



University of Groningen

Structural basis of Na+-independent and cooperative substrate/product antiport in CaiT

Schulze, Sabrina; Köster, Stefan; Geldmacher, Ulrike; Terwisscha van Scheltinga, Anke C.; Kühlbrandt, Werner

Published in: Nature

DOI: 10.1038/nature09310

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version Publisher's PDF, also known as Version of record

Publication date: 2010

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA): Schulze, S., Köster, S., Geldmacher, U., Terwisscha van Scheltinga, A. C., & Kühlbrandt, W. (2010). Structural basis of Na+-independent and cooperative substrate/product antiport in CaiT. *Nature*, *467*(7312), 233-237. https://doi.org/10.1038/nature09310

Copyright Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverneamendment.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

SUPPLEMENTARY INFORMATION

1. Background

L-carnitine is an essential metabolite in animals, plants, and prokaryotes. *Escherichia coli* and related bacteria, such as *Proteus mirabilis*, use L-carnitine (R(-)-3-hydroxy-4-trimethylaminobutyrate) as an electron acceptor under anaerobic growth conditions and convert it to γ -butyrobetaine¹⁻⁴. Mammalian cells have similar organic cation/carnitine (OCTN) transporters (fig. S1). *E. coli* CaiT is a constitutively active, highly specific L-carnitine/ γ -butyrobetaine antiporter, which works in both directions⁵. CaiT belongs to the BCCT (betaine, carnitine, choline transporter) family, which all transport substrates that contain a quaternary ammonium group.

1.1 Trimer architecture

PmCaiT and EcCaiT both form compact trimers of three identical protomers (fig. S3A). A hydrophobic cavity on the cytoplasmic side contains ordered detergent (fig. S3B) and would be filled with – most likely also ordered – lipid in the membrane. As in BetP, the long, curved α -helix 7 (H7) runs along the periplasmic membrane surface. This helix and the loop connecting it to TM8 (loop7, L7b), plus one residue in TM4, mediate tight trimer contacts. Arg299 in H7 forms a salt bridge to Asp288 in the neighbouring protomer. A hydrogen bond connects the hydroxyl of Thr304 in L7b to the backbone carbonyl oxygen of Asn284 the adjacent protomer, and a water molecule connects Asp305 to the backbone amide nitrogen of Gly308 in L7b to Glu132 in TM4. The strong, polar or ionic contacts in the CaiT trimer are thus very different from the hydrophobic trimer contacts in the BetP⁶, even though the order and arrangement of helices within the protomers is the same.

1.2 Inverted repeats in CaiT

TM3 to TM7 and TM8 to TM12 in CaiT define the two halves of an inverted repeat (figs. S2A, S4), as in many other secondary transporters⁶⁻¹². The inner core of the protomer is formed by TM3, TM4 of the first repeat and TM8, TM9 of the second repeat, arranged as an antiparallel four-helix bundle (figs. S2A, S4). This inner core is separated by a cytoplasmic

(L4, IH4) and a periplasmic helix-loop-helix motif (EH9a, L9, EH9b) from the supporting framework comprising TM5 to TM7 and TM10 to TM12. TM1 and the curved TM2 form a clamp-like scaffold for the helices of the inverted repeats (fig. S4).

1.3 Role of methionine in substrate coordination

Although the methionine side chain is usually thought of as hydrophobic, it can in fact participate in polar interactions, because the large, uncharged sulfur is more easily polarized than smaller atoms. Two types of interactions are possible¹³. The methionine sulfur is either negatively polarized and behaves as a nucleophile towards positively charged binding partners (e.g. Na⁺, Ni²⁺, H^{δ+}, C^{δ+}). Alternatively, the methionine sulfur can be positively polarized and behave as an electrophile towards anions or atoms with a partial negative charge (e.g. Cl⁻, O^{δ-}). In CaiT the sulfur of Met331 interacts with the negatively charged carboxyl group of γ -butyrobetaine, providing an elegant solution to the problem of recognizing and coordinating a hydrophilic substrate in the hydrophobic protein interior. It is known from the structures of small organic compounds such as 3-(methylthio) propanoic acid or norbornane endo-acid¹⁴ that sulfur atoms in a covalent bond interact with carboxylates¹⁴⁻¹⁶. Although this interaction does occur in other proteins¹⁷, a functionally important role of such a methionin-carboxylate bridge has not been reported up to now.

2 Tables

Table S1

Structure comparisons of PmCaiT and EcCaiT to each other and to the structurally related transporters BetP, vSGLT and LeuT.

