A NOVEL MEMBER OF SOLUTE CARRIER FAMILY 25 (SLC25A42) IS A TRANSPORTER OF COENZYME A AND ADENOSINE 3',5'-DIPHOSPHATE IN HUMAN MITOCHONDRIA*

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Running title: Mitochondrial transport of CoA by SLC25A42

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Mitochondrial carriers are a family of proteins that transport metabolites, nucleotides and cofactors across the inner mitochondrial membrane thereby connecting cytosolic and matrix functions. The essential cofactor coenzyme A (CoA) is synthesized outside the mitochondrial matrix and therefore must be transported into mitochondria where it is required for a number of fundamental processes. In this work we have functionally identified and characterized SLC25A42, a novel human member of the mitochondrial carrier family (Haitina, T., Lindblom, J., Renström, T., and Fredriksson, R., 2006, Genomics 88, 779-790). The SLC25A42 gene was overexpressed in Escherichia coli, purified and reconstituted into phospholipid vesicles. Its transport properties, kinetic parameters and targeting to mitochondria demonstrate that SLC25A42 protein is a mitochondrial transporter for CoA and adenosine 3',5'-diphosphate. SLC25A42 catalyzed only a counter-exchange transport, exhibited a high transport affinity for CoA, dephospho-CoA, ADP and adenosine 3',5'-diphosphate, was saturable and inhibited by bongkrekic acid and other inhibitors of mitochondrial carriers to various degrees. The main physiological role of SLC25A42 is to import CoA into mitochondria in exchange for intramitochondrial (deoxy)adenine nucleotides and adenosine 3',5'-diphosphate. This is the first time that a mitochondrial carrier for CoA and adenosine 3',5'-diphosphate has been characterized biochemically.

The mitochondrial carrier family, or the solute carrier family 25 (SLC25), comprises a large group of proteins that transport a variety of substrates across the inner mitochondrial membrane and, in a few cases, across other membranes (1,2). Common structural features of the mitochondrial carrier family members consist in a tripartite structure (three repeats of about 100 amino acids), the presence of two transmembrane α-helices separated by hydrophilic loops in each repeat and the presence of a signature motif at the C-terminus of the first helix in each repeat (3 and references therein). The SLC25 family is by far the largest of the currently known 43 SLC families. The Saccharomyces cerevisiae genome contains 35 members, that of Arabidopsis thaliana 58 and the human genome at least 48 SLC25 members. Until now, nearly 30 members and isoforms of this family have been identified in man. These include the uncoupling protein and the carriers for ADP/ATP, phosphate, 2-oxoglutarate/malate, citrate, carnitine/acylcarnitine, dicarboxylates, ornithine and other basic amino acids, oxodicarboxylates, deoxynucleotides and thiamine pyrophosphate, aspartate-glutamate, glutamate, S-adenosylmethionine, ATP-Mg/Pi, pyrimidine nucleotides, and adenine nucleotides in peroxisomes (see Ref. 1 for a review and Refs. 4-8). The present investigation was undertaken to identify the function of SLC25A42, a novel member of the SLC25 family recently found in the human genome (9). SLC25A42 is 318 amino acids long and is highly expressed in virtually all tissues, in most at higher levels than many other SLC25 family members (9).
In this study we provide direct evidence that SLC25A42 is a mitochondrial transporter for CoA and PAP. SLC25A42 was overexpressed in Escherichia coli, purified, reconstituted in phospholipid vesicles and shown to transport CoA, dephospho-CoA, PAP and (deoxy)adenine nucleotides with high specificity and by a counter-exchange mechanism. The main function of SLC25A42 is probably to catalyze the entry of CoA into the mitochondria in exchange for adenine nucleotides and PAP.

**Experimental Procedures**

**Materials** - Radioactive compounds were supplied from Moravek Biochemicals, Brea, CA, Perkin Elmer Life Science Products, Waltham, MA, and Campro Scientific, GmbH, The Netherlands.

**Sequence search and analysis** - Protein and genomic data bases (ensembl.ebi.ac.uk and www.ncbi.nlm.nih.gov) were screened with the protein sequence of SLC25A42 (Accession Number NM_178526), using BLASTP and TBLASTN. The amino acid sequences were aligned with ClustalW (version 1.8). The phylogenetic tree was computed using the PAM matrix and MacVector 7.1.1 software (Accelrys, Cambridge, UK).

