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The peroxisomal NAD^+ carrier of *Arabidopsis thaliana* transports coenzyme A and its derivatives

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Abstract The peroxisomal protein PXN encoded by the Arabidopsis gene At2g39970 has very recently been found to transport NAD^+, NADH, AMP and ADP. In this work we have reinvestigated the substrate specificity and the transport properties of PXN by using a wide range of potential substrates. Heterologous expression in bacteria followed by purification, reconstitution in liposomes, and uptake and efflux experiments revealed that PNX transports coenzyme A (CoA), dephospho-CoA, acetyl-CoA and adenosine 3′, 5′-phosphate (PAP), besides NAD^+, NADH, AMP and ADP. PXN catalyzed fast counter-exchange of substrates and much slower uniport and was strongly inhibited by pyridoxal 5′-phosphate, bathophenanthroline and tannic acid. Transport was saturable with a submillimolar affinity for NAD^+, CoA and other substrates. The physiological role of PXN is probably to provide the peroxisomes with the essential coenzymes NAD^+ and CoA.

Keywords *Arabidopsis thaliana* · At2g39970 protein · Coenzyme A transporter · Membrane transport · Mitochondrial carrier · Peroxisome

Abbreviations

CoA	coenzyme A
FMN	flavine mononucleotide
NMN	nicotinamide mononucleotide
PAP	adenosine 3′,5′-diphosphate
PXN	peroxisomal NAD^+ carrier

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Introduction

In both plants and mammals several cofactors such as NAD^+, FAD and CoA are synthesized outside the peroxisomes and must be imported into the peroxisomal matrix where they are essential for important processes. The *Arabidopsis thaliana* gene At2g39970 encodes a member of the mitochondrial carrier family that is localized in the peroxisomal membrane (Eubel et al. 2008; Reumann et al. 2009; Bernhardt et al. 2012). For a long time, the At2g39970 protein was thought to transport ATP into peroxisomes (Fukao et al. 2001), an assumption that was ruled out in 2008 (Linka et al. 2008). Recently, by means of uptake studies in liposomes reconstituted with the recombinant protein, Bernhardt et al. (2012) have shown that the At2g39970 protein, named PXN, is a peroxisomal NAD^+ transporter. The same authors found that, in addition to NAD^+, PXN transports AMP, ADP and NADH. Furthermore, consistently with the hypothesis that PXN provides peroxisomal β-oxidation with NAD^+, a retention of oil bodies and a delay in the degradation of storage oil-derived fatty acids was observed in Arabidopsis *pxn* null mutant seedlings (Bernhardt et al. 2012). In addition, another study (Mano et al. 2011) in which the Arabidopsis mutant for At2g39970 (APEM3) was also characterized concluded that the At2g39970 protein, called PMP38, plays an important role in peroxisomal proliferation. The closest relative of At2g39970 in humans is SLC25A17. We have recently found that SLC25A17 is a transporter of CoA, FAD and, to a lesser extent, NAD^+ (Agrimi et al. 2012).

In this work we have reinvestigated the substrate specificity of the At2g39970 protein, named PXN according to Bernhardt et al. (2012), using a wide range of potential substrates. PXN

was overexpressed in *Escherichia coli*, purified, reconstituted in phospholipid vesicles and shown to transport CoA, dephospho-CoA, acetyl-CoA and adenosine 3′,5′-diphosphate (PAP), besides NAD^+, AMP, ADP and NADH.

Materials and methods

Sequence search and analysis

Protein databases (http://plants.ensembl.org and www.ncbi.nlm.nih.gov) were screened with the protein sequence of PXN (Accession Number NP_181526.1) using BLASTP. Multiple sequence alignments of amino acid sequences from PXN and its plant homologues available at Aramemnon (http://aramemnon.botanik.uni-koeln.de/) were obtained using ClustalW *X*2 .