Mol A	Mol B	Z-Score	Aligned	RMSD	Sequence
			Residues	(Å)	Identity (%)
PmCaiT	EcCaiT	53.6	972	0.9	87
(Trimer)	(Trimer)				
PmCaiT	EcCaiT	60.5	496	0.7	87
(Protomer)	(Protomer)				
PmCaiT	BetP	43.1	1348	2.2	25
(Trimer)	(Trimer)				
PmCaiT	BetP	43.7	478	2.2	25
(Monomer)	(Monomer)				
EcCaiT	BetP	37.2	895	2.3	25
(Trimer)	(Trimer)				
EcCaiT	BetP	44.0	481	2.2	25
(Monomer)	(Monomer)				
PmCaiT	vSGLT	13.7	334	4.2	13
(Monomer)	(Monomer)				
EcCaiT	vSGLT	13.6	333	4.0	12
(Monomer)	(Monomer)				
PmCaiT	LeuT	14.8	342	4.1	10
(Monomer)	(Monomer)				
EcCaiT	LeuT	14.7	335	4.3	10
(Monomer)	(Monomer)				

Table S2

Lookup table for trans-membrane helices in LeuT-type transporters. TM3 to TM7 in CaiT or BetP are the equivalent of helices 1 to 5, and TM8 to TM12 are the equivalent of helices 6 to 10 in the other transporters.

	CaiT	BetP	vSGLT	LeuT	Mhp1	AdiC	АрсТ
Repeat 1							
Helix 1	87 – 118	137 – 169	52 - 80	10 - 38	28 - 55	11 – 37	9 – 37
Helix 2	127 – 163	177 – 212	83 - 109	40 - 72	57 - 86	43 - 67	40 - 66
Helix 3	186 - 224	234 - 268	123 – 158	87 – 125	99 - 137	81 - 112	83 - 117
Helix 4	228 - 249	275 – 296	161 – 178	165 – 185	142 – 159	122 – 143	122 – 141
Helix 5	251 - 277	300 - 325	185 – 213	189 - 214	161 – 191	146 – 172	145 – 172
Repeat 2							
Helix 6	311 - 340	358 - 390	249 - 277	240 - 269	208 - 234	195 – 217	183 - 214
Helix 7	343 - 377	393 - 427	279 - 314	275 - 306	241 - 278	226 - 248	218 - 247
Helix 8	403 - 435	448 - 482	348 - 385	336 - 371	295 - 331	277 - 310	269 - 305
Helix 9	445 - 467	488 - 511	391 - 418	374 - 396	335 - 351	323 - 342	320 - 337
Helix 10	469 - 502	513 - 546	422 - 448	398 - 425	359 - 383	351 - 376	339 - 364

Table S3

Kinetic and substrate binding analysis of wildtype (wt) PmCaiT, PmCaiT mutants, and wt EcCaiT.

		Pm	CaiT		EcCaiT
	wt	E111A	W316A	M331V	wt
Transport					
$K_{M}(\mu M)$	46 ± 6	inactive	159.2 ± 10.8	123.3 ± 12.2	81.1 ± 11.8
	119.7 ± 20^{a}				$100.4 \pm 14.1^{\ a)}$
v _{max} (nmol substrate/	4672 ± 205		1816 ± 353	478 ± 53	4921 ± 243
(min•mg protein))	$4824 \pm 301^{\ a)}$				$4975 \pm 243^{\ a)}$
k _{cat} (L-carnitine/min)	263 ± 12		69 ± 4	26 ± 3	279 ± 14
	272 ± 17^{a}				282 ± 14^{a}
Substrate binding					
(detergent solution)					
$K_D (\gamma$ -butyrobetaine)	3.1 ± 0.6		17.5 ± 6.0	11.7 ± 1.2	3.9 ± 0.7
(mM)	3.4 ± 0.2^{a}			11.8 ± 1.7^{a}	
B_{max} (γ -butyrobetaine)	42.6 ± 2.4		58.6 ± 7.8	51.1 ± 3.0	43.3 ± 3.2
(mM)	42.0 ± 1.0^{a}			50.2 ± 2.8^{a}	
Hill coefficient	1.0 ± 0.1		0.9 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
K _D (L-carnitine) (mM)	1.8 ± 0.3		9.0 ± 1.3	7.1 ± 1.1	1.9 ± 0.2
	$2.4\pm0.4^{\ a)}$			$7.0 \pm 0.8^{\ a)}$	
B _{max} (L-carnitine)	40.3 ± 2.5		33.7 ± 1.3	41.7 ± 2.7	39.1 ± 3.1
(mM)	39.1 ± 2.3^{a}			45.1 ± 1.5^{a}	
Hill coefficient	1.1 ± 0.1		1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Substrate binding					
(proteoliposomes)					
K_D (γ -butyrobetaine)	5.6 ± 0.8		11.2 ± 1.7	n.d. ^{b)}	5.3 ± 0.8
(mM)					
B_{max} (γ -butyrobetaine)	34.3 ± 2.9		21.8 ± 1.8	n.d.	22.8 ± 0.8
(mM)					
Hill coefficient	1.5 ± 0.1		1.7 ± 0.2	n.d.	1.4 ± 0.1
K _D (L-carnitine) (mM)	4.5 ± 0.8		7.5 ± 1.2	n.d.	5.8 ± 1.0
B _{max} (L-carnitine)	28.2 ± 1.9		24.4 ± 0.8	n.d.	30.5 ± 1.5
(mM)					
Hill coefficient	1.4 ± 0.1		1.6 ± 0.1	n.d.	1.5 ± 0.2