**Construction of expression plasmids** - The coding sequence for SLC25A42 was amplified by PCR from human brain cDNA (Clontech). The oligonucleotide primers were synthesized corresponding to the extremities of the coding sequence (Accession No. NM_178526), with additional NdeI and EcoRI sites. The amplified product was cloned into the pMW7 expression vector. The SLC25A42-pYES2 and SLC25A10-pYES2 plasmids were constructed by cloning the coding sequences of SLC25A42 and SLC25A10, respectively, into the yeast pYES2 expression vector (Invitrogen) under the control of the GAL10 promoter or the constitutive MIR1 promoter. The LEU5-pRS416 plasmid was constructed by cloning a DNA fragment consisting of the LEU5 open reading frame, of 500 bp upstream and of 260 bp downstream of the open reading frame (amplified from S. cerevisiae genomic DNA by PCR using primers with additional HindIII and BamHI sites) into the low copy centromeric vector pRS416 (10). The pMW7, pYES2 and pRS416 vectors, prepared as above, were transformed into E. coli DH5α cells. Transformants were selected on ampicillin (100 μg/ml) and screened by direct colony PCR and by restriction digestion of purified plasmids. The sequences of the inserts were verified.

**Bacterial expression and purification of SLC25A42** - The over-production of SLC25A42 as inclusion bodies in the cytosol of E. coli was accomplished as described previously (11), except that the host cells were E. coli CO214(DE3) (12). Control cultures with the empty vector were processed in parallel. Inclusion bodies were purified on a sucrose density gradient (11), washed at 4°C with a buffer containing Triton X-114 (3%, w/v), 1 mM EDTA and 10 mM PIPES-KOH pH 7.5, and twice with TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0). SLC25A42 was solubilized in 2% sarkosyl (w/v) and a small residue was removed by centrifugation (258,000 g, 20 min).

**Reconstitution of SLC25A42 into liposomes** - The recombinant protein in sarkosyl was diluted 11-fold with a buffer containing Tris-HCl 10 mM (pH 8) and 0.6% Triton X-114 and reconstituted into liposomes by cyclic removal of the detergent with a hydrophobic column of Amberlite beads (Fluka) (13). The composition of the initial mixture used for reconstitution was 90 μl of purified SLC25A42 (15 μg of protein), 70 μl of 10% Triton X-114, 90 μl of 10% phospholipids in the form of sonicated liposomes, 10 mM ADP (except where otherwise indicated), 10 mM Tris-HCl (pH 8.0), 0.6 mg of cardiolipin (Sigma), and water to a final volume of 700 μl. After vortexing, this mixture was recycled 13 times through the Amberlite column (3.5 x 0.5 cm) pre-equilibrated with a buffer containing 10 mM Tris-HCl, pH 8.0, and the substrate at the same concentration as in the starting mixture. All operations were

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1 Abbreviations: CoA, coenzyme A; EG(B)FP, enhanced green (blue) fluorescence protein; PAP, adenosine 3’,5’-diphosphate.
performed at 4°C, except the passages through Amberlite, which were carried out at room temperature.

*Transport measurements* - External substrate was removed from proteoliposomes on Sephadex G-75 columns pre-equilibrated with 50 mM NaCl and 10 mM Tris at pH 8.0 (buffer A). The eluted proteoliposomes were distributed in reaction vessels and used for transport measurements by the inhibitor-stop method (13). Transport at 25°C was started by adding $^{14}$C[ADP or other indicated labeled compounds to substrate-loaded proteoliposomes (exchange) or to empty proteoliposomes (uniport). In both cases, transport was terminated by addition of 20 mM pyridoxal-5'-phosphate and 20 mM bathophenanthroline, which in combination and at high concentrations inhibit the activity of several mitochondrial carriers completely and rapidly (see, for example, Refs. 7,11,12), though their mechanism of action has not been studied. In controls, the inhibitors were added at the beginning together with the radioactive substrate. All transport measurements were carried out at the same internal and external pH value (10 mM Tris, pH 8.0). Finally, the external radioactivity was removed from each sample of proteoliposomes by a Sephadex G-75 column; the proteoliposomes were eluted with 50 mM NaCl and their radioactivity was measured. The experimental values were corrected by subtracting control values. The initial transport rate was calculated from the radioactivity taken up by proteoliposomes after 2 min (in the initial linear range of substrate uptake). Alternatively, the initial transport rate was calculated from the time course of isotope equilibration (13). For efflux measurements, proteoliposomes containing 2 mM ADP were labeled with 5 µM $^{14}$C[ADP by carrier-mediated exchange equilibration (13). After 30 min, the external radioactivity was removed by passing the proteoliposomes through Sephadex G-75 pre-equilibrated with buffer A. Efflux was started by adding unlabeled external substrate or buffer A alone to aliquots of proteoliposomes and terminated by adding the inhibitors indicated above.

