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Crystal structure of the protein kinase domain of yeast AMP-activated protein kinase Snf1

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Abstract

AMP-activated protein kinase (AMPK) is a master metabolic regulator, and is an important target for drug development against diabetes, obesity, and other diseases. AMPK is a hetero-trimeric enzyme, with a catalytic (α) subunit, and two regulatory (β and γ) subunits. Here we report the crystal structure at 2.2 Å resolution of the protein kinase domain (KD) of the catalytic subunit of yeast AMPK (commonly known as SNF1). The Snf1-KD structure shares strong similarity to other protein kinases, with a small N-terminal lobe and a large C-terminal lobe. Two negative surface patches in the structure may be important for the recognition of the substrates of this kinase.

Keywords: Obesity; Diabetes; Metabolism; Protein structure

AMP-activated protein kinase (AMPK) is a master metabolic regulator and an important target for drug development against diabetes, obesity, and other diseases [1–5]. A rising AMP:ATP concentration ratio leads to the activation of AMPK, which in turn shuts off energy-demanding, biosynthetic processes and stimulates energy-producing, catabolic processes. For example, AMPK is a natural, dual inhibitor of acetyl-CoA carboxylase 1 (ACC1) and ACC2 in animals. ACC1 catalyzes the rate-determining step of fatty acid biosynthesis, whereas ACC2 is a negative regulator of fatty acid oxidation.

AMPK is a hetero-trimeric enzyme, with a catalytic (α) subunit, and two regulatory (β and γ) subunits. The protein kinase domain is located at the N-terminal end of the α subunit (Fig. 1A). AMP is believed to activate AMPK through an allosteric mechanism, by binding to the γ subunit. In addition, AMP enhances the activation of AMPK by its upstream kinases, as well as inhibits the dephosphorylation/inactivation of AMPK by phosphatases.

AMPK is found in all eukaryotes. Yeast AMPK is more commonly known as SNF1 [1,6,7]. SNF1 has important roles in transcription of genes repressed by glucose for growth on non-glucose carbon sources as well as other biological processes [1]. In fact, the name SNF1 is derived from sucrose non-fermenting, as the protein is required for yeast growth on sucrose. Like mammalian AMPK, yeast SNF1 also contains three subunits, the catalytic α subunit (Snf1), and the non-catalytic β and γ subunits.

To help understand the molecular basis for the function of AMPK, we have crystallized the kinase domain (residues 41–315) of Snf1 (Snf1-KD) and determined its structure at 2.2 Å resolution by the selenomethionyl single-wavelength anomalous diffraction (SAD) method (Table 1) [8]. Snf1-KD shares significant sequence homology with the kinase domain of mammalian AMPKs (Fig. 1B), and our structure of Snf1-KD therefore also provides an excellent framework for the structure of the kinase domains of mammalian AMPKs.

Materials and methods

Residues 41–315 of yeast Snf1 were sub-cloned into the pET28a vector and over-expressed in *Escherichia coli* at 20 °C. The soluble protein was

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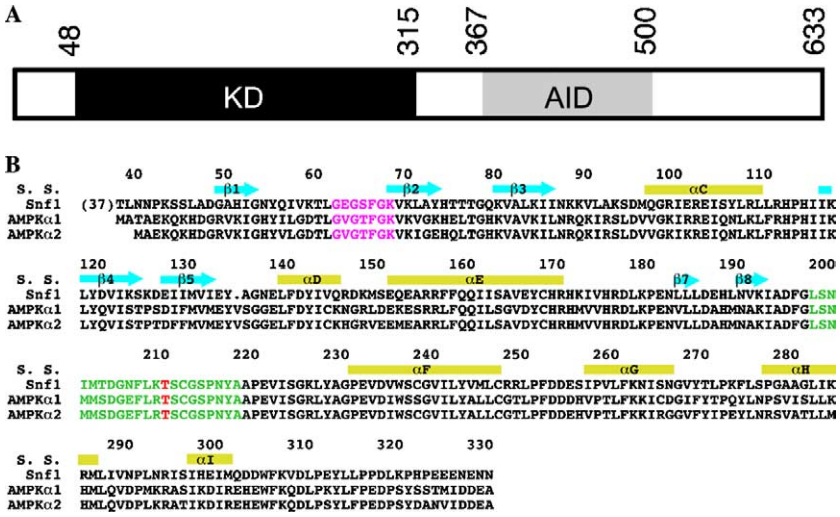

Fig. 1. Primary structure of the catalytic subunit of AMPK. (A) Domain organization of yeast Snf1. (B) Sequence alignment of the protein kinase domain of yeast Snf1 and the two α subunits of human AMPK. The secondary structure elements are indicated. The glycine-rich P-loop is shown in magenta and the activation loop in green. The Thr residue in the activation loop that is phosphorylated upon AMPK activation is shown in red.

