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AMPK β subunits display isoform specific affinities for carbohydrates

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ABSTRACT

AMP-activated protein kinase (AMPK) is a heterotrimer of catalytic (α) and regulatory (β and γ) subunits with at least two isoforms for each subunit. AMPK β1 is widely expressed whilst AMPK β2 is highly expressed in muscle and both β isoforms contain a mid-molecule carbohydrate-binding module (β-CBM). Here we show that β2-CBM has evolved to contain a Thr insertion and increased affinity for glycogen mimetics with a preference for oligosaccharides containing a single α-1,6 branched residue. Deletion of Thr-101 reduces affinity for single α-1,6 branched oligosaccharides by 3-fold, while insertion of this residue into the equivalent position in the β1-CBM sequence increases affinity by 3-fold, confirming the functional importance of this residue.

1. Introduction

The AMP-activated protein kinase (AMPK) is a heterotrimer of α, β and γ subunits, for each of which there are multiple isoforms. The isoforms, encoded by seven genes (α1, α2, β1, β2, γ1, γ2, γ3), give rise to 12 possible isoenzymes that differ in tissue and subcellular expression [1]. AMPK functions as a focal point for whole-body and cellular mechanisms of energy homeostasis adapting the body to new regimes of energy supply and demand, such as during exercise (reviewed in [2]).

AMPK β1 is widely expressed whilst AMPK β2 is the predominant and exercise-regulated β isoform in human skeletal muscle [3]. We and others previously identified a mid-molecule protease-resistant domain related to starch-binding modules in plants within the AMPK β subunit [4,5] that is a member of the carbohydrate-binding module-containing family 48 (β-CBM) [6]. In addition, the β subunit is N-terminally myristoylated, phosphorylated on multiple residues [7] and contains a C-terminal αγ-subunit-binding sequence essential for heterotrimer formation [8]. The β-subunit CBM, in mammalian AMPK, binds glycogen in vitro [5] and in cultured cells [4], and regulates the allosteric inhibition of AMPK activity by glycogen and branched oligosaccharides [9]. The crystal structure of the β1-CBM in complex with the glycogen mimetic β-cyclodextrin, reveals a unique carbohydrate-binding pocket that incorporates all known aspects of carbohydrate-binding observed in starch-binding domains into the one site. β-Cyclodextrin is held in a pincer-like grasp with two tryptophan residues cradling two β-cyclodextrin glucose units and a leucine residue piercing the β-cyclodextrin ring [10].

A molecular relationship between the activity and localization of AMPK and glycogen metabolism has been well established. For example, high muscle glycogen content is correlated with reduced activation of AMPK by the AMP mimic, AICA riboside in perfused rat muscle [11] and by exercise in human muscle [12]. In mammalian cells AMPK co-immunoprecipitates with proteins including glycogen synthase, glycogen phosphorylase and glycogen debranching enzyme [13,14].

Glycogen is a branched polymer of glucose comprising of α-1,4-glycosidic bonds with α-1,6-glycosidic linkages at branch

Abbreviations: AMPK, AMP-activated protein kinase; CBM, carbohydrate-binding module; ConA, Concanavalin A; NMR, nuclear magnetic resonance.

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points [15]. Glycogen is known to have markedly different structures in muscle and liver. For example, muscle glycogen consists of beta-particles with a diameter ranging in size from 20 to 50 nm, whilst liver glycogen consists of beta-particles that aggregate to form much larger particles (with a diameter of 150–300 nm) known as alpha-rosettes [16]. Liver and muscle glycogen also have different physiological roles; liver glycogen is important for maintaining blood glucose whilst muscle glycogen is important for providing glucose as a source of ATP for energy-requiring events like muscle contraction.

Previous studies to investigate the association of AMPK with glycogen [5,9,10] or oligosaccharides [6] have examined the ubiquitous β1 isoform, thus we decided to focus our attention on the muscle-specific β2 isoform. We found that the β2-CBM has evolved to bind oligosaccharides more tightly than the β1-CBM with a preference for singly branched oligosaccharides like that found during glycogen degradation. Recognition of branched oligosaccharides is facilitated by a β2-CBM specific threonine insertion. Collectively, these studies suggest that AMPK will differentially associate with glycogen particles, which is dependent on the β-subunit isoform, thus providing an opportunity to therapeutically target AMPK specifically in muscle to fight diseases like type 2 diabetes and obesity.

2. Materials and methods

2.1. Materials

Glycogen from bovine liver (Type IX), rabbit liver (Type III), oyster (Type II), slipper limpet (*Crepidula fornicata*; Type VIII), maltoheptaose and β-cyclodextrins were purchased from Sigma–Aldrich (St. Louis, MO, USA). Anti-6-His antibodies were purchased from Cell Signaling Technology (Beverly MA, USA). Secondary goat anti-rabbit peroxidase conjugated antibodies were from Calbiochem (San Diego, CA, USA).

