AMP-activated protein kinase β subunit requires internal motion for optimal carbohydrate binding

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AMP-activated protein kinase (AMPK) is an evolutionarily conserved heterotrimeric enzyme (α, β, γ subunits) essential in sensing and regulating metabolic processes. Two mammalian β-subunit isoforms exist, each containing a central carbohydrate-binding module (CBM) which share 80% sequence identity. We have shown that the muscle-specific β2-CBM isoform, either as an isolated domain or in the heterotrimer, binds glycogen mimetics up to ~20-fold more tightly than the ubiquitous β1-CBM (Koay *et al.*, 2010; Bieri *et al.*, 2012). Additionally, we observe that both β1- and β2-CBM bind optimally to single α,1-6 branched oligosaccharides, a conformation only observed during glycogen breakdown, suggesting ligand specificity.

Surprisingly, both NMR solution and X-ray crystal structures have not revealed any differences to account for these affinities, and indeed the ligand contact residues are strictly conserved. An apparent difference between the two isoforms is a threonine insertion in position 101 within a loop of β2-CBM which is not directly involved in ligand binding. Using both ^{15}N spin relaxation and relaxation dispersion experiments we find that β2-CBM, but not β1-CBM, shows significant μs motion. On binding ligand this motion is slowed, but becomes more widespread suggesting β2-CBM can adopt conformational states that are poorly accessed by β1-CBM. Deletion of Thr101 in β2-CBM results in a loss in both affinity for ligand and flexibility in both the free and ligand bound states. Conversely, insertion of a Thr into the equivalent position in β1-CBM, results in an increase in ligand affinity and flexibility. However, in neither case are wild-type dynamics observed, indicating that while the Thr may play a role in the affinity and dynamic differences, other residues must be involved.

In β2-CBM we noted Val134, which is Thr in β1-BM, is located at the base of the carbohydrate binding loop and shows μs motion in the bound state. Mutation of Val134 to Thr in both wild-type and Thr101-deleted β2-CBM respectively results in a 3-fold and 10-fold reduction in carbohydrate binding. The sidechain of Val134 is pointed into solution and plays no role in ligand-binding. The equivalent Thr in β1-CBM also does not contact ligand, however, the hydroxyl sidechain group of this residue forms a hydrogen bond with the peptide carbonyl of Gln132 which may constrain the mainchain of the protein near Trp133, which does contact ligand. We conclude that our results suggest the higher affinity of the β2-CBM may be attributed to the flexibility of residues that do not directly contact ligand and allow the protein to adopt conformations that effectively bind carbohydrate.

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