Construction of the expression plasmid

The coding sequence of At2g39970 (NM_129555) was amplified from flower cDNA by PCR using primers corresponding to the extremities of the coding sequence with additional BamHI and XhoI sites. The product was cloned into the pET-21b vector and the construct was transformed into *E. coli* TOP 10 cells. Transformants were selected on 2X TY (16 g/l tryptone, 10 g/l yeast extract, 5 g/l NaCl, pH 7.4) plates containing ampicillin (100 μg/ml) and screened by direct colony PCR. The sequence of the insert was verified.

Bacterial expression and purification

The protein was overproduced as inclusion bodies in the cytosol of *E. coli* strain BL21(DE3) (Fiermonte et al. 1993; Palmieri et al. 2006b; Lindhurst et al. 2006). Control cultures with the empty vector were processed in parallel. Inclusion bodies were purified on a sucrose density gradient and washed at 4 °C, first with Tris-EDTA buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.0), then twice with a buffer containing Triton X-114 (3 %, w/v), 1 mM EDTA, 20 mM Na_2SO_4 and 10 mM PIPES pH 7.0, and finally with the Tris-EDTA buffer pH 7.0 (Agrimi et al. 2004). The proteins were solubilized in 1.2 % sarkosyl (w/v). Eventual small residues were removed by centrifugation (20800 × g for 10 min at 4 °C).

Reconstitution of the recombinant PXN into liposomes

The recombinant protein in sarkosyl was reconstituted by cyclic removal of detergent as previously described (Palmieri et al. 1995; Hoyos et al. 2003) with some modifications. The reconstitution mixture consisted of protein solution (100 μl, about 6 μg), 10 % Triton X-114 (85 μl), 5 mM substrate except where otherwise indicated, cardiolipin (0.4 mg), 20 mM PIPES-NaOH pH 7.0 and water (final volume 700 μl). The mixture was recycled 13-fold through an Amberlite column pre-equilibrated with 20 mM PIPES-NaOH (pH 7.0) and substrate at the same concentration as in the reconstituted mixture. All operations were performed at 4 °C except the passages through Amberlite, which were carried out at room temperature.

Transport assays

External substrate was removed from proteoliposomes on Sephadex G-75 columns pre-equilibrated with 50 mM NaCl and 10 mM PIPES at pH 7.0 (buffer A). Transport at 25 °C was started by adding [^{3}H]NAD^+ (from PerkinElmer) or [^{14}C]AMP (from ARC) to proteoliposomes and terminated, after the indicated time, by addition of 20 mM pyridoxal 5′-phosphate and 20 mM of bathophenanthroline. In controls, inhibitors were added with the labeled substrate (Palmieri et al. 2008). The external substrate was removed by Sephadex G-75 columns pre-equilibrated with buffer A, and the entrapped radioactivity was counted (Palmieri et al. 1995). The experimental values were corrected by subtracting control values. The initial transport rate was calculated from the substrate taken up by proteoliposomes after 2 min (in the initial linear range of substrate uptake). For efflux measurements, proteoliposomes containing 2 mM NAD^+ or AMP were labeled with 5 μM [^{3}H]NAD^+ or [^{14}C]AMP, respectively, by carrier-mediated exchange equilibration (Marobbio et al. 2003; Fiermonte et al. 2009). After 45 min, external radioactivity was removed by passing the proteoliposomes through Sephadex G-75 columns. Efflux was started by adding unlabeled external substrate or buffer A alone and terminated by adding the inhibitors indicated above.

Other methods

Proteins were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and stained with Coomassie Blue dye. The identity of purified PXN was assessed by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry of trypsin digests of the corresponding band excised from a Coomassie-stained gel (Fiermonte et al. 2001). The amount of pure PXN was estimated by laser densitometry of stained samples, using carbonic anhydrase as protein standard (Palmieri et al. 2001a). The amount of protein incorporated into liposomes (about 20 % of the protein added to the reconstitution mixture) was measured as described (Fiermonte et al. 2003).