*Subcellular localization* - For the subcellular localization of SLC25A42 in CHO cells, the protein was fused to EGFP at the C-terminus. For this purpose, the SLC25A42 coding sequence was amplified without the termination codon and with additional HindIII and BamHI restriction sites. The PCR product was cloned into a modified pcDNA3 vector (14) in frame with the HA1-EGFP sequence (where HA1 is a nine-amino acid epitope derived from hemoagglutinin). CHO cells were grown on 24-mm coverslips to 50-70% confluence and co-transfected according to a standard calcium-phosphate procedure with 4 µg of mtEBFP/pcDNA1 (15) and 4 µg of the modified pcDNA3 plasmid containing the coding sequence of SLC25A42 fused with the EGFP sequence. EGFP and EBFP fluorescence were detected as described (15).

*Yeast strains, media and growth conditions* – The wild-type W303-1B strain (MATα, ade2-1 leu2-3,112 his3-11 trp1-1 ura3-1) was provided by the EUROFAN resource centre EUROSCARF (Frankfurt, Germany). The deletion strain ΔLEU5 was constructed using the PCR-mediated gene disruption technique by replacing the LEU5 open reading frame with the hygromycin B resistance cassette (hphMX3) in the W303-1B strain (16). LEU5 deletion was verified by PCR. The wild-type and deletion strains were grown in rich (YP) medium, containing 2% bactopeptone and 1% yeast extract, supplemented with 3% glycerol, 3% acetate, or 2% DL-lactate. The final pH was adjusted to 4.5.

*Other methods* - Proteins were analyzed by SDS-PAGE and stained with Coomassie Blue dye. N-terminal sequencing was carried out as described previously (17). The amount of pure SLC25A42 was estimated by laser densitometry of stained samples, using carbonic anhydrase as protein standard (17). The amount of protein incorporated into liposomes was measured as described (17) and varied between 20-30% of the protein added to the reconstitution mixture.

**RESULTS**

*Subcellular localization of SLC25A42* - Since some members of the mitochondrial carrier family are localized in non-mitochondrial
membranes (18-20), the intracellular localization of SLC25A42 was investigated. This aspect was addressed by constructing an expression plasmid with GFP fused to SLC25A42 (see Fig. 1). CHO cells were transfected with the pcDNA3-SLC25A42-EGFP plasmid, and images were acquired 40-45 hours after transfection. About 36% of cells were transfected, and green fluorescence revealed typical mitochondrial localization of SLC25A42. The green fluorescence of the GFP-tagged protein completely overlapped with the blue fluorescence of a mitochondrially targeted BFP (14) co-expressed in the same cells. Thus SLC25A42, which lacks the targeting N-terminal extension, contains in its amino acid sequence the structural information for import into mitochondria, in accordance with data available for other mitochondrial carriers (21,22).

Bacterial expression of SLC25A42 - The SLC25A42 gene was expressed at high levels in E. coli CO214(DE3) (Fig. 2, lane 4). The gene product accumulated as inclusion bodies and was purified by centrifugation and washing (Fig. 2, lane 5). The purified protein gave a single band by SDS-PAGE with an apparent molecular mass of 36.2 kDa in agreement with the calculated value with initiator methionine (35425 Da). Its identity was confirmed by N-terminal sequencing. The protein was not detected in bacteria harvested immediately before induction of expression (Fig. 2, line 2) nor in cells harvested after induction but lacking the coding sequence in the expression vector (Fig. 2, line 3). Approximately 55 mg of purified protein per liter of culture were obtained.

Functional characterization of recombinant SLC25A42 - SLC25A42 was reconstituted into liposomes and its transport activities for a variety of nucleotides were tested in homo-exchange experiments (i.e., with the same substrate internally and externally). Using external and internal substrate concentrations of 0.2 and 10 mM, respectively, the reconstituted protein catalyzed active [14C]ADP/ADP, [14C]AMP/AMP and [14C]ATP/ATP exchanges (Fig. 3) that were inhibited completely by pyridoxal-5'-phosphate and bathophenanthroline. By contrast, despite the long incubation period (i.e., 30 min), virtually no homo-exchange activity was observed for GMP, GDP, GTP, CMP, CDP, CTP, UMP, UTP, TMP and TTP (Fig. 3). No [14C]ADP/ADP and [14C]AMP/AMP exchange activities were detected when SLC25A42 had been boiled before incorporation into liposomes or if proteoliposomes were reconstituted with sarkosyl-solubilized material from bacterial cells either lacking the expression vector for SLC25A42 or harvested immediately before induction of expression. Likewise, no such activities were detected in liposomes reconstituted with the four unrelated mitochondrial carriers Sfc1p, Ort1p, Sam5p, and Ggc1 (2), which had been expressed and purified from E. coli using the same expression vector.