Table 1
Summary of crystallographic information

Structure	Snf1-KD	
	SeMet	Native
Resolution range (Å)	2.6	2.2
Number of observations	123,225	172,279
R_{merge}[a] (%)	7.3 (34.4)	5.5 (35.5)
Number of reflections	19,375[b]	42,125
Completeness (%)	95 (95)	99 (99)
R factor[c] (%)	—	25.3 (25.6)
Free R factor[c] (%)	—	28.7 (31.0)
rms deviation in bond lengths (Å)	—	0.010
rms deviation in bond angles (°)	—	1.3

[a] $R_{merge} = \sum_h \sum_i |I_{hi} - \langle I_h \rangle| / \sum_h \sum_i I_{hi}$. The numbers in parenthesis are for the highest resolution shell.
[b] The Friedel pairs are treated as independent reflections.
[c] $R = \sum_h |F_h^o - F_h^c| / \sum_h F_h^o$.

purified by nickel affinity and gel filtration chromatography. The protein was concentrated to 20 mg/ml in a solution containing 50 mM Tris (pH 8.5), 500 mM NaCl, and 10 mM DTT, and stored at −80 °C. The selenomethionyl protein was produced in B834(DE3) cells, grown in defined LeMaster media supplemented with selenomethionine [9], and purified following the same protocol as that for the native protein.

Crystals of Snf1-KD were obtained at 21 °C by the sitting-drop vapor diffusion method. The protein was at 10 mg/ml concentration. The reservoir solution contained 100 mM Tris (pH 8.5), 25% (w/v) PEG3350, and 300 mM $(NH_4)_2SO_4$. The crystals were cryo-protected by the introduction of 20% (v/v) glycerol and flash-frozen in liquid nitrogen for data collection at 100 K. The crystal belongs to space group $P2_12_12_1$, with cell parameters of $a = 71.0$ Å, $b = 75.1$ Å, and $c = 113.7$ Å. There are two molecules of the kinase domain in the asymmetric unit.

X-ray diffraction data were collected at the X4A beamline of the National synchrotron light source (NSLS). The diffraction images were processed with the HKL package [10]. A selenomethionyl single-wavelength anomalous diffraction (SAD) data set to 2.6 Å resolution were collected on a crystal of the kinase domain. The Se sites were located with the program SnB [11]. The reflection phases were determined with the program Solve/Resolve [12], which also automatically located about 80% of the residues.

For structure refinement, diffraction data set to 2.2 Å resolution were collected on a native crystal of the kinase domain at the X4A beamline. The structure refinement was carried out with the programs CNS [13] and Refmac5 [14]. The atomic model was built with the program O [15]. The crystallographic information is summarized in Table 1.

Results and discussion

To help understand the molecular basis for the function of AMPK, we have crystallized the kinase domain (residues 41–315) of the catalytic (α) subunit of yeast AMPK, more commonly known as SNF1 [1,6,7], and determined its structure at 2.2 Å resolution by the selenomethionyl single-wavelength anomalous diffraction (SAD) method (Table 1) [8]. The current atomic model contains residues 48–58, 67–89, 96–199, and 215–315, and residues 48–59, 68–90, 96–199, 215–224, and 230–315 for the two kinase domain molecules in the crystallographic asymmetric unit, respectively, with an R factor of 25.3%. The majority of the residues (89%) are in the most favored region, while none of the residues are in the disallowed region of the Ramachandran plot (data not shown).

The kinase domain of Snf1 (Snf1-KD) has the canonical protein kinase fold with a small N-terminal lobe (small lobe) and a large C-terminal lobe (large lobe) (Fig. 2A) [16,17]. The small lobe is composed of a five-stranded β-sheet (β1–β5) and a helix (αC). Residues connecting strands β1 and β2 (the P-loop) are disordered in the structure (Fig. 2A). This Gly-rich segment is expected to interact with the phosphates of ATP, whereas the current structure is in the apo form. Attempts at producing the structure of an ATP complex, by soaking and cocrystallization experiments, have so far been unsuccessful.