Results

Bacterial expression of PXN

The At2g39970 gene was expressed in *E. coli* BL21(DE3) (Fig. 1, lane 4). The gene product accumulated as inclusion

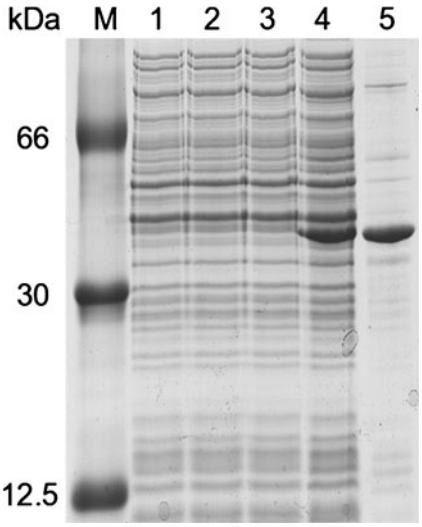

Fig. 1 Expression in *E. coli* and purification of PXN. Proteins were separated by SDS-PAGE and stained with Coomassie Blue dye. Markers in the left-hand column (bovine serum albumin, carbonic anhydrase and cytochrome *c*); lanes 1–4, *E. coli* BL21(DE3) containing the expression vector without (lanes 1 and 3) and with (lanes 2 and 4) the coding sequence of PXN. Samples were taken at the time of induction (lanes 1 and 2) and 4 h later (lanes 3 and 4). The same number of bacteria was analyzed in each sample. Lane 5, purified PXN protein (7 μg) derived from bacteria shown in lane 4

bodies and was purified by centrifugation and washing (Fig. 1, lane 5). The apparent molecular mass of the recombinant protein was approximately 36.5 kDa (the calculated value with the initiator methionine was 36213 Da). Its identity was confirmed by MALDI-TOF mass spectrometry, and the yield of the purified protein was approximately 15 mg per liter of culture. The protein was not detected in bacteria harvested immediately before induction of expression (Fig. 1, line 2) or in cells harvested after induction but lacking the coding sequence in the expression vector (Fig. 1, line 3).

Functional characterization of PXN

Recombinant, purified PXN was reconstituted into liposomes and its transport activity for a variety of potential substrates was tested. In Fig. 2 the initial rates of uptake of [^{3}H]NAD$^+$ and [^{14}C]AMP were measured in liposomes reconstituted with PXN and preloaded with many different substrates. The highest activities of [^{3}H]NAD$^+$ and [^{14}C]AMP uptake into proteoliposomes were found with internal AMP, ADP, NAD$^+$, NADH, CoA and dephospho-CoA. To a lesser extent, [^{3}H]NAD$^+$ and [^{14}C]AMP also exchanged with internal acetyl-CoA, PAP and FAD. By contrast, the uptake of both radioactives was very small with ATP, NMN, NADP$^+$, FMN, propionyl-CoA, cAMP, NaCl (Fig. 2) and phosphate, pyrophosphate, malate, oxoglutarate, citrate, carnitine, aspartate, glutamate, arginine, lysine, adenosine, adenosylmethionine, thiamine mono- and diphosphate, folate, GMP, GDP, GTP, CMP, CDP, CTP, UMP, UDP, UTP, TMP, TDP, TTP, nicotinamide, α-NAD$^+$, NADPH, ADP-ribose and pantothenic acid (data not shown). Furthermore, no [^{3}H]NAD$^+$/NAD$^+$ and [^{14}C]AMP/AMP exchange activities were detected when PXN 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 PXN or harvested immediately before induction of expression.