^{a)} Measurements in presence of 50 mM NaCl;. ^{b)} not determined

Table S4

Data collection and phasing statistics

	P. mirabilis CaiT	<i>E. coli</i> CaiT
Beamline	X10SA SLS	X10SA SLS
Wavelength (Å)	0.92	0.98
Resolution (Å) ^{a)}	19.7 – 2.3 (2.4 – 2.3)	24.7 - 3.4 (3.6 - 3.4)
Cell dimensions	a = b = 129.2 Å, $c = 160.3$ Å	<i>a</i> = <i>b</i> = 124.20 Å, <i>c</i> = 154.63 Å
	$\alpha = \beta = 90^\circ, \gamma = 120^\circ$	$\alpha = \beta = 90^\circ, \gamma = 120^\circ$
Number of	246 131	136 423
measured reflections		
Number of unique	43 243	71 232
reflections		
Completeness (%)	97.7 (91.2)	97.7 (87.1)
Redundancy	5.7	1.9
I/ <i>o</i> (I)	9.0 (1.4)	12.8 (1.3)
R_{merge} (%) ^{b)}	8.4 (55.7)	6.0 (90.0)
R_{work} (%) ^{c)}	20.9	23.7
$R_{\rm free}$ (%) ^d	23.8	27.1
r.m.s. deviations		
Bond length (Å)	0.007	0.011
Bond angles (°)	0.980	1.302

^{a)} Numbers in parentheses represent statistics for data in the highest resolution shell

^{b)}
$$R_{merge} = \Sigma_{hkl}\Sigma_i ||F_{hkl}| - |F_{hkl}(i)|| / \Sigma_{hkl}\Sigma_i |F_{hkl}(i)|$$

 $^{c)}$ $R_{work} = \Sigma_{hkl} ||F_{obs}|$ - $k|F_{calc}||$ / $\Sigma_{hkl}|F_{obs}|$

 $^{d)}$ R_{free} was calculated with 5% of reflections not used during refinement.

3 Supplementary figures

Figure S 1

1	0

PmCaiT	1		12
EcCaiT	1		12
StCaiT	1		12
SlCaiT	1		22
CgBetP	1	MTTSDPNPKPIVEDAQPEQITATEELAGLLENPTNLEGKLADAEEEIILEGEDTQASLNW	60
EcBetT	1	MTDLSHSREKDKINP	15
mOCTN3	1	MLDYDEVTAFLGE	13
hoctn2	1	MRDYDEVTAFLGE	13

		TM1		TM2			
		20	30	40	50	60	70
				I I			I
PmCaiT	13	KVFFPPLIIVGIL	WLTVRDLDA	SNEVINAVFS	YVTNVWGWAE	EWYMVIMFGG	WFWLVFG
EcCaiT	13	KVFFPPLIIVGIL	WLTVRDLDA	ANVVINAVFS	YVTNVWGWAF	EWYMVVMLF	WFWLVFG
StCaiT	13	KVFFPLLIIVGIL	WLTVRDLDA	ANVVINAVFS	YVTNVWGWAE	EWYMVVMLFS	WFWLVFG
SlCaiT	23	KIFFPSLIAVILLS	YLTVRDLDA	ANVVIKEVFH	YLTHSWGWAR	EWYMIALAIG	WGWLVWG
CgBetP	61	SVIVPALVIVLAT	VWGIGFKDS	FTNFASSALS	AVVDNLGWAF	ILFGTVFVFF	IVVIAAS
EcBetT	16	VVFYTSAGLILLFS	LTTILFRDF	SALWIGRTLD	WVSKTFGWYY	LLAATLYIVE	VVCIACS
mOCTN3	14	WGTFQRLIFFLLS	SIIPNGFTG	LSAVFLTAIP	EHRCRIPDTV	NLSSAWRNHS	IPMETKD
hOCTN2	14	WGPFQRLIFFLLS	SIIPNGFTG	LSSVFLIATP	EHRCRVPDAA	NLSSAWRNHT	VPLRLRD

