

Supplementary Material for the Submission “Identification of the Citrate-Binding Site of Human ATP-Citrate Lyase Using X-ray Crystallography” by Tianjun Sun, Koto Hayakawa, Katherine S. Bateman, Marie E. Fraser.

Supplementary Table S1. Residues of human ATP-citrate lyase identified by peptide mass spectrometry.

Experimental relative molecular weight (Da)	Calculated relative molecular weight (Da)	Sequence, including the identities of the two residues bordering the detected peptide	Residue range in hACL
Peptides from the 50 kDa band			
1059.5214	1059.4985	R.VTPDTDWAR.L	34 - 42
2596.4763	2596.4741	R.LLQDHPWLLSQNLVVKPDQLIK.R	43 - 64
2256.9955	2257.0288	R.EGDYVLFHHEGGVDVGDVDAK.A	128 - 148
1416.6961	1416.6826	K.WGDIEFPPPFGR.E	233 - 244
1696.7515	1696.7831	R.EAYPEEA YIADLDAK.S	245 - 259
1949.0752	1949.0986	K.ILIIGGSIANFTNVAATFK.G	337 - 355
1929.9719	1929.9949	R.DYQGPLKEHEVTIFVR.R	363 - 378
1128.6050	1128.5928	K.EHEVTIFVR.R	370 - 378
1245.5997	1245.6214	R.RGGPNYQEGLR.V	379 - 389
1089.5353	1089.5203	R.GGPNYQEGLR.V	380 - 389
1992.9752	1993.0415	K.AKPAMPQDSVPSRSLQGK.S	470 - 488
Peptides from the 35 kDa band			
1060.5544	1060.5488	K.AIVWGMQTR.A	499 - 507
1076.5366	1076.5437	K.AIVWGMQTR.A Oxidation of M	499 - 507
1878.8405	1878.8458	R.DEPSVAAMVYPFTGDHK.Q Oxidation of M	522 - 538
1092.5622	1092.5505	K.QKFYWGHEK.E	539 - 546
836.3939	836.3970	K.FYWGHEK.E	541 - 546
1736.9589	1736.9573	R.KHPEVDVLINFASLR.S	562 - 576
1608.8706	1608.8624	K.HPEVDVLINFASLR.S	563 - 576
1895.8115	1895.8029	R.SAYDSTMETMNYAQIR.T Oxidation of M	577 - 592
1911.7670	1911.7979	R.SAYDSTMETMNYAQIR.T Oxidation of two M's	577 - 592
1566.8956	1566.8981	R.TIAIIAEGIPEALTR.K	593 - 607
1366.7288	1366.7357	K.LYRPGSVAYVSR.S	651 - 662
1506.7124	1506.7096	R.SGGMSNELNNIISR.T Oxidation of M	663 - 676
2928.3391	2928.3713	R.TTDGVYEGVAIGGDRYPGSTFMDHVLRL.Y Oxidation of M	677 - 703
1437.6672	1437.6711	R.YPGSTFMDHVLRL.Y Oxidation of M	692 - 703

Supplementary Figure S1. 9% SDS-PAGE gels showing the presence of two polypeptide chains in crystals of truncated human ATP-citrate lyase. Lane 1 contains the molecular weight markers: 200, 116, 97, 66 and 45 kDa. Lane 2 contains human ATP-citrate lyase. Lane 3 contains human ATP-citrate lyase incubated overnight with chymotrypsin. Lane 4 contains dissolved crystals.

Supplementary Figure S2. Sequence alignment of *E. coli* and pig GTP-specific succinyl-CoA synthetase with the truncated human ATP-citrate lyase, based on their structures. Only the residues of *E. coli* or pig GTP-specific succinyl-CoA synthetase whose C α atoms superpose within 2.5 Å of the C α atoms of ATP-citrate lyase are included. Residues of the *E. coli* enzyme are listed above the sequence of ATP-citrate lyase and residues of pig GTP-specific succinyl-CoA synthetase are listed below. Vertical lines denote identical residues. A. Alignment of residues of domains 1 and 2 (chain A or the α -subunit in succinyl-CoA synthetase). The five loops that may be involved in binding CoA are underlined. B. Alignment of residues of domains 3-5 (chain B or the β -subunit in succinyl-CoA synthetase).

Figure S1

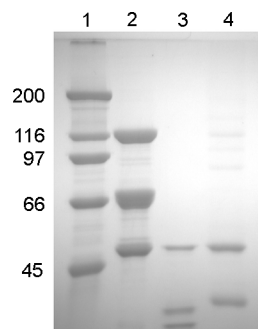


Figure S2A

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B1 MNLHEYQAKQLFARY B15   B21 VGYAC B25
      |   |   |
1  MSAKAISEQTGKELLYKFICTTSAIQNRFKYARVTPDPTDWARLLQDHPWLLSQ
      |   |   |
B1 MNLQEYQSKKLMSDN B15   B21 RFFVAD B26

B42 PWVVKCQVHAGGRG   GVKV B62EDIR B70
      |||   |   |
54  NLVVKPDQLIKRRGKLGVLGVNLTLDGVKSWLKPRLGQEATVGKATG
      |   |   |   |   |   |   |
B42 EIVLKAQILAGGRG   GGVHL   DPEVVGQLAK B81