The effects of inhibitors of other mitochondrial carriers on the [^{3}H]NAD$^+$/NAD$^+$ exchange reaction catalyzed by reconstituted PXN were also examined. The NAD$^+$/NAD$^+$ exchange was inhibited nearly completely by pyridoxal-5′-phosphate, bathophenanthroline and tannic acid, strongly by mersalyl, mercuric chloride and bromocresol purple, and only poorly by *p*-hydroxymercuribenzoate, *N*-ethylmaleimide and α-cyano-4-hydroxycinnamate (Fig. 3). In contrast, little effect was observed with butylmalonate, 1,2,3-benzenetricarboxylate and the two powerful inhibitors of the ADP/ATP carrier, carboxyatractyloside and bongkrekic acid.

Kinetic characteristics of recombinant PXN

The time-course was compared for the uptake of [^{3}H]NAD$^+$ or [^{14}C]AMP into reconstituted liposomes measured either as uniport (in the absence of internal substrate) or as exchange (in the presence of one of the indicated substrates) (Fig. 4). The uptake of both [^{3}H]NAD$^+$ and [^{14}C]AMP by exchange with internal NAD$^+$, AMP, CoA, PAP, acetyl-CoA or FAD substantially increased with time, quasi-equilibrium being approached within 60 min incubation. In comparison, the uniport uptake of [^{3}H]NAD$^+$ and [^{14}C]AMP was very low as it was with internal NMN (Fig. 4).

The mode of the PXN-mediated transport was further investigated by measuring the efflux of [^{3}H]NAD$^+$ or [^{14}C]AMP from proteoliposomes preloaded with these compounds. This experimental approach provides a more sensitive assay for unidirectional transport (Palmieri et al. 1995). In the absence of external substrates a low efflux of both radioactives from proteoliposomes was observed which was prevented completely by the presence of the inhibitors bathophenanthroline and pyridoxal 5′-phosphate (Fig. 5). In contrast, the addition of NAD$^+$, AMP, CoA, PAP, acetyl-CoA and dephospho-CoA caused a substantial efflux of radiolabeled NAD$^+$ or AMP. This efflux was also impeded completely by the same inhibitors (data not shown). Notably, upon addition of external FAD only a marginal efflux of radioactivity occurred as compared to that observed in the absence of external substrates (Fig.5). These results indicate that under the experimental conditions used i) reconstituted PXN is able to catalyze a low unidirectional transport besides a fast counter-exchange reaction of substrates, and ii) NAD$^+$, AMP, CoA, PAP, acetyl-CoA and dephospho-CoA are transported by

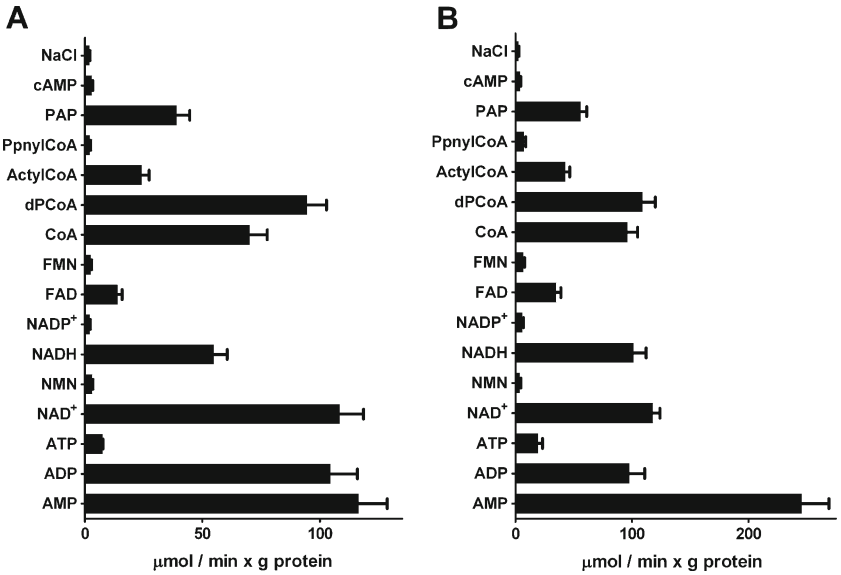

Fig. 2 Dependence of PXN activity on internal substrate. Liposomes were reconstituted with PXN and preloaded internally with various substrates or NaCl (concentration, 5 mM). Transport was initiated by adding 125 μM [^{3}H]NAD$^+$ in A and 150 μM [^{14}C]AMP in B. The reaction time was 2 min. Results are means±S.E. for at least three independent experiments. Abbreviations: NMN, nicotinamide mononucleotide; FMN, flavine mononucleotide; dPCoA, dephospho-CoA; ActylCoA, acetyl-CoA; PpnylCoA, propionyl-CoA; PAP, adenosine 3′,5′-diphosphate

