for the nucleosome remodelling activity of CHD4.

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2. Materials and methods

2.1. Expression and purification of CHD4 constructs

C-terminal 8xHis-tagged constructs were generated by PCR using human CHD4 cDNA (Mammalian Gene Collection) as a template and appropriate sets of primers. The amplified PCR products were transferred into the expression pTriEx2 (Novagen) vector, verified by DNA sequencing, and used to transform *Escherichia coli* BL21 (DE3) cells. Expression in LB or TB media was induced with 0.7 mM of isopropyl-thiogalactopyranoside (IPTG) when culture reached OD_{600} = 0.6 and incubated overnight at 20 °C. Cells were lysed in 50 mM sodium phosphate pH 7.5, 500 mM NaCl, 5 mM imidazole, 0.2% Tween 20, protease inhibitor cocktail tablets (Roche) and the obtained supernatant mixed with Talon™ resin (Clontech). The recombinant proteins were eluted with imidazole gradients and further purified by size exclusion chromatography in 20 mM Tris pH 7.5, 200 mM NaCl, 1 mM DTT.

2.2. MALS

MALS experiments were carried out using an analytical Superdex S200 or S75 10/30 column (GE Healthcare) with online static light scattering (DAWN HELEOS II, Wyatt Technology, Santa Barbara, CA), differential refractive index (Optilab rEX, Wyatt Technology) and Agilent 1200 UV (Agilent Technologies) detectors.

2.3. EMSA

DNA binding and its dependence on the probe length was simultaneously probed using molecular weight markers (Gene Ruler 100 bp Plus DNA ladder, Fermentas; MW marker XIII 50–750, Roche; MW marker Gene Ruler 1 Kb and 100 bp Plus DNA ladder, Invitrogen) while 500 nt long ssRNA transcript was used for RNA. Probes were mixed with increasing amounts of CHD4 constructs in 20 μl of binding buffer (20 mM Tris pH 7.5, 200 mM NaCl), incubated for 5 min at room temperature and then run on a 2% agarose gel. In the case of the titration of tPHD into tCHD, the proteins were mixed in 20 mM Tris pH8, 200 mM NaCl buffer and incubated at RT for 30 min.

2.4. Nucleosome core particle mobility shift assay

Isolated radiolabeled mononucleosomes (50 fmol) reconstituted by salt gradient dialysis from recombinant *X. laevis* histones and a 168 bp fragment of 601-DNA (as described in [15] were incubated with increasing amount of CHD4 constructs for 15 min on ice in 10 μl of binding buffer (20 Tris–HCl pH 8, 200 mM NaCl, 10% sucrose). To control for nucleosome dissociation, any DNA released from the nucleosomes was chased with a 200-fold excess of λ-DNA. Nucleosome core particles were then separated by native gel electrophoresis in 5% polyacrylamide gels containing 10% glycerol run in 20 mM Hepes pH 8, 1 mM EDTA at 15 mA for 3 h. Gels were dried and nucleosome–protein complexes were visualized by autoradiography.

2.5. Surface plasmon resonance (SPR)

SPR binding studies were performed using a Biacore 2000/3000 and T100 (GE Healthcare) at 25 °C in 20 mM Tris pH 7.5, 200 mM NaCl, 0.05% (v/v) polysorbate 20. Biotinylated histone peptides (Millipore) were immobilized onto streptavidin sensor (GE Healthcare) [16]. Different concentrations of CHD4 construct were injected onto the sensor chip (flow rate 100 μl/min to minimize mass transport effects). Experiments were repeated three times. Sensorgrams were corrected for bulk solute and when appropriate, globally fitted to a second-order association reaction. The equilibrium dissociation constants K_d values were estimated from the concentration dependence of the steady-state response.

2.6. ATPase activity assay

The steady-state rate of ATP hydrolysis was determined using the phosphate release assay EnzCheck (Invitrogen) in a standard buffer (40 mM Tris pH 7.5, 50 mM NaCl, 5 mM $MgCl_2$) at 30 °C as described in [17].

2.7. SAXS data collection and processing

The solution small angle X-ray scattering (SAXS) data were collected using synchrotron radiation at ESRF beamline ID14-3 (Grenoble, France). Ten frames were collected and processed by in-house software. Background subtraction and data quality appraisal were done using PRIMUS program package [18]. Indirect transformation method was used to estimate the maximum dimensions (D_{max}) in GNOM [19–21]. Ab initio shape reconstruction was done using programs DAMMIN [21] and DAMMIF [22]. Multiple models were averaged (DAMAVER) and filtered (DAMFILT) to the estimated excluded volume [23] and superimposed (SUPCOMB [24,25]). SASREF was used to fit the tPHD, tCHD ab inito models and the ATPase atomic model (PDB: 3MWY [26]) into the tPHDtCHD/ATPase scattering data [27].

3. Results

In order to dissect the individual roles of the tPHD, tCHD and ATPase of the human CHD4 chromatin remodelling factor, we created a panel of domain constructs (Fig. 1a). All the proteins were purified to homogeneity and were assessed to be monodisperse by dynamic light scattering (DLS) (Supplementary Fig. 1).

3.1. The nucleic acid binding activity of the tCHD is modulated by the adjacent tPHD

We tested whether a minimal construct containing all three domains (tPHDtCHD/ATPase) of human CHD4 is able to bind dsDNA, whether the tCHD is responsible for such binding, and if this interaction is influenced by the adjacent tPHD and ATPase domains. The ability of different constructs to bind dsDNA was probed by mobility shift assays using a DNA ladder as a way of probing binding to differently sized molecules. The tPHDtCHD/ATPase retarded larger (>0.5 kbp) DNA fragments irrespective of ATP (Fig. 1b). In contrast, the ATPase exhibited weak binding, detectable only at high protein concentrations. The gradual increase of the observed DNA probe shift with protein concentration indicates that binding is not sequence specific and that more than one protein may bind to the same DNA fragment.

We next tested the binding affinity of isolated tPHD and tCHD for dsDNA. When incubated with the tCHD, the dsDNA probe was retained in the loading well, forming complexes too large to enter the gel (Fig. 1c) unless very low concentrations were used. The tCHD also exhibited ssDNA (data not shown) and ssRNA binding (Supplementary Fig. 2). In contrast, tPHD failed to bind nucleic acids (Fig. 1c, Supplementary Fig. 2).

A construct, containing both tPHD and tCHD (tPHDtCHD) exhibited dsDNA binding similar to that of tPHDtCHD/ATPase without aggregation (Fig. 1c). In contrast, a construct consisting of the ATPase domain and tCHD (tCHD/ATPase) aggregated in presence of DNA unless very low tCHD/ATPase concentrations were used, i.e. similar to tCHD alone. These results suggest that while the tCHD