				TM3			
		80	90	100	110	120	130
							1
PmCaiT	73	RYAKKRLGDEKPEF	STASWIFM	FASCISAAVI	FWGSIEIYY	ISSPPFGMEG	YSAPA 130
EcCaiT	73	PYAKKRLGNEPPEF	STASWIFM	FASCISAAVI	FWGSIEIYY	ISTPPFGLEP	NSTGA 130
StCaiT	73	PYAKKRLGDEKPEF	STASWIFM	FASCISAAVI	FWGSIEIYYY	ISTPPFGLEP	NSTGA 130
SlCaiT	83	PYANNRLGQEKPEF	STGSWIFL	FASCISAAVI	YWGSLEAYYY	(LTYPPFEIQS	MSVQA 140
CgBetP	121	KFGTIRLGRIDEAPEF	RTVSWISM	FAAGMGIGLN	FYGTTEPLTI	YRNGVPG	HDEHN 177
EcBetT	76	RFGSVKLGPEQSKPEF	SLLSWAAMI	FAAGIGIDLM	(FFSVAEPVT)	QYMQPP-EGAG	QTIEA 134
mOCTN3	74	GPEVPQKCRRY	RLATIANFS	ELGLEPGRDV	DLEQLEQEN	LDGWEYDKDI	FLSTI 128
hoctn2	74	GREVPHSCRRY	RLATIANFS	ALGLEPGRDV		LDGWEFSQDV	YLSTI 128
					<u> </u>		

			ГМ4				
		140	150	160	170	180	190
				1			11
PmCaiT	131	KEIGLAYSLFHW GP	LPWATYSFLS	VAFAYFFFV	RKMEVIRPSSTL	TPLVGEKHV	NGLFGT 190
EcCaiT	131	KELGLAYSLFHW GP	LPWATYSFLS	VAFAYFFFV	RKMEVIRPSSTL	VPLVGEKHA	KGLFGT 190
StCaiT	131	KEIGLAYSLFHW GP	LPWATYSFLS	VAFAYFFFV	RKMDVIRPSSTL	VPLVGEKHA	KGLFST 190
SlCaiT	141	KELGVAYSLFHW GP	LPWMGYGFFT	VALGYFLFV	KKMDVVRPSGTL	APVLG-KHH	KGILGT 199
CgBetP	178	VGVAMSTTMFHWTL	HP WAIYAIV G	LAIAYSTFR	VGRKQLLSS-AF	VPLIGEKGA	EGWLGK 236
EcBetT	135	ARQAMVWTLFHYGL	TGWSMYALMG	MALGYFSYR	YNLP-LTIRSAL	YPIFG-KRI	NGPIGH 192
mOCTN3	129	VTEWDLVCKDDWKA	PLTTSFFYVG	VLLGSFISG	QLSDRFGRKNIL	FLTMAMHTG	FSFIQV 188
hoctn2	129	VTEWNLVCEDDWKA	PLTISLFFVG	VLLGSFISG	QLSDRFGRKNVL	FVTMGMQTG	FSFLQI 188