PXN not only when present inside liposomes but also when added externally.

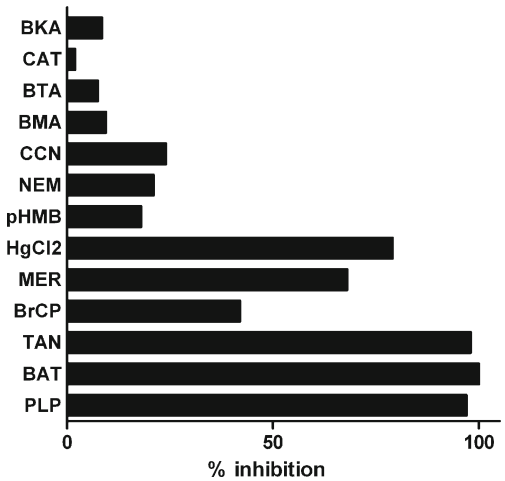

Fig. 3 Effect of inhibitors on the [^{3}H]NAD$^+$/NAD$^+$ exchange by reconstituted PXN. Proteoliposomes were preloaded internally with 5 mM NAD$^+$. Transport was initiated by adding 125 μM [^{3}H]NAD$^+$ to proteoliposomes and terminated after 2 min. Thiol reagents were added 2 min before the labeled substrate; the other inhibitors were added together with the labelled substrate. The final concentrations of the inhibitors were 10 mM (PLP, pyridoxal 5′-phosphate; BAT, bathophenanthroline), 0.2 % (TAN, tannic acid), 0.2 mM (BrCP, bromocresol purple; MER, mersalyl; $HgCl_2$, mercuric chloride; p-HMB, p-hydroxymercuribenzoate), 1 mM (NEM, N-ethylmaleimide, CCN, α-cyanocinnamate), 2 mM (BMA, butylmalonate; BTA, 1,2,3-benzenetricarboxylate), and 10 μM (CAT, carboxyatractyloside; BKA bongkrekic acid). The extents of inhibition (%) from a representative experiment are given. Similar results were obtained in at least three experiments

The kinetic constants of recombinant PXN were determined from double-reciprocal plots of the rates of [^{3}H]NAD$^+$ or [^{14}C]AMP uptake at various external NAD$^+$ or AMP concentrations into proteoliposomes containing 10 mM NAD$^+$ or AMP. The half-saturation constant (K_m) and specific activity (V_{max}) values at 25 °C were 102.5±13.2 μM and 166.6±33.4 μmol/min/g protein for the NAD$^+$/NAD$^+$ exchange and 119.2±9.6 μM and 416.0±66.5 μmol/min/g protein for the AMP/AMP exchange, respectively. The V_{max} of the AMP/AMP exchange was therefore more than 2-fold greater than that of the NAD$^+$/NAD$^+$ exchange, whereas the K_m values of PXN for external NAD$^+$ and AMP were rather similar. The inhibition constants (Ki) of competitive inhibitors of the NAD$^+$/NAD$^+$ exchange were the following: CoA 108±9 μM, dephospho-CoA 76±10 μM, ADP 146±18 μM, PAP 291±36 μM and acetyl-CoA 589±83 μM.

