Supplementary Information: Long-chain fatty acyl-CoA esters regulate metabolism via allosteric control of AMPK β1-isoforms. Stephen L. Pinkosky, John W. Scott et al.

Supplementary Information Table 1. Basal activities of AMPK expressed in COS7 mammalian cells

AMPK complex	immobilization	basal activity (nmol/min/mg lysate) ± s.e.m.	relates to
α1β1γ1	myc IP (***β***-subunit)	0.1094 ± 2.1	Fig. 1f
α1β2γ1	myc IP (***β***-subunit)	0.1689 ± 17.7	Fig. 1h
α2β1γ1	myc IP (***β***-subunit)	0.238 ± 0.5	Fig. 1f
α2β2γ1	myc IP (***β***-subunit)	0.814 ± 6.7	Fig. 1h
α1β1γ1	myc IP (***β***-subunit)	0.613 ± 7.5	Fig. 2a
α1β1(N-Δ71)γ1	myc IP (***β***-subunit)	0.502 ± 4.0	Fig. 2a
α1β1(N-Δ145)γ1	myc IP (***β***-subunit)	0.327 ± 2.5	Fig. 2a
α1β1γ1	flag IP (***α***-subunit)	0.1063 ± 5.9	ED Fig. 2a
α1β1γ1	GST (***α***-subunit)	0.2789 ± 20.2	ED Fig. 2b
α1β1γ1	myc IP (***β***-subunit)	0.1433 ± 5.3	ED Fig. 3a
α1β1(S108A)γ1	myc IP (***β***-subunit)	0.724 ± 1.4	ED Fig. 3a

Supplementary Table 2. Basal activities of AMPK expressed in *E. coli*

AMPK complex	basal activity (nmol/min/mg enzyme) ± s.e.m.	relates to
α1β1γ1	424.0 ± 14.8	Fig. 1i & 2b
α1β2γ1	335.6 ± 9.1	Fig. 1i
α1β1(S108A)γ1	406.1 ± 24.6	Fig. 2b

In silico modelling

Our experimental data indicated that (1) palmitoyl-CoA allosterically activates AMPK forms containing the β1-subunit but not those containing the β2 isoform, (2) both the α-catalytic (isoforms 1 or 2) and β1-regulatory subunits are required for palmitoyl-CoA activation, (3) β1-CBM deletion abolishes palmitoyl-CoA allosteric activation and (4) S108 data indicates the ADaM site may be involved in palmitoyl-CoA binding. Taken together, the data suggested that palmitoyl-CoA was binding to residues in both the α-catalytic and β1-regulatory subunits and therefore our *in silico* modeling studies focused on potential LCFA-CoA binding pockets involving residues from both subunits.

There are five active human AMPK crystal structures in the protein data bank (PDB, http://rscb.org; August 2018) containing α2-catalytic and β1-regulatory subunits (PDB IDs: 4CFE, 4CFF, 4ZHX, 5ISO and 6B1U) [1-6]. The five AMPK structures also contain the γ1-regulatory subunit. There were no active human AMPK structures containing both the α1- and β1-subunits (existing α1β1 structures all use the rat α1-subunit). The conformational plasticity of AMPK was taken into consideration, at least in part, by selecting the structures with the greatest conformational variance for the palmitoyl-CoA docking studies (*i.e.* PDB IDs: 4CFF, 5ISO and 6B1U; when aligned to 4CFF ABE chains via Cα atoms the root-mean-square deviation = 1.1 and 0.7 for 5ISO ABE chains and 6B1U CDF chains respectively). The channel running across the interface of the AMPK α2- and β1-subunits encompasses two

largely hydrophobic pockets, the well described ADaM site [2,6] and a novel pocket located directly below the cyclodextrin binding groove in the β1-CBM (Extended Data Figure 4a-c). Palmitoyl-CoA was flexibly docked into this channel, while also allowing the side-chains of the residues lining the channel to be flexible. The docked palmitoyl-CoA poses were ranked using C-Score [7] and the top 30 ranked poses for each AMPK structure were retained for analysis. Any palmitoyl-CoA pose not interacting with residues from both the α2- and β1-subunits was discarded.