The large lobe is mostly helical, with 6 α-helices (αD–αI) and a few short 3_{10} helices (Fig. 2A). The activation loop of the kinase (residues 200–215), enclosed between the highly conserved DFG and APE sequence motifs (Fig. 1B), is

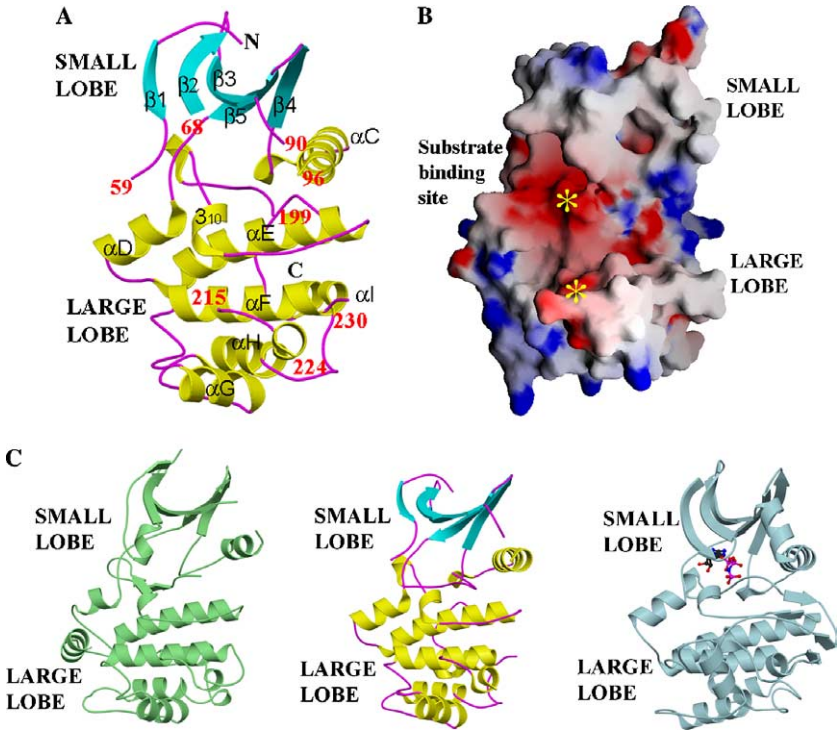

Fig. 2. Structure of Snf1-KD. (A) Ribbon representation of the Snf1-KD structure. The α-helices are shown in yellow, β strands in cyan, and the connecting loops in magenta. Prepared with Molscript [26] and rendered with Raster3D [27]. (B). The molecular surface of Snf1-KD. The putative substrate binding site is labelled, and the asterisks point to two negatively charged surface patches that may help recognize the substrate. Prepared with Grasp [28]. (C). Structure comparison with other protein kinases. Structure of Snf1-KD (middle) shown in the same orientation as those of CaMK (left) and cAPK (right). Prepared with Molscript [26] and rendered with Raster3D [27].

disordered in the current structure. The activation of Snf1 requires the phosphorylation of Thr 210 in this loop by upstream kinases (AMPK kinases), which can stabilize the conformation of this loop as well as the interactions between the two lobes. The current structure of Snf1-KD is in the unphosphorylated form. It has been reported that mutating the Thr residue in the activation loop to an Asp residue can confer partial activity to AMPK [18,19]. We have therefore produced crystals of the T210D mutant of yeast Snf1-KD and determined its structure at 2.7 Å resolution. This structure showed that the activation loop is still disordered in the T210D mutant.

Previous studies of Snf1 substrate preference demonstrated a core recognition motif that includes basic residues at the P-6 (6 residues N-terminal to the phosphorylation site) and P+3 positions [19]. The electrostatic surface potential of Snf1-KD has two negatively charged patches near the substrate-binding site that are created by highly conserved acidic residues among AMPKs (Glu 138, Asp 141, Glu 181, Asp 253–254, and Glu 255) (Fig. 2B). These may complement the positively charged basic residues in the substrate peptide. In comparison, these residues are more divergent among the other protein kinases.

Comparison with other protein kinase structures shows that the current structure of Snf1-KD is mostly in an open conformation, with the small lobe located away from the large lobe. The closest structural homologs of Snf1-KD, as defined by Dali [20], include the cAMP-dependent protein kinase (cAPK) [21,22], calcium/calmodulin-dependent protein kinase (CaMK) [23], checkpoint kinase (CHK1) [24], and cyclin-dependent kinase 2 (CDK2) [25] (Fig. 2C). The rms distance for the aligned Cα atoms ranges from 2.5 to 3 Å between the structure of Snf1-KD and those other kinases, and the sequence identity for these structurally equivalent residues varies from 20% to 37%. The highest sequence identity is to that of human CHK1. The strong structural homology between Snf1-KD and cAPK, which is in the closed conformation, is due mostly to the alignment of the large lobes of the two structures (Fig. 2C).