To investigate the substrate specificity of SLC25A42 further, we measured the uptake of [14C]ADP into proteoliposomes that had been preloaded with a variety of potential substrates (Fig. 4). The highest activity of [14C]ADP uptake into proteoliposomes was found with internal AMP, ADP, ATP, dADP, PAP, CoA and dephospho-CoA (Fig. 4). To a lesser extent [14C]ADP also exchanged with internal dAMP, dATP, ADP-ribose, ADP-glucose, 3'-AMP, FAD and pyrophosphate, and to an even lesser extent with NAD+7. In contrast, the uptake of [14C]ADP was negligible in the absence of internal substrate (NaCl present) or in the presence of internal cAMP, adenosine, pantothenate, NMN, NADP+7, FMN, thiamine pyrophosphate, thiamine monophosphate, phosphate, sulfate, oxoglutarate, glutamate, ornithine (Fig. 4) and pyruvate, malate, citrate, aspartate, carnitine, lysine, reduced glutathione, tetrahydrofolate, S-adenosylmethionine, inosine monophosphate and inosine triphosphate (data not shown). Similar results were obtained by measuring the uptake of [14C]AMP instead of [14C]ADP into proteoliposomes preloaded with different substrates (data not shown). Therefore, the substrate specificity of SLC25A42 is confined essentially to adenine nucleotides, PAP, CoA and dephospho-CoA.
The effects of inhibitors of other mitochondrial carriers on the \[^{14}C\]ADP/ADP exchange reaction catalyzed by reconstituted SLC25A42 were also examined. The ADP/ADP exchange was inhibited markedly by pyridoxal-5’-phosphate, bathophenanthroline, tannic acid, bromocresol purple and mersalyl and only partially by p-hydroxymercuribenzoate, \(\alpha\)-cyano-4-hydroxycinnamate, butylmalonate, phenylsuccinate, 1,2,3-benzenetricarboxylate and bongkrekate (Fig. 5). In contrast, little effect was observed with \(N\)-ethylmaleimide and carboxyatractyloside. Interestingly, bongkrekic acid inhibited the activity of SLC25A42 partially (51%), whereas carboxyatractyloside had little effect (13%) at a concentration (10 \(\mu\)M) that completely inhibits the ADP/ATP carrier (23,24). In addition, the \[^{14}C\]ADP/ADP exchange in reconstituted liposomes was inhibited strongly by externally-added PAP, ADP, CoA and dephospho-CoA, to a lesser extent by AMP, ATP and dADP and only slightly by dAMP, dATP, ADP-ribose, ADP-glucose, 3’-AMP and FAD (Fig. 6).

**Kinetic characteristics of recombinant SLC25A42** - In Fig. 7, the kinetics are compared for the uptake by proteoliposomes of 40 \(\mu\)M \[^{14}C\]ADP either as exchange (in the presence of internal 2 mM CoA or dephospho-CoA) or as uniport (in the absence of internal substrate). The uptake of ADP by exchange followed a first order kinetics (rate constant, 0.04 and 0.03 min\(^{-1}\); initial rate, 42.7 and 55.6 mmol/min/g protein for the ADP/CoA and ADP/dephospho-CoA exchanges, respectively), isotopic equilibrium being approached exponentially (Fig. 7). In contrast, the uniport uptake of \[^{14}C\]ADP was negligible suggesting that SLC25A42 does not catalyze a unidirectional transport of ADP. The addition of 2 mM unlabeled CoA or dephospho-CoA after incubation for 100 min, when radioactive uptake by proteoliposomes had almost reached equilibrium, caused an extensive efflux of radiolabeled ADP. This efflux shows that the \[^{14}C\]ADP taken up by proteoliposomes is released in exchange for externally added CoA or dephospho-CoA. Therefore, CoA and dephospho-CoA are transported by reconstituted SLC25A42 not only when present inside liposomes but also when added externally. The possibility for SLC25A42 to function also as uniporter was further investigated by measuring the efflux of \[^{14}C\]ADP from prelabeled active proteoliposomes, as this provides a more sensitive assay for unidirectional transport (13). In the absence of external substrate, no significant efflux was observed even after incubation for 40 min (Fig. 8). However, upon addition of external PAP, ADP, CoA or dephospho-CoA an extensive efflux of radioactivity occurred, which was prevented by the presence of pyridoxal-5’-phosphate and bathophenanthroline. These results demonstrate that, at least under the experimental conditions employed, reconstituted SLC25A42 catalyzes an obligatory exchange reaction of substrates.

