Functional interplay between protein acylation and cellular metabolism in metabolic disorders
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Increased protein propionylation contributes to mitochondrial dysfunction in Propionyl-CoA carboxylase deficiency

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Introduction

Mitochondria are intracellular organelles which are crucial for maintaining metabolic homeostasis. Among numerous biochemical functions, mitochondria carry out oxidative phosphorylation (OXPHOS), which is the major source of cellular ATP. Tissues with high energy demand, including heart, brain and skeletal muscle are therefore dependent on a properly functioning OXPHOS system. Accordingly, disorders that result in mitochondrial dysfunction are characterized by neurological defects such as encephalopathy, movement disorders and developmental retardation, along with cardiac and skeletal muscle abnormalities. In the past decades the contribution of mitochondrial defects to human diseases are being increasingly recognized (Leonard and Schapira, 2000a, b; Schapira, 2006). Mitochondrial dysfunction can be primary, which is caused by defects in nucleic or mitochondrial DNA encoding OXPHOS subunits or secondary, which is caused by endogenous or exogenous toxins.

Propionyl-CoA carboxylase (PCC) deficiency is a genetic metabolic disorder in lipid and amino acid catabolism that leads to accumulation of propionyl-CoA (Fenton., 2001). PCC deficiency belongs to the group of inborn errors known as propionic acidemias (PA). Since the first patient was described in 1961 (Childs et al., 1961), the clinical presentation of PA was associated with neuropathological symptoms such as encephalopathy and developmental retardation, metabolic acidosis and cardiomyopathy (de Keyzer et al., 2009; Fenton., 2001; Mardach et al., 2005). Age of clinical onset and disease course varies between patients, with an acute neonatal form accompanied by severe metabolic decompensation and a chronic progressive form with a later onset (Fenton., 2001). Not all of the clinical manifestations of PA can be explained by metabolic acidosis and there are strong indications that mitochondrial dysfunction is involved in the pathological mechanism of the disorder. Indeed, PA patients present with OXPHOS deficiency which results in decreased ATP production and have abnormally shaped mitochondria (Schwab et al., 2006). Furthermore, biochemical parameters including lactic acidosis, hyperketosis and hypoglycemia point to metabolic deregulation in these patients (Fenton., 2001). Collectively, this suggests that secondary mitochondrial dysfunction contributes to the pathogenesis of this disorder.

There is evidence that the endogenous toxin causing the mitochondrial dysfunction in PCC deficient cells is propionyl-CoA as several studies have demonstrated that propionyl-CoA accumulation results in disrupted mitochondrial metabolism. Propionyl-CoA was shown to inhibit the activity of pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (KGDH), OXPHOS complex III and succinate-CoA ligase (SCL) (Schwab et al., 2006; Stumpf et al., 1980). Incubation of rat liver mitochondria with propionate causing accumulation of propionyl-CoA in the matrix resulted in reduced mitochondrial oxidation of glutamate and α-ketoglutarate (Matsuishi et al., 1991; Stumpf et al., 1980). Also, propionate was shown to inhibit palmitate and pyruvate oxidation in isolated rat hepatocytes (Brass et al., 1986). Interestingly, the actual mechanism through which propionyl-CoA inhibits mitochondrial enzymes and metabolic pathways has not been identified.

We have recently demonstrated that fibroblasts from PCC deficient patients
have elevated levels of protein propionylation (Pougovkina et al., 2014). Protein propionylation is a reversible post translational modification of a protein, which involves covalent attachment of a propionyl group to the lysine residue. Protein propionylation is a member of large group of modifications known as protein acylation. Protein acetylation is the most extensively studied acylation modification which is involved in various cellular processes ranging from epigenetic control to the regulation of metabolism (Choudhary et al., 2009; Hebert et al., 2013; Kim et al., 2006b; Kouzarides, 2007; Zhao et al., 2010). Recent advances in proteomics enabled to identify a wide spectrum of lysine acylation modifications including among others propionylation, butyrylation, malonylation, succinylation and glutarylation (Chen et al., 2007; Peng et al., 2011b; Tan et al., 2014; Zhang et al., 2011) all of which use a corresponding acyl-CoA as a donor. Protein propionylation was first identified on human histones by proteomic analysis (Chen et al., 2007). Subsequent studies have shown propionylation to be present on yeast histones and also on non-histone proteins in human cells, including p53, p300 and CBP (Cheng et al., 2009; Zhang et al., 2009b). Due to lack of further functional and proteomic analysis, the physiological function of protein propionylation has not been identified.

Given that elevated levels of propionyl-CoA were shown to disrupt mitochondrial metabolism (Matsuishi et al., 1991), we hypothesized that this was an effect of aberrant protein propionylation. This could account for the mitochondrial dysfunction observed in PA patients. To determine whether excessive lysine propionylation causes OXPHOS dysfunction, we set-up a method to induce propionylation in control fibroblasts and liver cells by exposing them to propionate in concentrations comparable to that found in the plasma of PA patients. We then analyzed whether induced propionylation would lead to respiratory defects observed in the PCC deficient cells.

