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Oligosaccharide recognition and binding to the carbohydrate binding module of AMP-activated protein kinase

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Abstract The AMP-activated protein kinase (AMPK) contains a carbohydrate-binding module (β1-CBM) that is conserved from yeast to mammals. β1-CBM has been shown to localize AMPK to glycogen in intact cells and in vitro. Here we use Nuclear Magnetic Resonance spectroscopy to investigate oligosaccharide binding to ^{15}N labelled β1-CBM. We find that β1-CBM shows greatest affinity to carbohydrates of greater than five glucose units joined via α,1 → 4 glycosidic linkages with a single, but not multiple, glucose units in an α,1 → 6 branch. The near identical chemical shift profile for all oligosaccharides whether cyclic or linear suggest a similar binding conformation and confirms the presence of a single carbohydrate-binding site.

Keywords: AMP-activated protein kinase; Carbohydrate-binding module; NMR; Oligosaccharide; Glycogen

1. Introduction

The AMP-activated protein kinase (AMPK) is an evolutionarily conserved energy sensor found in mammals and known to coordinate cellular metabolism and systemic energy balance in response to energy demands and a range of stimuli [1]. AMPK is a highly conserved heterotrimeric complex comprising a catalytic α subunit and regulatory β and γ subunits that have been identified in plants, yeast, nematodes, flies and mammals.

AMPKβ1 subunit was recently shown to contain a glycogen-binding domain. Using secondary structure predictions a protease-resistant domain within residues 68–163 of AMPKβ1 was identified that associated with glycogen in cell-free assays [2]. Furthermore fluorescently tagged AMPK heterotrimers associated with glycogen-containing granules in the cytoplasm of cultured human cells that was abolished when glycogen-binding domain was deleted [3]. The glycogen-binding domain has been reclassified as a member of the carbohydrate-binding module-containing family 48 (CBM48) (http://afmb.cnrs-mrs.fr/CAZY) based on amino acid sequence, alongside other carbohydrate-binding proteins such as isoamylase and glycogen branching enzymes. To date, the structures of both rat β1-CBM, in complex with the unnatural cyclic sugar β-cyclodextrin [4], and human apo β2-CBM (unpublished, PDB ident code 2F15) have been elucidated by X-ray crystallography. AMPKβ1-CBM folds into a β sandwich consisting of two anti-parallel β sheets similar to structures of N-isoamylase or starch-binding domains [4].

Glycogen, a branched polymer of glucose, is a cellular store of energy and is important for whole body glucose metabolism with largest stores found in liver and skeletal muscle [5]. Glycogen is stored in cells in the form of granules that not only contain glucose, but also bound proteins that are involved in glycogen metabolism like glycogen synthase and glycogen phosphorylase [6]. Several studies link AMPK to glycogen metabolism. For example, AMPK co-immunoprecipitates with glycogen synthase [7], glycogen phosphorylase [7] and glycogen debranching enzyme [8] and mutations in the AMPK γ subunit result in a novel cardiac glycogen storage disease in human individuals [9,10]. To further investigate the carbohydrate-binding nature of β1-CBM we used Nuclear Magnetic Resonance (NMR) spectroscopy to identify the residues important for oligosaccharide binding to β1-CBM and to determine affinity for this association. We find that β1-CBM preferentially binds sugars with 6–7 glucose units and ideally with one type of branched sugar.

2. Materials and methods

2.1. Expression and purification of isotopically labeled β1-CBM

AMPK β1-CBM was cloned into pProEX HT and expressed as a His-tag fusion protein in BL21 *Escherichia coli* cells [11]. AMPK β1-CBM was ^{13}C and ^{15}N or ^{15}N only labelled by growing cultures in a 2 L Braun Biostat Fermentor containing 1 L of minimal media with $^{15}NH_4Cl$ and ^{13}C-D-glucose (Spectral Gases Laboratory) as the sole nitrogen and carbon sources [12]. β1-CBM expression was induced by the addition of 1 mM IPTG until the nutrients were depleted after which cells were harvested, pelleted and purified as previously described [11].

2.2. Nuclear magnetic resonance (NMR) experiments and data processing

Purified ^{13}C and ^{15}N or ^{15}N only labelled β1-CBM were dissolved into NMR buffer (25 mM Na_2HPO_4–NaH_2PO_4, 0.02% sodium azide, pH 7.5, 10% D_2O) and placed into NMR sample tubes. For resonance assignment NMR spectra were acquired on 0.5 mM β1-CBM samples at 25 °C using Varian 600 MHz INOVA or Bruker 800 MHz

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Abbreviations: AMPK, AMP-activated protein kinase; CBM, carbohydrate-binding module; β1-CBM, AMPK β1 carbohydrate-binding module; NMR, nuclear magnetic resonance spectroscopy; HSQC, heteronuclear single quantum correlated

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AVANCE spectrometers. All experiments utilized cryogenically cooled probes of the spectrometers to maximize sensitivity. Heteronuclear 2D and 3D resonance spectra were acquired and the data was processed with the program NMR Pipe [13] using a Gaussian function in the direct H^N dimension and cosine squared functions in the indirect dimensions. All data were zero-filled once prior to Fourier transformation. Data was analysed using NMR View, v5.2.2 [14]. For sequence specific assignment of resonances 3D HNCACB [15] and CBCA(CO)NH [16] experiments were acquired and analysed, 3D C(CO)NH [17] experiments were performed to confirm assignments.