Discussion

The At2g39970 protein, named PXN, belongs to a very large family of solute carriers called the mitochondrial carrier family, which includes 53 members in *H. sapiens*, 58 in *Arabidopsis thaliana* and 35 in *Saccharomyces cerevisiae* (see Palmieri et al. 2006a; Palmieri et al. 2011; Palmieri 2012 for reviews). Each of these functionally characterized carriers transports a diverse set of substrates, some of which are accepted by more than one carrier. Therefore, complete mitochondrial carrier substrate specificity is difficult to determine without comprehensive experimentation using a

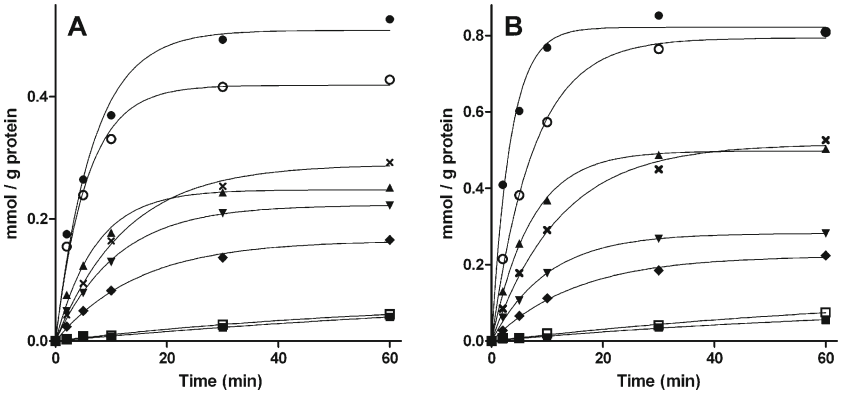

Fig. 4 Uptake of [^{3}H]NAD$^+$ or [^{14}C]AMP into liposomes reconstituted with PXN. 125 μM [^{3}H]NAD$^+$ in A or 150 μM [^{14}C]AMP in B was added to proteoliposomes preloaded internally with 5 mM NAD$^+$ (○), 5 mM AMP (●), 5 mM CoA (▲), 5 mM PAP (**X**), 5 mM acetyl-CoA (▼), 5 mM FAD (♦), 5 mM NMN (□) or 5 mM NaCl (■). Data from representative experiments are given. Similar results were obtained in three independent experiments

wide range of potential substrates. The direct transport measurements in liposomes reconstituted with PXN, reported in this work, demonstrate that recombinant PXN is able to transport NAD$^+$, NADH, CoA, dephospho-CoA, AMP, ADP and, to a lesser extent, acetyl-CoA and PAP. Notably none of the many other compounds tested, including thiamine diphosphate and nucleotides of the bases G, C, U and T, is transported to any significant extent. To our knowledge, PXN is the first plant peroxisomal carrier shown to be capable of transporting CoA and some of its derivatives. Furthermore, our data confirm the results of Bernhardt et al. (2012) regarding NAD$^+$, NADH, AMP and ADP. They also agree with the conclusion of Bernhardt et al. (2012) that FAD is not a good substrate for PXN. Indeed, despite the fact that intraliposomal FAD promotes uptake of externally added NAD$^+$ and AMP in time, the low initial rates of the FAD/[^{3}H]NAD$^+$ and FAD/[^{14}C]AMP exchanges and the inability of FAD to cause efflux of NAD$^+$ and AMP from proteoliposomes clearly indicate that FAD is not efficiently transported by PXN.

Because NAD$^+$ and CoA are produced outside proteoliposomes, the main function of PXN is to catalyze the uptake of NAD$^+$ (as discussed by Bernhardt et al. (2012)) and CoA into peroxisomes. In the peroxisomal matrix of Arabidopsis CoA functions as a substrate of two acyl-CoA synthetases, LACS6 and LACS7 (Fulda et al. 2002), three 3-keto-acyl-CoA thiolases (Carrie et al. 2007) and an acetoacetyl-CoA thiolase (Carrie et al. 2007). It is interesting that Arabidopsis null mutants for both LACS6 and LACS7 or for one of the three 3-keto-acyl-CoA thiolases (*kat2*) display defects in the degradation of fatty acids and in germination (Fulda et al. 2004; Germain et al. 2001), which resemble those observed in the *pxn* null mutant (Bernhardt et al. 2012; Mano et al. 2011). Aside from CoA, some dephospho-CoA might also be imported into the peroxisomal matrix where it would be converted to CoA