The two molecules of Snf1-KD in the crystallographic asymmetric unit have essentially the same conformation. The rms distance for equivalent Cα atoms of the two molecules is 0.48 Å. Interestingly, the two Snf1-KD molecules form a dimer in the crystal where residues 253–260 (helix αG and the preceding loop) of one monomer are inserted into the groove between the small and large lobes (i.e., the active site) of the other monomer (Fig. 3A). There is an ion pair between residue Asp 254 of one monomer and Arg 99 of the other monomer (Fig. 3B). Most importantly, the side chain of Ser 256 from one monomer is directly hydrogen-bonded to the side chain of Asp 177 of the other monomer (Fig. 3B). Asp 177 is the catalytic base of the protein kinase, as it is expected to extract the proton

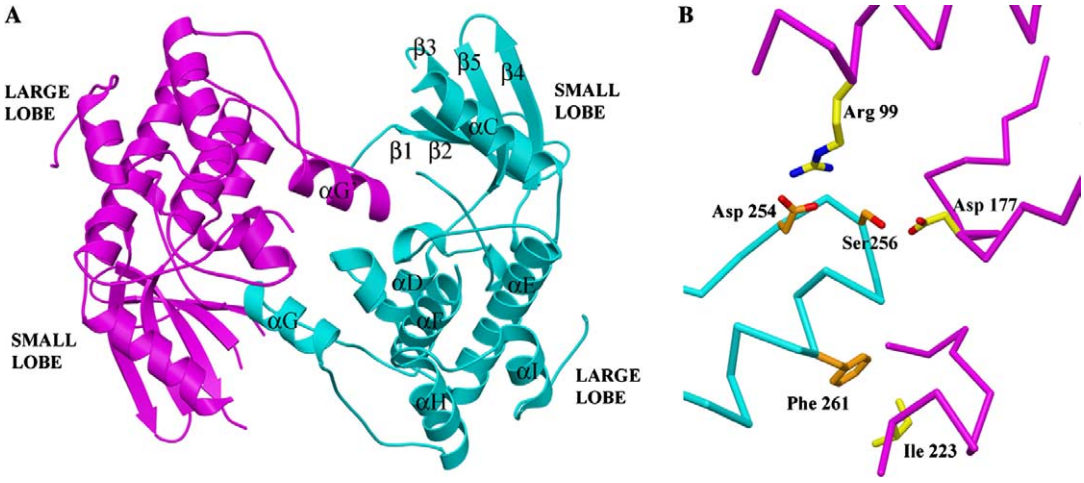

Fig. 3. Structure of the dimer of Snf1-KD. (A). Structure of an auto-inhibited Snf1-KD dimer that is observed in the crystal. (B). Some of the interactions in this dimer interface. Prepared with Molscript [26] and rendered with Raster3D [27].

from the phosphoacceptor residue of the substrate to initiate the phosphorylation reaction. Therefore, such a dimeric association is expected to disrupt the catalysis and to block the access of substrates to the active site of the Snf1 kinase. However, gel filtration and light scattering studies demonstrate that the Snf1-KD is monomeric in solution (data not shown). The dimer observed in the crystal is likely a crystallization artifact. It remains to be seen whether the observed dimerization is relevant for regulating the activity of this kinase domain in the AMPK holoenzyme.

Previous studies have identified an auto-inhibitory domain (AID) that follows the KD in the catalytic subunit of AMPK [18]. The boundaries of this domain have been defined as residues 313–392 for the mammalian α1 subunit, which may correspond to residues 367–500 in yeast Snf1 (Fig. 1A). The sequence conservation between mammalian and yeast AMPK in this domain is however much weaker. In an attempt to elucidate the molecular basis of this auto-inhibition, we have created several bacterial expression constructs for Snf1 that cover the AID, containing residues from 41 up to 500. We were able to purify a large amount of protein for most of these constructs and were also able to obtain crystals of the proteins. Unfortunately, all of these crystals displayed poor X-ray diffraction quality, and the best diffraction extended only to approximately 3.6 Å resolution at a synchrotron radiation source. Therefore, we were not able to define the conformation of residues in the AID. However, the fact that all of these crystals have poor diffraction suggests that the AID might be flexible in structure. Interestingly, we observed the same dimer of the kinase domain in the crystal of residues 41–440, which diffracted to 3.6 Å resolution, but we did not observe significant density for the AID in the electron density map for this crystal.

In summary, we have determined the crystal structure of the protein kinase domain (KD) of the catalytic subunit (Snf1) of yeast AMPK at 2.2 Å resolution. The structure of Snf1-KD shares strong similarity to those of many other protein kinases, including checkpoint kinase (CHK1), calcium/calmodulin-dependent protein kinase (CaMK), and cAMP-dependent protein kinase (cAPK). Two negative surface patches in the structure may be important for the recognition of the substrates of this kinase. Structural studies of Snf1 containing both the KD and the putative auto-inhibitory domain (AID) suggest that the AID might be flexible in structure.

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