		TM5			TM6	
	200	210	220	230	240	
		1 1		1		
191	VVDNFYLVALILAM	GTSLGLATPL	VTECIQYLFG	IPHTLQL	DAIIISCWILLNA	244
191	IVDNFYLVALIFAM	GTSLGLATPL	TECMOWLFG	IPHTLQL	DAIIITCWIILNA	244
191	IVDNFYLVALIFAM	GTSLGLATPL	TECMOWLFG	IPHTLQL	DAIIITCWIILNA	244
200	FIDNIYVVALILAM	GTSLGLATPL	VTECIQWLFG	IERTIEV	-DTFVISVWIIFNA	253
237	LIDILAIIATVFGT	ACSLGLGALQ	IGAGLSAANI	IEDPS	DWTIVGIVSVLTL	288
193	SVDIAAVIGTIFGI	ATTLGIGVVQ	LNYGLSVLFD	IP	DSMAAKAALIALS	241
189	FSVNFEMFTLLYTL	VGMGHISNYV	AAFVLGTEML	SKSVRIIFATL	SVCIFFAFGFMVLPL	248
189	FSKNFEMFVVLFVL	VGMGQISNYV	AAFVLGTEIL	GKSVRIIFSTL	SVCIFYAFGYMVLPL	248
	191 191 200 237 193 189	200 II 191 VVDNFYLVALIFAM 191 IVDNFYLVALIFAM 191 IVDNFYLVALIFAM 200 FIDNIYVVALIFAM 237 LIDILAITATVFGT 193 SVDIAAVIGTIFGT 189 FSVNFEMFTLLYTL 189 FSKNFEMFVVLFVL	TM5 200 210	TM5 200 210 220 191 VVDNFYLVALILAMGTSLGLATPLVTECIQVLFG 191 IVDNFYLVALIFAMGTSLGLATPLVTECMQWLFG 191 IVDNFYLVALIFAMGTSLGLATPLVTECMQWLFG 200 FIDNIYVVALIFAMGTSLGLATPLVTECMQWLFG 200 FIDNIYVVALIFAMGTSLGLATPLVTECMQWLFG 201 SVDIAAVIGTIFGIATTLGIGVQLNYGLSVLFD 193 SVDIAAVIGTIFGIATTLGIGVQLNYGLSVLFD 189 FSVNFEMFTLLYTLVGMGHISNYVAAFVLGTEML 189 FSKNFEMFVVLFVLVGMGQISNYVAAFVLGTEIL	TM5 200 210 220 230 iiiii. 191 VVDNFYLVALILAMGTSLGLATPLVTECIQYLFGIPHTLQL 191 IVDNFYLVALIFAMGTSLGLATPLVTECMQWLFGIPHTLQL 191 IVDNFYLVALIFAMGTSLGLATPLVTECMQWLFGIPHTLQL 200 FIDNIYVVALIFAMGTSLGLATPLVTECMQWLFGIPHTLQL 201 FIDNIYVVALILAMGTSLGLATPLVTECIQWLFGIPHTLQL 202 FIDNIYVVALILAMGTSLGLATPLVTECIQWLFGIPHTLQL 203 LIDILAIIATVFGTACSLGLGALQIGAGLSAANIIEDPS 193 SVDIAAVIGTIFGIATTLGIGVVQLNYGLSVLFDIP	TM5 TM6 200 210 220 230 240 191 VVDNFYLVALILAMGTSLGLATPLVTECIQYLFGIPHTLQLDAIIISCWILLNA 191 IVDNFYLVALIFAMGTSLGLATPLVTECMQWLFGIPHTLQLDAIIIITCWIILNA 191 IVDNFYLVALIFAMGTSLGLATPLVTECMQWLFGIPHTLQLDAIIIITCWIILNA 200 FIDNIYVVALIFAMGTSLGLATPLVTECMQWLFGIPHTLQLDAIIIITCWIILNA 201 FIDNIYVVALIFAMGTSLGLATPLVTECMQWLFGIPHTLQLDAIIIITCWIILNA 202 FIDNIYVVALILAMGTSLGLATPLVTECIQWLFGIPHTLQLDAIIIITCWIILNA 203 FIDNIYVVALILAMGTSLGLATPLVTECIQWLFGIERTIEVDAIIIITCWIILNA 204 FIDNIYVVALILAMGTSLGLATPLVTECIQWLFGIERTIEVDAIIIITCWIILNA 205 FIDNIYVVALILAMGTSLGLALQIGAGLSAANIIEDPSDWTIVGIVSVLTL 206 SVDIAAVIGTIFGIATTLGIGVVQLNYGLSVLFDIPDSMAAKAALIALS 217 LIDILAIIATVFGTACSLGLGALQIGAGLSAANIIEDPSDSMAAKAALIALS 218 FSVNFEMFTLLYTLVGMGHISNYVAAFVLGTEMLSKSVRIIFATLGVCIFFAFGFMVLPL 218 FSKNFEMFVVLFVLVGMGQISNYVAAFVLGTELIGKSVRIIFSTLGVCIFYAFGYMVLPL

				TM7			H7	
		250	2	60	270	280	290	
		11 .	I	1	I I	I I	I I	
PmCaiT	245	ICVAFGL	Q KGVKIA S	DVRTYLSF	LMLGWVFIVG	GASFIVNYFT	DSVGTLLMYMPR	299
EcCaiT	245	ICVACGL	Q KGVRIA S	DVRSYLSF	LMLGWVFIVS	GASFIMNYFT	DSVGMLLMYLPR	299
StCaiT	245	ICVACGL	Q KGVRIA S	DVRSYLSF	LMLGWVFIVS	GASFIMNYFT	DSVGMLLMHLPR	299
SlCaiT	254	VCVAFGL	TKGIKIAS	DLRSYLSI	IMLFWVFLIG	ATSFTVNYFT	ESVGVMLAYLPR	308
CgBetP	289	AFIFSAISGV	GKGIQYLS	NANMVLAA	LLAIFVFVVG	PTVSILNLLP	GSIGNYLSNFFQ	346
EcBetT	242	VIIATISVTSGV	DKGIRVLS	ELNVALAL	GLILFVLFMG	DTSFLLNALV	LNVGDYVNRFMG	301
mOCTN3	249	FAYFI	-REWRRLL	LAITLPGV	LCGALWWFIP	ESPRWLISQG	RIKEAEVIIRKA	300
hoctn2	249	FAYFI	-RDWRMLL	VALTMPGV	LCVALWWFIP	ESPRWLISQG	RFEEAEVIIRKA	300