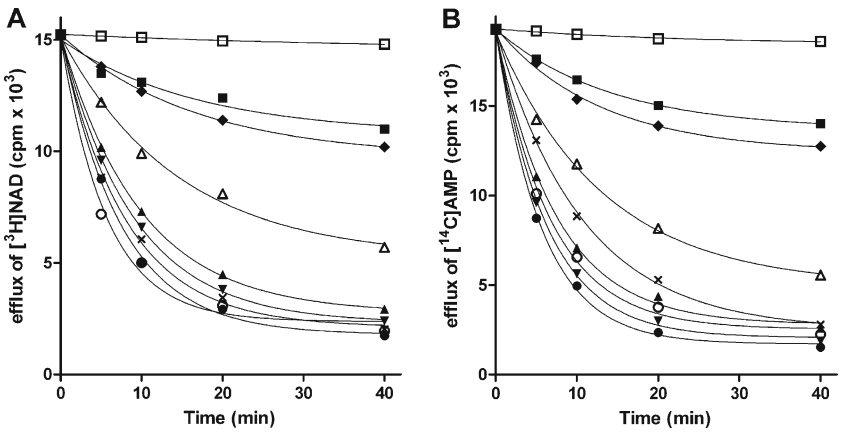

Fig. 5 Efflux of [^{3}H]NAD$^+$ or [^{14}C]AMP from liposomes reconstituted with PXN. The internal substrate pool was labeled with [^{3}H]NAD$^+$ in A or [^{14}C]AMP in B by carrier-mediated exchange equilibration. Then the proteoliposomes were passed through Sephadex G-75. The efflux of [^{3}H]NAD$^+$ or [^{14}C]AMP was started by adding buffer A alone (■), 5 mM NAD$^+$ (○), 5 mM AMP (●), 5 mM CoA (▲), 5 mM PAP (**X**), 5 mM acetyl-CoA (▼), 5 mM dephospho-CoA (△), 5 mM FAD (♦), 20 mM pyridoxal-5′-phosphate and 20 mM bathophenanthroline (□) in buffer A. Data from representative experiments are given. Similar results were obtained in three independent experiments

by the dephospho-CoA kinase found to be associated to Arabidopsis peroxisomes but not shown to be in their matrix (Reumann et al. 2009). For the exchange mode of transport, our measurements in reconstituted liposomes suggest that AMP, produced in the peroxisomal matrix by LACS6 and LACS7, is a good counter-substrate of PXN for CoA. Acetyl-CoA, the end-product of fatty acid oxidation in peroxisomes, believed to exit via citrate synthase and a yet unidentified citrate transporter (Pracharoenwattana et al. 2005), could also be exported, at least in part, in exchange for CoA. In addition, dephospho-CoA and PAP could also serve as counter-substrates of CoA by PXN if CoA phosphatase and Nudix hydrolases (which produce PAP from CoA and CoA-derivatives) are present in the peroxisomal matrix. Given that uncertainties still exist as to whether these enzymes are located inside Arabidopsis peroxisomes, the latter possibilities require further investigation. Finally, the rate of the PXN-mediated exchange is much higher than that of uniport. However, the uniport reaction can be essential under special conditions, for example when cells divide, or in specific metabolic states.