Results

Propionyl-CoA carboxylase deficient cells have lower OXPHOS capacity

Propionyl-CoA is formed through catabolism of several essential amino acids (isoleucine, valine, methionine and threonine), odd-chain fatty acids and cholesterol. Downstream metabolism of propionyl-CoA includes its carboxylation to methylmalonyl-CoA by propionyl-CoA carboxylase (PCC), which is then isomerized to succinyl-CoA (Beck et al., 1957; Flavin and Ochoa, 1957; Tietz and Ochoa, 1959). In PCC deficient cells propionyl-CoA cannot be converted to methylmalonyl-CoA, which leads to accumulation of propionyl-CoA (Fig. 1A). We used a set of three independent PCC deficient patient cell lines, in which PCC activity was severely reduced to undetectable levels (Fig. 1B).

PCC deficient cells were shown to have a less active OXPHOS, due to lower activity of specific OXPHOS complexes (Schwab et al., 2006). To assess mitochondrial respiration in our PCC deficient cells we used Seahorse respirometry to measure OCR of these cells in regular medium. All of the three patient cell lines had lower mitochondrial spare capacity than the controls (Fig. 1C). To determine whether lower spare capacity was caused by defective OXPHOS we set-up a method to
measure mitochondrial respiration in digitonin-permeabilized cells. The use of an optimized amount of digitonin to permeabilize the cellular membrane while leaving the mitochondrial membrane intact, allowed the use of substrates that would otherwise not be able to enter the cell (Fig. 1D). To this end, we used pyruvate as substrate for complex I driven respiration. Interestingly, we observed that complex I driven respiration was significantly lower in all of the three patient cell lines compared to the controls, which is evident from the lower rate of oxygen uptake (OCR) after injection of digitonin and the substrates (Fig. 1E) and decreased state 3 respiration in the patient cells (Fig. 1F). These results confirm that the three PCC deficient cell lines that we are using have mitochondrial dysfunction.

**Inducing protein propionylation in the control cells leads to mitochondrial respiration defects observed in PCC deficient cells**

Previously, we have shown that PCC deficiency results in a striking increase of protein propionylation due to accumulation of propionyl-CoA (Pougovkina et al., 2014). To analyze whether aberrant protein propionylation in PA patient cells would affect mitochondrial respiration, we developed a cell model in which we could induce cellular lysine propionylation. Because propionyl-CoA elevation simultaneously induces propionylation, altering propionyl-CoA levels in cells experimentally would also alter propionylation. Propionate is a precursor for propionyl-CoA generation. Propionate in healthy mammals is mainly formed by bacterial activity in the gut (Leonard, 1997; Thompson et al., 1990). In PCC patients propionate accumulates and was shown to reach a plasma concentration as high as 5.4 mmol/L in a PA patient (Hommes et al., 1968), which is over thousand fold higher than in controls (Tanaka et al., 1966). We reasoned that exposing control cells to propionate could mimic PCC deficiency of the patients. We therefore cultured control and patient fibroblasts in medium containing 4 mM propionate for 3 weeks followed by propionyllysine western blot analysis of the cells lysates.

When cultured in regular medium, control cells had almost undetectable propionylation levels, whereas the patient cells clearly exhibited increased protein propionylation (Fig. 2A). Interestingly, after propionate exposure the propionylation levels in controls increased significantly and the protein band profile closely resembled that of the patients (Fig. 2A). Also, in patient cells exposed to propionate, propionylation was increased substantially. Interestingly, the propionylation profile of propionate-exposed patient cells overlapped with the profile observed in the absence of added propionate, suggesting that only the levels of propionylation increased and not the distribution of propionylation on different proteins (Fig. 2A). We therefore hypothesized that increased protein propionylation caused by elevated levels of propionyl-CoA in the patient cells plays a key role in the pathophysiology of the disorder. To determine whether aberrant protein propionylation contributes to defective OXPHOS observed in PCC deficient cells, we analyzed mitochondrial respiration in control cells exposed to propionate. To eliminate the effect of increased propionyl-CoA levels due to additional propionate in the medium, the cells were cultured for one day in normal medium prior to the assay. To assess mitochondrial respiration we measured pyruvate-driven OCR in permeabilized cells.
Figure 1. Propionyl-CoA carboxylase deficient (PCCD) cells have defective OXPHOS. (A) Schematic representation of propionyl-CoA metabolism and its accumulation in PCCD cells. (B) PCC activity in controls and PCCD cells (mean ± SD). The graph presents the minimum and the maximum PCC activity range (0.31-1.18 nmol/min*mg protein). In the three patient PCCD cell lines, PCC activity was reduced below the detection limit (<0.01 nmol/min*mg protein). (C) Mitochondrial respiration in PCCD and control fibroblasts, assessed through oxygen consumption rate (OCR) measured with XF-96 Seahorse Analyzer. Oligomycin (ATP synthase inhibitor), FCCP (uncoupler) and Rotenone with antimycin A (AA) (complex I and III inhibitors) were injected during the run. (D) Schematic representation of mitochondria.
We performed this assay with two independent control human fibroblast lines. Strikingly, propionate exposure resulted in decreased OXPHOS capacity of the control cells (Fig. 2B and C). The control cell lines that we tested had 55.2% and 21.7% (pyruvate-driven OCR) of OXPHOS capacity after propionate exposure, as compared to the cells cultured over-time in regular medium. These results confirm our hypothesis that increased protein propionylation in PCC deficient cells due to propionyl-CoA accumulation is at least in part responsible for the mitochondrial dysfunction.