2.2. Expression and purification of AMPK β1-CBM and AMPK β2-CBM

AMPK β1-CBM was prepared as previously described [6]. AMPK β2-CBM (67–163) cDNA was amplified by PCR using the oligonucleotides: sense, 5′-GATTCTGTGAAGCCCACCCA-3′; antisense 5′-TCAATCAAATACTTCAAA-3′. The amplified cDNA was cloned into EcoR1 and Xho1 sites of the pProEX HT vector and positive clones confirmed by sequencing. AMPK β2-CBM was expressed and purified as for AMPK β1-CBM [6].

2.3. Site-directed mutagenesis

AMPK β1-CBM Thr-102 insertion and β2-CBM Thr-101 deletion mutants were made by site-directed mutagenesis according to manufacturer's instructions (Stratagene) by using the template described above. The sequences of the mutagenesis primers are available from the authors upon request.

2.4. Oligosaccharide synthesis

6-*O*-α-Glucosyl-β-cyclodextrin (glucosyl-β-cyclodextrin) and 6-*O*-α-maltosyl-β-cyclodextrin (maltosyl-β-cyclodextrin) were purchased from Wako (Wako Pure Chemical Industries Ltd., Tokyo, Japan). 6-*O*-α-Maltotetraosyl-β-cyclodextrin (maltotetraosyl-β-cyclodextrin) was enzymatically synthesized as previously described [17]. Branched maltoheptaoses were obtained as described previously [18].

2.5. Fluorescence spectroscopy

Fluorescence was measured using a Cary Eclipse fluorescence spectrophotometer. Titrations were conducted at 25 °C with CBM concentrations in the range of 0.5–35 μM, in 25 mM sodium phosphate, pH 7.5. Aliquots of oligosaccharide were sequentially added to CBM until further addition no longer elicited a quenching of the fluorescence (final concentrations of ligands were 4–17 mM). The volume change due to addition of ligand was not more than 10% of the initial volume. Quintuplicate emission spectra were recorded from 300 to 380 nm, using an excitation wavelength of 295 nm. The emission intensity at 356 nm was extracted from the spectrum and plotted as a function of the total ligand concentration. Three titration datasets were performed on each ligand, each at three different protein concentrations. The characteristic intensity of the CBM–oligosaccharide complex in the three titrations was then globally fitted (GraphPad Prism v4.0a, GraphPad Software Inc., San Diego, CA, USA) to extract a dissociation constant (K_d), assuming a single ligand recognition site.

2.6. Nuclear magnetic resonance (NMR) spectroscopy

The binding of sugars to the CBMs were monitored by acquiring heteronuclear multiple quantum correlation spectra (SOFAST-HMQC [19]) for each titration point of increasing sugar concentration to protein. The initial concentration of protein used was 100 μM in 25 mM sodium phosphate, pH 7.5 and did not vary by more than 10% over the titration. Final concentrations of ligands varied between 3 and 15 mM. Titrations were carried out at 25 °C on Bruker 800 MHz Avance II or 600 MHz Avance III spectrometers. The ^{1}H, ^{13}C, ^{15}N assignments of the β1-CBM have been determined using triple resonance methods [6]. The resonances of the β2-CBM were similarly assigned (Koay et al., unpublished). Dissociation constants were calculated using non-linear regression by fitting the titration data for individual ^{1}H and ^{15}N resonances and assuming a single ligand recognition site

2.7. Phylogenetics

Sequences similar to AMPK β-subunits were collected using BLAST and curated entries from Ensembl, the Arabidopsis Information Resource TAIR, The Joint Genome Institute and GeneDB. Multiple sequence alignment was carried out using MAFFT-L-INS-i [20], and then manually adjusted and trimmed using JalView to produce an alignment of 199 residues. Distance, parsimony and maximum likelihood tree making methods were then used to infer trees using PHYLIP PROTDIST, PROTPARS [21], and PhyML [22], respectively, automated using custom-built BioRuby scripts [23].

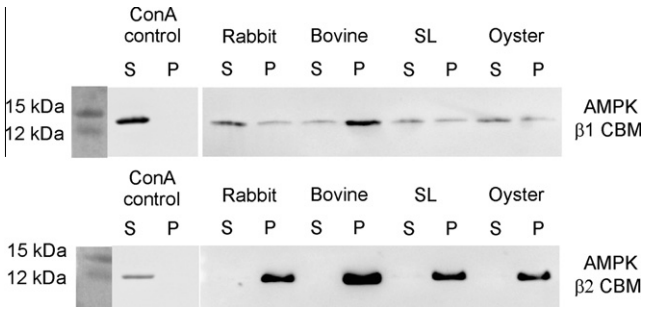

Fig. 1. AMPK β2-CBM associates tightly with glycogen. Thirty micrograms samples of either AMPK β1- or β2-CBM were incubated alone or with rabbit, bovine, slipper limpet (SL) or oyster glycogen bound to ConA–Sepharose for 60 min. Sepharose beads were recovered by centrifugation and samples of the supernatant (S) and pellet (P) analyzed by Western blot using an anti-His monoclonal antibody.