The kinetic constants of reconstituted SLC25A42 were determined by measuring the initial transport rate at various external \[^{14}C\]ADP concentrations in the presence of a fixed saturating concentration of internal PAP, ADP, CoA or dephospho-CoA (Table I). Several external substrates were competitive inhibitors of \[^{14}C\]ADP uptake as they increased the apparent \(K_m\) without changing the Vmax (not shown). The \(K_i\) values of these substrates for SLC25A42 are the following: 27 ± 3.5 \(\mu\)M (PAP), 54 ± 8 \(\mu\)M (ADP), 112 ± 10 \(\mu\)M (CoA), 108 ± 11 \(\mu\)M (dephospho-CoA), 383 ± 55 \(\mu\)M (AMP), 435 ± 51 \(\mu\)M (ATP), and 457 ± 62 \(\mu\)M (dADP). These results show that CoA and dephospho-CoA have a high affinity for SLC25A42; their \(K_i\) values are lower than those of AMP and ATP and 2-fold higher than that of ADP. Of note, the affinity of SLC25A42 for PAP is higher than that for any other substrate transported by this carrier. In addition, it is worth noting that the \(K_i\) value of CoA for SLC25A42 is similar to the physiological concentrations of cytosolic-free CoA, which in animal tissues can be estimated to range from 15 to 100 \(\mu\)M (25).

In view of the different charges carried out by the SLC25A42 substrates at physiological pH values, we thought to investigate whether the hetero-exchanges catalyzed by SLC25A42 are affected by the
membrane potential or if their charge imbalance is H⁺-compensated. To generate a K⁺ diffusion potential or a pH difference across the proteoliposomal membrane with the K⁺ uniporter valinomycin or the K⁺/H⁺ exchanger nigericin, respectively, we applied a K⁺ gradient (mM/mM; in/out or out/in) by adding 50 mM KCl to either side of the membrane in the presence of 1 mM KCl on the opposite side. Unfortunately, under both conditions (i.e., in the presence of 50 mM KCl inside or outside the proteoliposomes) the activity of SLC25A42 measured as ADP/ADP homo-exchange was either abolished with 50 mM K⁺ inside or drastically diminished (by about 90%) with 50 mM K⁺ outside; thus, the effect of valinomycin or nigericin could not be quantified. We were therefore technically unable to assess the effect of these ionophores on the kinetic constants of SLC25A42 or to answer the question as to whether SLC25A42 is electrogenic or electroneutral as was the case for the NAD⁺ carrier (26) but not for other reconstituted transporters (see, for example, Refs. 4,12,27,28).

**DISCUSSION**

In this work, the function of the SLC25A42 protein was investigated by direct transport assays upon expression in *E. coli*, purification and incorporation of the protein into phospholipid vesicles. The same strategy has in recent years enabled the definitive identification and detailed characterization of several novel mitochondrial carriers (see Refs. 1,2 and 30 for reviews).

The transport properties and kinetic characteristics of recombinant SLC25A42, together with its targeting to mitochondria, demonstrate that this protein is a mitochondrial transporter for CoA and PAP. To our knowledge, SLC25A42 is the first mitochondrial protein shown to be capable of transporting CoA and PAP. In the past, contrary to earlier observations (31-33), two studies have provided evidence for the existence of a CoA-transporting system into rat heart and potato mitochondria (34,35). In both cases, exogenous CoA was taken up by isolated mitochondria with saturable kinetics and in a manner sensitive to temperature. As yet, there is no proof that SLC25A42 is closely related to the transporters for CoA detected in rat heart and potato mitochondria which have not been analyzed by protein sequencing, although this is probably the case.