				TM8				
	3	00		310	320	330	340	
		11.					1	
PmCaiT	300	MLFYTDP		IGKGGFPQ	WTVFYWAWW	VIYAIQMSIF	LARISKGR	34
EcCaiT	300	MLFYTDP		IAKGGFPQC	WTVFYWAWW	VIYAIQMSIF	LARISRGR	34
StCaiT	300	MLFYTDA		IGKGGFPQC	WTVFYWAWW	VIYAIQMSIF	LARISRGR	34
SlCaiT	309	MLFYTSS		ISADSWPQE	WTVFYWAWW	VYGIQMCIF	LAKISRGR	35
CgBetP	347	MAGRTAMSA		-DGTAGEWLGS	WTIFYWAWW	ISWSPFVGMF	LARISRGR	39
EcBetT	302	MTLNSFAF		-D-RPVEWMNN	WTLFFWAWW	VAWSPFVGLF	LARISRGR	34
mOCTN3	301	AKINGIVAPSTI	DPSETNKLQ	DDSSKKPQSHF	IYDLVRTPN:	IRILTIMSII	LWLTISVG	36
hOCTN2	301	AKANGIVVPSTI	DPSELQDL-	SS KK QQSHN			LWMTISVG	35
			TM9					
		350	360	370	380	390	400	
							1 1	
PmCaiT	343	TVRELCLGMVSGI	TAGTWLIWT	YSGGNTLQLI	QNILNIPQL	IDQYGVPRAI	IETWAALP	40
EcCaiT	343	TVRELCFGMVLGI	TASTWILWT	VLGSNTLLLI	KNIINIPNL	IEQYGVARAI	IETWAALP	40
StCaiT	343	TVRELCFGMVMGI	TASTWILWT	VLGSNTLLLM	KNILNIPQL	IEQHGVARAI	IETWAALP	403
SlCaiT	352	TVRELCLTMVLGI	TASTWFLWT	ILGSNTVNLM	ESIINMGQL	IQDHGAPRAI	IETWAALP	41
CgBetP	393	SIREFILGVLLVI	AGVSTVWFS	IFGGTAIVFE	NGESI	GDGAAEEQL	FGLLHALP	44

 EcBett|
 346
 TIRQFVLGTLIIPFTFTLLWLSVFGNSALYEIIHGGAAFAEEAMVHP-ERGFYSLLAQYP
 404

 mOCTN3|
 361
 YFGLSLDTPNLNGNIYVNCFLLAAVEVPAYVLAWLLLQHVSRRYSMAGSLFLGGSVLLLV
 420

 hOCTN2|
 358
 YFGLSLDTPNLHGDIFVNCFLSAMVEVPAYVLAWLLLQYLPRRYSMATALFLGGSVLLFM
 417

			TM10			TM1	1	
		410	420	430	440	450	460	
				111				
PmCaiT 4	03	LSTATMWGFFILC	FIATVTLIN	ACSYTLAMSTC	RSMKEGAEP	PLLVRIGWSVI	VGIIGI 46	51
EcCaiT 4	03	LSTATMWGFFIL	FIATVTLVN	ACSYTLAMSTC	REVRDGEEP	PLLVRIGWSII	VGIIGI 46	51
StCaiT 4	03	LSTATMWGFFIL	FIATVTLIN	ACSYTLAMSTC	REVRDGEEP	PLLVRIGWSVI	VGIIGI 46	61
SlCaiT 4	13	MSTITMWGF-ILC	FLATVILIN	ACSYTLAMSTC	KGADADNEP	PVWIRVGWSVI	VGVIGI 47	1(
CgBetP 4	49	GGQI-MGIFIAMI	LLGTFFITS	ADSASTVMGTM	SQHG-QLEA	N KWV T AAWGVI	TAAIGL 50)(
EcBetT 4	05	AFTFSASVATIT(LLFYVTSAD	SGALVLGNFTS	QLKDINSDA	PGWLRVFWSVI	IGLLTL 46	57
mOCTN3 4	21	QLVPSDLHYLST	LVMVGKFGI	TSAYSMVYVYT	AELYPT-	VVRNMGVGV	SSTASR 47	14
hoctn2 4	18	QLVPPDLYYLATV	LVMVGKFGV	TAAFSMVYVYI	AELY PT-	VVRNMGVG	SSTASR 47	73