The palmitoyl-CoA poses fell into distinct conformational clusters, some clusters were common to all three AMPK structures while others were unique to a particular AMPK conformation. For the 4CFF AMPK conformation seven clusters were observed, there were five unique palmitoyl-CoA clusters for the 5ISO AMPK conformation and two unique clusters for the 6B1U AMPK conformation (Extended Data Figure 5). In every case, the fatty-acyl chain of palmitoyl-CoA was located in the hydrophobic ADaM site and the palmitate chain appears to be optimum for spanning the length of the site. The majority of the docked poses placed the palmitoyl-CoA adenine ring in various orientations within the hydrophobic pocket lying directly below the cyclodextrin binding groove in the β1-CBM (Extended Data Figure 5). The pocket is formed by β1-subunit residues F82, W84, T85, L93, Y125, F127, T134, D136, P137, S138, E139, N151 and I153. The polar residues (T85, T134, D136, S138, and E139) form a border around the hydrophobic residues (F82, L93, Y125, F127 and I153) in the interior of the pocket. It is also possible for the adenine ring to make π-π interactions with the aromatic residues F82, W84, Y125 and F127. β1-subunit residues F82-W84 form part of β-strand 1, including the critical ADaM site residue β1-R83 [6], while residues T134-E139 lie on the flexible loop between β-strand 5 and 6. These palmitoyl-CoA poses also have the ribose ring (in varying orientations) located near the start of the α-B helix in the α2-subunit. Residues surrounding the ribose ring are R83, T85, S138 and E139 from the β1-subunit and N48, R49, Q50, K51 and T87 from the α2-subunit. Although the di-phosphate moiety is largely solvent exposed it can form interactions with nearby β1-subunit residues R83, T85, G86, N111 and S138 and α2-subunit residues N48, Q50 and K51. For the 5ISO AMPK structure, palmitoyl-CoA conformational clusters 2 and 5 have the adenine ring located near the C-interacting helix (β1-CBM) whereas it was located near β-strand 6 (β1-CBM) for cluster 4; the di-phosphate moiety for all three clusters is located near the start of the α-B helix (α2-subunit) and the ribose ring is found in the region between the C-interacting helix and β-strand 6 (both in the β1-CBM). Although the palmitoyl-CoA docked poses form distinct clusters, when all of the poses for the 4CFF, 5ISO and 6B1U AMPK structures are overlaid (except for those in Clusters 2, 4 and 5 for the 5ISO AMPK structure) it is clear that they all fall under a general binding mode (Extended Data Figure 5d). From the 4CFF AMPK structure docked poses, palmitoyl-CoA pose 1 of Cluster 1 was selected to illustrate in detail the putative general palmitoyl-CoA binding mode.

To understand the β1-isoform selectivity we identified the non-conserved residues in close proximity (6.0 Å radius) to the general binding mode of palmitoyl-CoA. Based on a structural alignment of the β-subunits the non-conserved residues are: β1-F82 (β2-I81) and β1-T85 (β2-S84) in the adenine ring binding pocket; β1-G86 (β2-E85) in the di-phosphate moiety binding region; and β1-T106 (β2-I106), β1-R107 (β2-K107) and β1-N111 (β2-D111) in the ADaM site. Although located within the ADaM site, β1-N111 (β2-D111) is also in the di-phosphate moiety binding region. Depending upon the specific palmitoyl-CoA conformation, β1-T85 (β2-S84) can also interact with the ribose ring or the di-phosphate moiety. The isoform difference β1-F82 and β2-I81 maintains the hydrophobic character of the adenine ring binding pocket but removes the ability of the β2-subunit residue to form π-π interactions with the adenine ring. The side-chains of β1-T85 and β2-S84 are both capable of hydrogen

bonding and are similar in size, while only the backbone atoms of β1-T106 (β2-I106) and β1-R107 (β2-K107) participate in the ADaM site. Residues β1-F82 (β2-I81), β1-T85 (β2-S84), β1-T106 (β2-I106) and β1-R107 (β2-K107) are therefore considered unlikely to influence β1-isoform selectivity. The introduction of negatively charged residues, β2-E85 (β1-G86) and β2-D111 (β1-N111), may result in an unfavourable charge match (*i.e* electronic repulsion) with the negatively charged di-phosphate moiety of palmitoyl-CoA. However, the loop containing β2-subunit residues S84-E85-G86-G87 would need to flip downwards from the orientation observed in the crystal structure conformations (human β2-subunit PDB IDS: 6B2E and 4RER) for the side-chain of β2-E85 to be located near the palmitoyl-CoA di-phosphate moiety. It was also observed in the human AMPKα2β2γ1 structure (PDB ID: 6B2E) that β2-E85 is able to hydrogen bond with the β2-W83 ring nitrogen and also make charge-π interactions with the tryptophan ring. Such interactions between E85 and W83 would likely make the S84-E85-G86-G87 loop less mobile and preclude activation of β2, while allowing binding. Using a similar approach, we have docked palmitoyl-CoA to AMPKα1β2γ1 (PDB 4RER) and AMPKα2β2γ1 (PDB 6B2E) and found it can adopt similar binding modes to those in our palmitoyl-CoA/β1 models. However, we note palmitoyl-CoA is unable to hydrogen bond with the side-chain of β2-D111, which we previously showed to be essential for β2 activation by the synthetic compound SC4 [2]. This may offer an alternative explanation for our observations that palmitoyl-CoA is able to bind to, but not activate, β2 AMPK.

Supplementary references

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