The closest relatives of SLC25A42 in yeast are YPR011c (29% identity and 41% similarity) and Leu5p (25% identity and 42% similarity), and in man are SLC25A16 (34% identity and 46% similarity) and the four isoforms of the ATP-Mg/Pi carrier (28-32% identity and 48-53% similarity). Nothing is known about YPR011c. On the other hand, a role was assigned to Leu5p in the cellular distribution of CoA (29). More precisely, it was proposed that either CoA, or a precursor of CoA, is the substrate of Leu5p based on the decreased CoA level in mitochondria and decreased activity of mitochondrial CoA-dependent enzymes in ΔLEU5 cells (29). Moreover, SLC25A16, previously known as Graves’ disease carrier (36), was thought to be the human ortholog of Leu5p (29). The direct demonstration that SLC25A42 transports CoA provides strong support to the evidence...
whereby Leu5p is a CoA carrier and makes it unlikely that Leu5p is a carrier for pantothenate-like CoA precursors. It may be that also YPR011c and SLC25A16 are capable of transporting CoA. However, it is worth noting that SLC25A42 and SLC25A16 are not isoforms of the same carrier since the isoforms of several mitochondrial transporters, which have been characterized so far in man, exhibit an identity not less than 55% (1,4). On this basis, it is likely that SLC25A42, SLC25A16, Leu5p and YPR011c may display differences in the substrates they transport. Indeed, surprisingly, we have shown that SLC25A42 catalyzes a highly efficient transport of PAP which is produced from CoA in the reaction of phosphopentetheinylation of acyl carrier protein (ACP) mediated by the mitochondrial 4’-phosphopantetheinyl transferase.

Apart from the relatively low homology that SLC25A42 displays with Leu5p, YPR011c and SLC25A16 (25-34% identical amino acids), SLC25A42 does not exhibit significant sequence homology with any other known mitochondrial carrier greater than the homology existing among the different members of the SLC25 family. However, several protein sequences available in data bases are likely to be orthologs of SLC25A42 in other organisms. These sequences include: XP_580755 from B. taurus (92% identity); XP_852174 from C. familiaris (92%); NP_001007571 from M. musculus (91%); XP_424684 from G. gallus (73%); NP_001038918 from D. rerio (71%); NP_001088738 from X. laevis (70%); CAG04865 from T. nigroviridis (66%); NP_732519 from D. melanogaster (49%); XP_311055 from A. gambiae (48%); NP_492333 from C. elegans (47%); XP_396993 from A. mellifera (45%); XP_001897134 from B. malayi (44%); XP_001649538 from A. aegypti (43%); XP_001945408 from A. pisum (42%); and XP_822738 from T. brucei (40%). To our knowledge, none of these proteins has been characterized biochemically.

Besides CoA, SLC25A42 transports dephospho-CoA, PAP and (deoxy)adenine nucleotides. The substrate specificity of SLC25A42 is distinct from that of any other previously characterized member of the mitochondrial carrier family including the nucleotide transporters. Interestingly, adenine nucleotides but not pyrimidine nucleotides are transported by SLC25A42, in agreement with the fact that this carrier displays a higher homology with the ATP-Mg/Pi and the ADP/ATP carrier isoforms than with PNC1 (SLC25A33), the human pyrimidine nucleotide transporter (7), and its closest relative SLC25A36 (17.9 and 17.6% identical amino acids, respectively).

Because in man the biosynthesis of CoA takes place entirely outside the mitochondrial matrix (25,37,38), a primary function of SLC25A42 is to transport CoA into the mitochondria. In the mitochondrial matrix CoA has an essential role in major processes occurring in the organelles, such as the β-oxidation of fatty acids, the tricarboxylic acid cycle at the level of pyruvate and α-oxoglutarate dehydrogenases, the biosynthesis of heme at the level of δ-aminolevulinate synthase, branched-chain amino acid catabolism, the urea cycle at the level of acetylglutamate synthase and protein acetylation. In addition, inside the mitochondria of mammals and other organisms the phosphopantetheine moiety of CoA is transferred to ACP with production of PAP (39-41). ACP is a key component of type II fatty acid synthase which is present in the matrix (39 and references therein) and whose role is to provide octanoate, the precursor of lipoic acid (another basic cofactor of aerobic metabolism) and long-chain fatty acids that play an essential role in mitochondrial function. Since SLC25A42 functions exclusively by a counter-exchange mechanism, the carrier-mediated uptake of CoA requires efflux of a counter-substrate. Our transport measurements in reconstituted liposomes indicate that adenine nucleotides and PAP are the most likely counter-substrates of SLC25A42 for CoA. Among the adenine nucleotides, the best candidates to exchange with external CoA are ADP, which is one of the preferred reactants of SLC25A42, and to a lesser extent ATP, which is more abundant than AMP in the mitochondrial matrix (42,43). It is probable that under some conditions
SLC25A42 can substitute the ADP/ATP carrier. In the AAC1 knock-out mice (44) and in the human disease named AAC1 deficiency (45), which manifest exercise intolerance, lactic acidosis and hypertrophic cardiomyopathy, as well as in mice lacking their two AAC isoforms in liver (46), loss of AAC function is compatible with adult life. The possibility exists for lack of AAC activity to be compensated in part by SLC25A42 activity.