					TM12	
			470	480	490	500
PmCaiT	462	ILLALGG	LKPIQTA	IIAGGCPL	FVNIMVTLSF	IKDAKVH-WKD
EcCaiT	462	VLLALGG	LKPIQTA	IIAGGCPL	FVNIMVTLSF	IKDAKQN-WKD
StCaiT	462	VLLALGG	LKPIQTA	IIAGGCPL	FVNIMVTLSF	IKDAKVH-WKD
SlCaiT	471	VLLSLGG	LKPLQTA	IIAGGAPL	VIVNILVIIS <mark>F</mark>	LKDARKNNWAS
CgBetP	507	TLLLSGG	DNALSNL(QNVTIVAA:	PFLFVVIGLM	FALVKDLSNDV
EcBetT	468	GMLMTNG	ISALQNT'	TVIMGLPF	FVIFFVMAGL	YKSLKVEDYRR
mOCTN3	475	LGSILSP	YFVYLGA	YDRRLPYI	MGSLTILTAI	ITLFFPESSGV
hoctn2	472	LGSILSP	YFVYLGA	YDRFLPYI	MGSLTILTAI	LTLFLPESFGT

		570	580	590	
PmCaiT	504				5(
EcCaiT	504				50
StCaiT	505				50
SlCaiT	514				51
CgBetP	566	ARERRVHNEHR	KRELAAKRRR	ERKASGAGK	59
EcBetT	528	DRLSWKKRLSR	LMNYPGTRYT	KOMMETVCYPAMEEVAQELRLRGAYVELKSLPPEEGQQL	58
mOCTN3	534	KOROSLSKKGS	PKESKGNVSR	TSRTSEPKGF	56
hoctn2	531	KHRKTPSHTRM	IKDGQERPTI	LKSTAF	55

Amino acid sequence alignment of CaiT from Proteus mirabilis (PmCaiT), E. coli (EcCaiT), Salmonella typhimorium (StCaiT), Shewanella loihica (SlCaiT), BetP from Corynebacterium glutamicum (CgBetP), E.coli BetT (EcBetT), and organic cation transporters from mouse (mOCTN3) and human (hOCTN2). Red triangle: Met331 which coordinates the carboxyl group of the bound substrate in the transport site of CaiT. Orange triangles: fully or partly conserved hydrophobic residues in the central transport site. Blue triangle: Trp316 in the regulatory site of CaiT. Red asterisk: Glu111, which coordinates the network of hydrogen bonds linking the two inverted repeats in the inside-open conformation of CaiT (Fig. 4a,b). Residues 588 – 677 of EcBetT were omitted.



(a) Inverted helix repeats in CaiT. Repeat 1 (TM3 to TM7) is shown in dark colours, repeat 2 (TM8 to TM12) in lighter colours.

(b) The 10 TM helices of the two inverted repeats (left) are coloured as in (a). Repeat 2 (right) is rotated relative to repeat 1 (centre) by $\sim 180^{\circ}$ around the internal pseudo-twofold axis of the protomer (black line), and by $\sim 20^{\circ}$ in the perpendicular direction.



(a) The PmCaiT trimer. In the periplasmic view (left), close contacts between protomers (arrow) are mediated by an ion pair (yellow sidechains) and hydrogen bonds. The side view (right) shows a deep cavity between protomers on the cytoplasmic side (arrow) that contains bound detergent (yellow). (b) Detailed views of the ion and water bridges that connect protomers in the trimer (left), and the Cymal-5 detergent head group interacting with the aromatic sidechain of Phe67 (right) in the hydrophobic cavity.





Stereo diagrams of the EcCaiT protomer with two bound γ -butyrobetaine substrates. (a) cytoplasmic view; (b) side view. The arrows in B point to the bound substrate molecules in the transport site (centre) and in the regulatory site on the periplasmic side.



Binding of γ-butyrobetaine or L-carnitine to wildtype (wt) PmCaiT (black), PmCaiT M331V (blue) and PmCaiT W316A (green) solubilised in the detergent Cymal-5 in sodium-free buffer (top) or with 50 mM NaCl, monitored by Trp fluorescence.



Oligomeric state of CaiT in detergent solution. Wildtype PmCaiT (wt) forms stable trimers that run at about 230 kDa in the blue-native gradient gel (4 - 16%). Mutation of Glu111 to alanine (E111A) destabilizes the trimer, and results in the appearance of dimers and monomers.



(a) Cytoplasmic (left) and extracellular view (right) of the PmCaiT trimer (yellow) superposed onto the EcCaiT trimer (blue) in stereo. The superposition indicates a 3° tilt of the substrate-bound EcCaiT protomer relative to PmCaiT, towards the threefold axis in the extracellular view. (b) Stereo view of the superimposed regulatory sites of EcCaiT (coloured) and PmCaiT (grey). Substrate binding in the external binding site replaces two water molecules and disrupts the hydrogen bond network coordinated by them. As a result, the extracellular end of TM12 moves by ~1.3Å.



Stereo drawing of superposed protomers of PmCaiT and BetP, showing an iris-like movement of the cytoplasmatic part of the helices TM3, TM7, TM8 and TM10 (yellow for PmCaiT, red for BetP) in the transition from the fully inside-open conformation of CaiT to the occluded state of BetP.