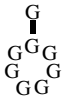
2.3. Oligosaccharide titrations

To study carbohydrate recognition and binding to β1-CBM, individual titrations using 12 different oligosaccharides shown in Table 1 were obtained from Supelco (USA) and WAKO (Japan) and performed with 0.2 mM ^{15}N β1-CBM in NMR buffer. Each oligosaccharide was prepared as a stock concentration ranging from 50 to 400 mM, except for β-cyclodextrin which was prepared as a 20 mM stock due to low solubility. Eight to 10 oligosaccharide titration points with increasing concentration from 0 to 10 mM were performed. For each titration point, a ^1H–^{15}N heteronuclear single quantum correlation (HSQC) spectrum was acquired at 800 MHz and 25 °C. The final sample dilution was less than 10% of the original volume.

2.4. Dissociation constant determination

Dissociation constants for oligosaccharides binding to β1-CBM were calculated by fitting the titration data for individual resonances, assuming a single ligand recognition site, in the programs xcrvfit, (v3.0.6, Robert Boyko and Brian Sykes) and xmgrace (v 5.1.2 Paul J. Turner). Maximum H^N and ^{15}N chemical shift differences were scaled according to the following equation [18]: $\Delta\delta_{Total} = ((\Delta\delta_{HN})^2 + (\Delta\delta_N \times 0.154)^2)^{1/2}$ where $\Delta\delta_{HN}$ and $\Delta\delta_N$ are the chemical shift differences for the H^N and ^{15}N resonances between the free and titrated protein. Molecular visualization and generation of figures were performed using MOLMOL v2k.2 [19].

Table 1
Oligosaccharide structures and dissociation constants (K_d) for the binding of soluble oligosaccharides to β1-CBM of AMPK at 25 °C and pH 7.5

Ligand	Structure	K_d (mM)
Maltoheptaose	GGGGGGG	0.36 ± 0.033
Maltohexaose	GGGGGG	0.67 ± 0.057
Maltopentaose	GGGGG	3.69 ± 0.34
Maltotetraose	GGGG	4.21 ± 0.12
Maltotriose	GGG	9 to 18
Maltose	GG	N.D.
Glucose 6-phosphate	pG	N.D.
β-Cyclodextrin		0.33 ± 0.025
Glucosyl β-cyclodextrin		0.15 ± 0.016
Maltosyl β-cyclodextrin		0.51 ± 0.04
Glucosyl maltotriose		8 to 14
Isomaltose		N.D.

Glucose units (G) connected via an α, $1 \rightarrow 6$ glycosidic bond are indicated by a solid line. All other glucose units are linked via α,$1 \rightarrow 4$ glycosidic bonds. pG represents glucose 6-phosphate.

3. Results

3.1. Assignment of the ^1H–^{15}N HSQC NMR spectrum of β1-CBM

The ^1H–^{15}N HSQC spectrum obtained for apo β1-CBM showed a pattern of well-dispersed cross peaks (Fig. 1S, Supplementary data). A total of 13 H^N correlations (M53, D54, N70, E71, K72 T85, G86, K102, Q109, N110, Q145, L146 and E161) out of an expected 95 could not be unambiguously assigned, or were not observed.

3.2. NMR titrations of oligosaccharides with β1-CBM

For each oligosaccharide shown in Table 1, 8–10 ^1H–^{15}N HSQC spectra were collected after progressive addition of oligosaccharide and overlayed to track peak chemical shift perturbations. Each of the titrated oligosaccharides except isomaltose and glucose-6-phosphate were found to cause significant chemical shift perturbations to the β1-CBM spectrum. An example of the overlayed spectra for maltoheptaose is shown in Fig. 1A. Saturation of the protein was possible for the linear maltodextrins: maltoheptaose and maltohexaose, and the cyclodextrins: β-cyclodextrin, glucosyl β-cyclodextrin and maltosyl β-cyclodextrin. Under the conditions of this study we could not saturate the protein sample with maltopentaose, maltotetraose, maltotriose and glucosyl-maltotriose. However, all oligosaccharides that induced chemical shift changes in β1-CBM showed similar patterns of change upon ligand binding suggesting a single carbohydrate-binding site (Figs. 1B and C, 2 and 2S, Supplementary data). A large number of resonances exhibited no chemical shift perturbation upon ligand binding. In titrations with β-cyclodextrin, glucosyl β-cyclodextrin, maltosyl β-cyclodextrin, maltoheptaose, maltohexaose and maltopentaose, a number of cross peaks were rapidly broadened upon the progressive addition of ligand until they completely vanish from the spectra, only to reappear as the oligosaccharide concentration increased.

Dissociation constants (K_d) were independently determined for individual resonances that showed significant chemical shift deviation in either or both the ^1H and ^{15}N dimension upon oligosaccharide binding. Peaks that exhibited peak broadening were excluded from K_d calculations [20]. The obtained K_d values were averaged to obtain an overall K_d value for each titrated oligosaccharide as reported in Table 1. K_d values for maltotriose and glucosyl-maltotriose could not be accurately determined because titrations with these ligands did not result in sufficient saturation of the β1-CBM even at very high concentrations of oligosaccharide, suggesting weak binding to β1-CBM. For these oligosaccharides a range of K_d values were determined (Table 1). We find β1-CBM binds with higher affinity to long chain α,$1 \rightarrow 4$ linked oligosaccharides and this affinity diminishes with maltodextrin length (Table 1). A distinct decrease in the values of K_d is found between maltohexaose and maltopentaose where the affinity of β1-CBM for maltopentaose is approximately five times weaker than maltohexaose.

4. Discussion

In this present study, we have identified key determinants in the association of oligosaccharides with the AMPK β1 carbohydrate-binding module (β1-CBM). The oligosaccharide K_d