Aside from CoA, some dephospho-CoA might also be imported into the matrix where it would be converted to CoA by the dephospho-CoA kinase found to be associated to mitochondria in *S. cerevisiae* (47). Another possibility is that CoA is imported, and inside the matrix a CoA phosphatase would convert CoA to dephospho-CoA which would serve as a counter-substrate for the uptake of CoA by SLC25A42. In this case, we would have a combination of SLC25A42-mediated uptake of CoA in exchange for adenine nucleotides, PAP and dephospho-CoA. Given that the concentration of dephospho-CoA in the cytosol is not known and uncertainties still exist as to whether a dephospho-CoA kinase and/or a CoA phosphatase is present in the mitochondrial matrix, these possibilities require further investigation.

**REFERENCES**


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This paper is dedicated to Prof. Martin Klingenberg on the occasion of his 80th birthday.

FIGURE LEGENDS
Fig. 1. Subcellular localization of SLC25A42. CHO cells were transiently co-transfected with pcDNA3 vector carrying the DNA sequence coding SLC25A42 in frame with the GFP DNA sequence and pcDNAI plasmid carrying the DNA sequence of mitochondrially targeted BFP (see experimental procedures). Images were acquired by a fluorescence microscope equipped with appropriate filters, and identical fields are presented. SLC25A42-GFP, fluorescence of GFP fused to SLC25A42; mt-BFP, fluorescence of the mtBFP (here shown in red); overlay, merged image of mtBFP fluorescence with SLC25A42-GFP fluorescence.

Fig. 2. Expression in Escherichia coli and purification of SLC25A42. Proteins were separated by SDS-PAGE and stained with Coomassie Blue. Markers in the left-hand column (bovine serum albumin, carbonic anhydrase and cytochrome c); lanes 1-4, E. coli CO214(DE3) containing the expression vector without (lanes 1 and 3) and with (lanes 2 and 4) the coding sequence of SLC25A42. Samples were taken at the time of induction (lanes 1 and 2) and 5 h later (lanes 3 and 4). The same number of bacteria was analyzed in each sample. Lane 5, purified SLC25A42 protein (5 µg) derived from bacteria shown in lane 4.

Fig. 3. Homo-exchanges of nucleotides in proteoliposomes containing recombinant SLC25A42. Transport was initiated by adding radioactive substrate (final concentration, 0.2 mM) to proteoliposomes preloaded internally with the same substrate (concentration, 10 mM). The reaction was terminated after 30 min. Values are means ± S.D. of at least three independent experiments.

Fig. 4. Substrate specificity of SLC25A42. Liposomes reconstituted with SLC25A42 were preloaded internally with various substrates (concentration, 10 mM). Transport was started by addition of 60 µM [14C]ADP and terminated after 2 min. Values are means ± S.D. of at least three independent experiments. ADP-rib, ADP-ribose; ADP-gluc, ADP-glucose; PAP, adenosine 3',5'-diphosphate; Adenos, adenosine; CoA, coenzyme A; dPCoA, dephospho-CoA; Pan, pantothenate; ThPP, thiamine pyrophosphate; ThMP, thiamine monophosphate; PPI, pyrophosphate; OG, oxoglutarate; Glu, glutamate; Orn, ornithine.

Fig. 5. Effect of inhibitors on the [14C]ADP/ADP exchange by SLC25A42. Proteoliposomes were preloaded internally with 10 mM ADP and transport was initiated by adding 40 µM [14C]ADP to liposomes reconstituted with SLC25A42. The incubation time was 2 min. Thiol reagents and α-cyanocinnamate were added 2 min before the labeled substrate. The final concentrations of the inhibitors were 10 µM (CAT, carboxyatractyloside; BKA, bongkrekic acid), 20 mM (PLP, pyridoxal-5'-phosphate; BAT, bathophenanthroline), 0.2 mM (pHMB, p-hydroxymercuribenzoate; MER, mersalyl), 1 mM (NEM, N-ethylmaleimide; CCN, a-cyanocinnamate) and 0.1% (TAN, tannic acid) and 0.1 mM (BrCP, bromocresol purple). The values are expressed as a percentage of the [14C]ADP/ADP exchange activity, which was 98 µmol/min x g of protein. Similar results were obtained in at least three independent experiments.