Stereo drawings comparing EcCaiT helices TM3, TM8 and TM12 with key residues in the inside-open conformation (a, blue) to a model of EcCaiT in the outside-open conformation (b, pink), based on the LeuT structure. In the model (b), the second, regulatory substrate-binding site on the extracellular side defined by residues Tyr114, Trp316 and the carbonyl oxygen of Gly315 remains intact, so that substrate is likely to remain bound throughout the transport cycle. (c) Superposition of A and B, indicating the helix movements that accompany the transition from the inside-open to the outside-open conformation on the extracellular side of CaiT. (d) Corresponding helix regions of the outside-open LeuT (TM1, TM6 and TM10,

green) in complex with tryptophan. The Trp in the extracellular channel of LeuT is in a different position compared to the substrate in the regulatory site of CaiT.

4. References

- 1. Jung, H., Jung, K. & Kleber, H. P. L-carnitine metabolization and osmotic stress response in Escherichia coli. *J Basic Microbiol* **30**, 409-413 (1990).
- 2. Jung, H., Jung, K. & Kleber, H. P. L-carnitine uptake by Escherichia coli. *J. Basic. Microbiol.* **30**, 507-514 (1990).
- 3. Jung, K., Jung, H. & Kleber, H. P. Regulation of L-carnitine metabolism in Escherichia coli. *J. Basic. Microbiol.* **27**, 131-137 (1987).
- 4. Kleber, H. P. Bacterial carnitine metabolism. *FEMS Microbiol. Lett.* **147**, 1-9, doi:S0378-1097(96)00412-0 [pii] (1997).
- 5. Jung, H. *et al.* CaiT of Escherichia coli, a new transporter catalyzing Lcarnitine/gamma -butyrobetaine exchange. *J Biol Chem* **277**, 39251-39258, doi:10.1074/jbc.M206319200M206319200 [pii] (2002).
- Ressl, S., Terwisscha van Scheltinga, A. C., Vonrhein, C., Ott, V. & Ziegler, C. Molecular basis of transport and regulation in the Na(+)/betaine symporter BetP. *Nature* 458, 47-52, doi:nature07819 [pii]10.1038/nature07819 (2009).
- 7. Faham, S. *et al.* The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na+/sugar symport. *Science* **321**, 810-814, doi:1160406 [pii]10.1126/science.1160406 (2008).
- 8. Gao, X. *et al.* Structure and mechanism of an amino acid antiporter. *Science* **324**, 1565-1568, doi:1173654 [pii]10.1126/science.1173654 (2009).
- 9. Sevilla, A. *et al.* Design of metabolic engineering strategies for maximizing L-(-)carnitine production by Escherichia coli. Integration of the metabolic and bioreactor levels. *Biotechnol. Prog.* **21**, 329-337, doi:10.1021/bp0497583 (2005).
- 10. Weyand, S. *et al.* Structure and molecular mechanism of a nucleobase-cation-symport-1 family transporter. *Science* **322**, 709-713, doi:1164440 [pii]10.1126/science.1164440 (2008).
- Yamashita, A., Singh, S. K., Kawate, T., Jin, Y. & Gouaux, E. Crystal structure of a bacterial homologue of Na+/Cl--dependent neurotransmitter transporters. *Nature* 437, 215-223, doi:nature03978 [pii]10.1038/nature03978 (2005).
- 12. Fang, Y. *et al.* Structure of a prokaryotic virtual proton pump at 3.2 A resolution. *Nature* **460**, 1040-1043, doi:nature08201 [pii]10.1038/nature08201 (2009).
- 13. Rosenfield, J., R.E., Parthasarathy, R. & Dunitz, J. D. Directional Preferences of Nonbonded Atomic Contacts with Divalent Sulfur. 1. Electrophiles and Nucleophiles. *J. Am. Chem. Soc.* **99**, 4860-4862 (1977).
- 14. Mahling, S., Asmus, K. D., Glass, R. S., Hojjatie, M. & Wilson, G. S. Neighboring group participation in radicals: pulse radiolysis studies on radicals with sulfur-oxygen interaction. *J. Org. Chem.* **52**, 3717-3724 (1987).
- 15. Burling, F. T. & Goldstein, B. M. A database study of nonbonded intramolecular sulfur-nucleophile contacts. *Acta Crystallogr. B* **49** (**Pt 4**), 738-744 (1993).
- 16. Pal, D. & Chakrabarti, P. Non-hydrogen bond interactions involving the methionine sulfur atom. *J. Biomol. Struct. Dyn.* **19**, 115-128 (2001).
- 17. Pal, D. & Chakrabarti, P. Non-hydrogen bond interactions involving the methionine sulfur atom. *J Biomol Struct Dyn* **19**, 115-128 (2001).