In a phylogenetic tree of *H. sapiens, S. cerevisiae* and *A. thaliana* mitochondrial carriers (Palmieri et al. 2011), PXN clusters with the carriers for NAD^+ (Todisco et al. 2006; Palmieri et al. 2009), pyrimidine nucleotides (Marobbio et al. 2006; Floyd et al. 2007), FAD/folate (Tzagoloff et al. 1996; Titus and Moran 2000; Bedhomme et al. 2005), peroxisomal Ant1p (Palmieri et al. 2001b) and its homologs At3g05290 and At5g27520 (Linka et al. 2008), and peroxisomal SLC25A17 (Agrimi et al. 2012). With all these transporters, except yeast Ant1p and its homologs in Arabidopsis, PXN shares the distinct feature of having a tryptophan instead of an acidic residue in the mitochondrial carrier signature motif PX(D/E)XX(R/K) present in the second repeat of these proteins (Cappello et al. 2007; Fig. 2 of Palmieri et al. 2011; Lawrence et al. 2011). Nevertheless, the substrate specificity of PXN is distinct from that of any other carrier present in the above-mentioned cluster, although some overlapping functions (i.e., substrates transported) occur, as well as from that of any other previously characterized members of the mitochondrial carrier family (Palmieri and Pierri 2010a; Palmieri et al. 2011; Palmieri 2012). PXN is also different from its closest relative in humans, i.e., the peroxisomal SLC25A17. Indeed, PXN tranports NAD^+ and CoA efficiently and FAD poorly, whereas SLC25A17 transports CoA and FAD efficiently and NAD^+ poorly (Agrimi et al. 2012).

Being three-fold pseudo-symmetric, mitochondrial carriers display symmetry-related triplets of amino acids when their three repeat sequences are aligned (Robinson et al. 2008; Palmieri and Pierri 2010b; Palmieri et al. 2011). Furthermore, the great majority of amino acids which have been found essential for function particularly by site-directed mutagenesis studies (Heidkämper et al. 1996; Briggs et al. 1999; Echtay et al. 2001; Stipani et al. 2001; Indiveri et al. 2002; Tonazzi et al. 2005; De Lucas et al. 2008; Cappello et al. 2006, 2007; Aluvila et al. 2010; Miniero et al. 2011; Giangregorio et al. 2010; Lawrence et al. 2011; Monné et al. 2012; Tonazzi et al. 2012) belong to the mitochondrial carrier signature motif or protrude into the carrier's internal cavity, i.e., into the substrate translocation path. The residues of triplets 23, 30, 33, 77 and 80 protruding into the carrier cavity differ between PXN (QVT, QWL, NVK, STK and GMQ, respectively) and SLC25A17 (MVT, DWQ, RNQ, VSK and SLQ, respectively). It is likely that the differences in these triplets between PXN and SLC25A17 account for the preference of SLC25A17 for FAD and of PXN for NAD^+. Carrier subfamilies defined on the basis of function (substrate specificity) are also characterized by specific and conserved sets of amino acid triplets (Palmieri et al. 2011). Several available plant protein sequences show identical triplets 23, 30, 33, 77 and 80 and a high percentage of identical amino acids (56–97 %) to PXN throughout the entire sequence. These sequences include XP_002881687.1 from *A. lyrata*; XP_002526854.1 from *Ricinus communis*; XP_002315175.1 and XP_002301880.1 from *Populus trichocarpa*; XP_003558284.1 and XP_003558450.1 from *Brachypodium distachyon*; XP_002279488.2 and XP_002278260.2 from *Vitis vinifera*; XP_003616573.1 from *Medicago truncatula*; XP_003552504.1, XP_003538450.1 and NP_001242427.1 from *Glycine max*; NP_001049647.1 from *Oryza sativa*; NP_001152063.1 from *Zea mays*; XP_002468133.1 and XP_002439245.1 from *Sorghum bicolor*; XP_001767175.1, XP_001779190.1 and XP_001780370.1 from *Physcomitrella patens*; and XP_002965581.1 from *Selaginella moellendorfii*. To our knowledge, none of these proteins has been characterized biochemically. In view of the high degree of identity between these proteins and PXN, we conclude that they are all transporters of NAD^+, CoA and their derivatives.

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