Fig. 6. Inhibition of the rate of [14C]ADP/ADP exchange by external substrates. Liposomes reconstituted with SLC25A42 were preloaded internally with 10 mM ADP. Transport was started by adding 40 µM [14C]ADP and stopped after 2 min. External substrates (concentration, 0.4 mM) were added together with [14C]ADP. The values are expressed as a percentage of the [14C]ADP/ADP exchange activity, which was 111 µmol/min x g of protein. Similar results were obtained in at least three independent experiments. ADP-rib, ADP-ribose; ADP-gluc, ADP-glucose; PAP, adenosine 3',5'-diphosphate; Adenos, adenosine; CoA, coenzyme A; dPCoA, dephospho-CoA; PPI, pyrophosphate; ThPP, thiamine pyrophosphate; ThMP, thiamine monophosphate.

Fig. 7. Time-course of reconstituted [14C]ADP/CoA and [14C]ADP/dephospho-CoA exchanges and efflux of [14C]ADP from proteoliposomes after addition of unlabeled CoA or dephospho-CoA. 40 µM [14C]ADP was added to liposomes reconstituted with SLC25A42 and preloaded internally with 2 mM CoA or dephospho-CoA (exchange, ▲ and ■, respectively) or 2 mM NaCl and no substrate (uniport, ○). The arrow indicates the addition of 2 mM nonradioactive CoA (△) or dephospho-CoA (□). Similar results were obtained in three independent experiments.

Fig. 8. Efflux of [14C]ADP from proteoliposomes reconstituted in the presence of 2 mM ADP. The internal substrate pool was labeled with [14C]ADP by carrier-mediated exchange equilibrium. Then the
proteoliposomes were passed through Sephadex G-75. The efflux of $[^{14}\text{C}]}\text{ADP}$ was started by adding buffer A alone (■), 0.5 mM PAP in buffer A (◆), 0.5 mM ADP in buffer A (○), 0.5 mM CoA in buffer A (□) or 0.5 mM dephospho-CoA in buffer A (●). Similar results were obtained in three independent experiments.

Fig. 9. Effect of complementing ΔLEU5 cells with human SLC25A42 and SLC25A10 proteins on growth. Four-fold serial dilutions of wild-type (WT), ΔLEU5 cells and ΔLEU5 cells transformed with the SLC25A42-pYES, SLC25A10-pYES or LEU5-pRS plasmid (ΔLEU5 + A42, ΔLEU5 + A10 or ΔLEU5 + LEU5, respectively) were plated on YP medium supplemented with glycerol (A), lactate (B), or acetate (C) for 72 hours at 30°C. In (A) and (B) the plates contained additional 0.2% galactose and the pYES plasmids the coding sequence of SLC25A42 or SLC25A10 under the control of the GAL10 promoter; in (C) the pYES plasmid contained the coding sequence of SLC25A42 under the control of the MIR1 promoter.
Table I. Kinetic parameters of reconstituted SLC25A42. Km and Vmax values were calculated from double-reciprocal plots of the rates of [14C]ADP uptake at various external ADP concentrations into proteoliposomes containing the indicated substrates (2 mM). The data represent the means ± S.D. of at least four independent experiments.

<table>
<thead>
<tr>
<th>external substrate</th>
<th>internal substrate</th>
<th>Vmax (µmol/min/g protein)</th>
<th>Km (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C]ADP</td>
<td>ADP</td>
<td>267 ± 47</td>
<td>55 ± 8</td>
</tr>
<tr>
<td>[14C]ADP</td>
<td>PAP</td>
<td>333 ± 50</td>
<td>50 ± 7</td>
</tr>
<tr>
<td>[14C]ADP</td>
<td>CoA</td>
<td>116 ± 32</td>
<td>71 ± 10</td>
</tr>
<tr>
<td>[14C]ADP</td>
<td>dephospho-CoA</td>
<td>129 ± 25</td>
<td>64 ± 9</td>
</tr>
</tbody>
</table>
Figure 8

Efflux of [14C] ADP (cpm x 10^3)

- Time (min)
Figure 9

A

WT

ΔLEU5

ΔLEU5 + A42

ΔLEU5 + A10

B

WT

ΔLEU5

ΔLEU5 + A42

ΔLEU5 + A10

ΔLEU5 + LEU5

C

WT

ΔLEU5

ΔLEU5 + A42

ΔLEU5 + LEU5
A novel member of solute carrier family 25 (SLC25A42) is a transporter of coenzyme a and adenosine 3',5'-diphosphate in human mitochondria

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