      B95 QILVEAATDI   KELYLGAVV   RRVVFMASTEGG B130   IHKVAL B149
            |   |   |           |   |           |||
101  FLKNFLIEPFVPHSQAEFFYVCIYATREGDYVLFHHEGGVDVGDVDAKAQKLLVGVDEKLNPEDIKKHLLVHAPEDK
            |   |   |   |   |   |   |   |   |   |
B99  KVNKVMVAEALDI   RETYLAILM   NGPVLVGSPOGG B137   IFKEQI   GIKDS B165

      B176 QFTKIFMGLATIFLERDLALIEINPLVIT   DLICLDGKLGAD B219
            |   |           |||||   |   |
180  KEILASFISGLFNFYEDLYFTYLEINPLVVTKDGVVYVLDLAAKVDATADYICKVKWGDIEFPPPFGR
            |   |   |   |   |   |   |   |   |   |
B181  QNQAADQIKKLYNLFLKIDATQVEVNPFGF   QVVCFDAKINFD B226

      B238 EDPREAQAA   B250 LNYVALD   NIGCMVN   LAMGTMDIVKLH B279
            |   |   |           |   |           |   |
245  EAYPEEAYIADLDAKSGASLKLTLNPKGRIWTMVAGGGASVVYSDTICDLGGVNE
            |   |   |   |   |   |   |   |   |   |
B244  ENAPIENEAAKYD B257 LKYIGL   NIACFVN   AGLAMATCDIIFL B285

B283 PANFLDV B289           B312 KAVLVNIF B319
      |   |
301  LANYGEYSGAPSEQQTYDYAKTILSLMTREKHPDGKILIIIGGSIAN
      |   |   |   |
B290 PANFLDL GGVKES VYQAFKLL B312   B319 EAILVN B324

      B336 VAEVG B340   VPVVVRLE B350           B367 NIIA B370   DAAQQVVA B383
            |   |           |   |           |
347  FTNVAATFKGIVRAIRDYQGPLKEHEVTIFVRRGGPNYQEGLRVMGEVGKTTGIPIHVFGTETHMTAIVGMALGHRPIP 425
            |   |   |   |   |   |   |   |   |
B329  IVNCAIIANGITKACRELE   B350 VPLVVRLEGTNVHEAQNIL B368           B381 LEDAAK B386

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Figure S2B

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      A3 LIDKNTKVICG   GTFHSEQAIAYG   A33 KMVGGVTP   GTH A47
      |   |   |   |   |   |   |   |   |   |
487 GKSTTLFSRHTKAIVWGMQTRAVOGMLDFDYVCSRDEPSVAAMVYPFTGDHKQKQFYWGHEILI 550
      |   |   |   |   |   |   |   |   |   |
      A12 VDKNTKVICQG   GTFHSQOALEYG   A41 NLVGGTTP   A48

A51 PVFNTVREAVAATG ATASVIYVPPAPFCCKDSILEAIDA A87
   ||| | | | | | | | | | | | | | | | | | | | |
551 PVFKNMADAMRKHPEVDVLINFASLRSAYDSTMETMNYAQ 590
   ||| | | | | | | | | | | | | | | | | | | | |
A59 PVFN   EAKEQT GATASVIYVPPPFAAAAINEAIDA A95

A89 IKLIITITEGIPTLDMLTVKVKLDEAGVRMIGPNCPGVITPGECKI A134
   | | | | | | | | | | | | | | | | | | | | | | |
591 IRTIAIIAEGIPEALTRKLIKKADQKGVTIIGPATVGGIKPGCFKI 636
      | | | | | | | | | | | | | | | | | | | | | | |
A97 VPLVVCITEGIPQQDMVRVKHRLLRQ A123
      | | | | | | | | | | | | | | | | | | | | | | |
      A125 TRLIGNCPGVINPGECKI A143

      A144 PGKVGIVSRSGTLTYEAVKQTTDY A167
      | | | | | | | | | | | | | | | | | | | | | | |
637 GNTGGMLDNILASKLYRPGSVAYVSRSGGMSNELNNIISRTT 678
      | | | | | | | | | | | | | | | | | | | | | | |
      A153 KGRIGIVSRSGTLTYEAVHQTTQV A176

A169 FGQSTCVGIGGDPIPGSNFIDILEMFEKDPQTEAIVMIGEIGGSAAE A215
      | | | | | | | | | | | | | | | | | | | | | | |
679 DGVYEGVAIGGDRYPGSTFMDHVLRYQDTPGVKMIVVLGEIGGTEEYKICRGIKEG 734
      | | | | | | | | | | | | | | | | | | | | | | |
A178 LGQSLCVGIGGDPFNGTDFTDCLEIFLNDPATEGIILIGEIGGNAEEN A225

A224 HVTKPVVGYIAGVT A237   A256 GTADEKFA A263
      ||| | | | | | | | | | | | | | | | | | | | |
735 RLTKPIVCWCIGTCATMFSSEVQFGHAGACANQASETAVAKNQALKEA 782
      ||| | | | | | | | | | | | | | | | | | | | |
A240   KPVVSFIAGLT A250   A270   GAKEKIT A276

A269 GVKTV A281 GEALKTV A286
      || | | | | | | | | | | | | | | | | | | | | |
783 GVFVPRSFDELGEIIQSVYEDLVANGVIVPAQEVPPPT 820
      || | | | | | | | | | | | | | | | | | | | | |
A282 GVVVS A294 TTIYKE A299

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