Structure and Autoregulation of the Yeast Hst2 Homolog of Sir2

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Introduction

Yeast Sir2 (ySir2) is the founding member of the Class III histone deacetylases (HDACs) [1], and unlike other HDACs, its deacetylase activity is NAD^+-dependent [2-4]. The Sir2 proteins are extensive and broadly conserved from bacteria to human organisms, and the eukaryotic homologs appear to play roles in gene silencing, DNA repair, genome stability, and longevity [5-6].

Sequence homology among the Sir2 proteins is restricted to a roughly 270-residue domain that has been shown to be sufficient for catalytic activity [2, 4]. Interestingly, the intact prokaryotic homologs are not much larger than the 270-residue enzymatic domain, and prokaryotic organisms typically contain one or two Sir2 proteins. In contrast, the eukaryotic homologs usually contain more extended N- and C-terminal sequences, and eukaryotic organisms often have several Sir2 homologs, with yeast and human organisms having five and seven Sir2 homologs, respectively [1]. This observation suggests that the N- and C-terminal regions of the Sir2 proteins may impart an additional protein-specific level of regulation of the eukaryotic enzymes [1]. In order to understand how Sir2 proteins might be differentially regulated by regions outside the conserved catalytic domain, we have determined the x-ray crystal structure of the full-length Hst2 protein from yeast (yHst2) [7].

Methods and Materials

The full-length *Hst2* gene from *Saccharomyces cerevisiae* was cloned into the pRSET-A expression vector and overexpressed as an N-terminal 6 × His tagged-fusion protein (His-yHst2) in *Escherichia coli* BL21 (DE3). The protein was purified by using a combination of Ni-NTA resin (Qiagen) and Superdex-75 gel filtration chromatography. Selenomethionine-derivatized His-yHst2 protein was overexpressed from pRSET-yHst2-transformed bacterial strain B834 (DE3) (Novagen) and grown in a MOPS-based minimal medium as described elsewhere [8], and the protein was purified essentially as described for the underivatized protein.

Crystals of native and selenomethionine-derivatized His-yHst2 were grown at room temperature by using the hanging-drop vapor-diffusion method by equilibrating 4 mg ml^{-1} protein against a reservoir solution containing 1.8-1.9 M $(NH_4)_2SO_4$, 100 mM HEPES (pH 7.5), and 2% (volume/volume or v/v) PEG 400. Crystals formed in the space group R32 with one molecule per asymmetric unit cell, and they were flash-frozen in a reservoir solution supplemented with 25% glycerol for storage in solid propane prior to data collection. The His-yHst2 structure was determined by multiwavelength anomalous diffraction (MAD). Native data and a three-wavelength (peak, inflection, and remote) MAD dataset for selenium and zinc were collected on beamline 19-BM at the APS by using an ADSC Quantum-4 charge-coupled device (CCD) detector at 100K. The data were processed with the HKL 2000 suite (HKL Research Inc.). Four selenium sites were identified by using CNS [9] and SOLVE [10], and the zinc position was identified by using SHELX [11]. The phases from one Zn and four Se sites were combined and refined by using CNS and SOLVE, and the resulting electron density map was improved by solvent flipping with the program SHARP [12]. The program O [13] was used to build the model of the protein, with the selenomethionine and zinc sites used as guides. Model refinement employed simulated annealing and torsion angle dynamic refinement protocols in CNS, and iterative manual adjustments of the model were made by using the program O. Toward the later stages of refinement, individual atomic B-factors were adjusted, and solvent molecules were modeled into the electron density map. The final model was checked for errors with composite-simulated annealing omit maps, and a final round of refinement resulted in a model with excellent refinement statistics and geometry [7].

Results

The structure of the conserved yHst2 catalytic core is architecturally similar to the structures of the previously reported Sir2 homologs, Af1 Sir2-NAD [14] and Af2 Sir2-p53 peptide [15] from the archaean *Archaeoglobus fulgidus* and to the structure of human SIRT2 (hSIRT2) [16] (Fig. 1). What is particularly novel is that the intact yHst2 protein contains the structural elements (N-terminal and C-terminal) to the catalytic core domain that are not present in the isolated catalytic core domain of the Sir2-related structures. The C-terminal region contains two α-helices; α12 packs against the large domain of the catalytic core, and α13 binds within a cleft between the large and small domains. What is particularly interesting about the α13 helix of yHst2 is that it occupies the NAD^+-binding site of the catalytic domain, suggesting that this helix of yHst2 may

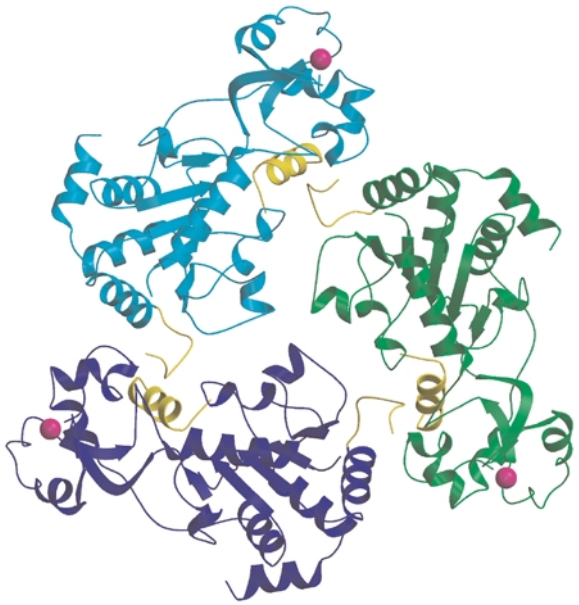

FIG. 1. Structure of the intact yHst2 homotrimer. Highlighted in yellow are the C-terminal helix and N-terminal strand that are proposed to autoregualte NAD^+ binding and acetyl-lysine binding, respectively.

autoregulate NAD^+ binding. The N-terminal's seven residues of the native protein form an extended loop that interacts with residues in the cleft between the small and large domains and the α13 helix of a symmetry-related molecule, mediating the formation of a symmetrical homotrimer in the crystal lattice. What is particularly interesting about the N-terminal stand and homotrimer formation is that it blocks the acetyl-lysine-binding site. These structural observations suggest that within yHst2, the N-terminal strand and homotrimer formation may autoregulate acetyl-lysine binding. Biochemical studies are consistent with the hypothesis that the C- and N- terminal regions of yHst2 autregulate NAD^+-binding and acetyl-lysine binding, respectively [7].

Discussion

Our studies with yHst2 suggest a mechanism by which both intramolecular regulation by the C-terminus and intermolecular regulation by trimer formation may occur. Since the regions N-terminal and C-terminal to the catalytic core domain of yHst2, which mediate this regulation, show no detectable sequence conservation with other Sir2 homologs, the mechanism for yHst2 autoregulation may differ from the mode of regulation by other Sir2 proteins. This difference may contribute to the different biological function and/or substrate specificities of the Sir2 proteins.

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