Exposure of liver cells to propionate induces protein propionylation and leads to decreased OXPHOS.

Propionate exposure in fibroblasts induces protein propionylation phenocopying PCC deficiency. To obtain more evidence that propionylation directly controls mitochondrial function we exposed liver cells to propionate. To test whether we can induce protein propionylation in liver cells, we exposed Fao rat hepatoma cells to 4 mM propionate in culture medium. To achieve substantial propionylation levels we cultured Fao cells for five days in propionate-containing medium (Fig. 3A). We also included one day of recovery in regular medium after the exposure before harvesting the cells (Fig. 3A). This was done to ensure that propionyl-CoA and propionate levels in the cells were not elevated, so that in subsequent experiments we would be analyzing the effect of increased protein propionylation and not of increased propionate or propionyl-CoA.

Because propionylcarnitine profiles accurately reflect cellular propionyl-CoA levels (Wikoff et al., 2007) and our propionyl-CoA analysis was not sensitive enough to analyze propionyl-CoA levels in cultured cells, we monitored propionylcarnitine levels in cells cultured in regular medium, in cells after five days of propionate exposure and in cells that were exposed to 5-day propionate followed by one day culture in propionate-free medium. After 5-day propionate exposure propionylcarnitine levels were increased (Fig. 3B). Notably, after one day of recovery on medium without propionate, propionyl-carnitine levels decreased and normalized to the same levels as in the cells that were not exposed to propionate (Fig. 3B). This implies that one day of culturing on regular medium is sufficient to remove excess cellular propionate.

Next, we analyzed the levels of protein propionylation in the Fao cells exposed to propionate by western blotting. Exposure to propionate gave rise to a substantial increase in protein propionylation levels (Fig. 3C). Interestingly, one day recovery on propionate-free medium after the exposure did not lower protein propionylation levels (Fig. 3C), indicating that the induced propionylation is stable, at least for 24 h. Thus, our experimental set-up yields cells with increased protein propionylation and the OXPHOS analysis in permeabilized cells. The cells are in MAS buffer containing no substrates. Digitonin is injected to permeabilize the cell membrane. Together with digitonin, ADP and complex specific substrates are injected. OCR measured after this injection represents mitochondrial state III respiration. (E) OXPHOS in control and PCCD fibroblasts assessed in digitonin-permeabilized cells with complex I substrates (pyruvate and malate). (F) State III OCR calculated from the data in 1E (mean ± SD, * indicates p<0.05).
Figure 2. Induced protein propionylation in the control cells leads to mitochondrial respiration defects observed in PCCD cells. (A) Anti-propionyllysine western blot analysis of three PCCD patient cell lines and three independent control cell lines. The cells were either cultured in regular medium or exposed to 4 mM propionate. 37.5 μg of protein was loaded in each lane. (B) OXPHOS capacity of the two control cell lines exposed to propionate (the same cell lines as in 2A). XF-96 Seahorse analyzer was used to measure the changes in OCR. The cells were permeabilized with digitonin and ADP, pyruvate and malate were added to the wells (first injection) to induce mitochondrial state III respiration. (C) Quantification of OXPHOS capacity in the two control cell lines, with and without exposure to propionate (mean ± SD, * indicates p < 0.05 and *** indicates p<0.001). The table represents the percentage of OXPHOS capacity in cells exposed to propionate compared to cells cultured in regular medium.

base-line propionyl-CoA levels. This is essential for analyzing the effect of increased propionylation on mitochondrial respiration as propionyl-CoA or propionate by itself may potentially be able to inhibit mitochondrial enzymes.

We then investigated the functional aspect of increased protein propionylation in Fao cells. For this we analyzed OXPHOS in permeabilized Fao cells using complex I (pyruvate and malate), complex II (succinate) and complex IV (TMPD and ascorbate)
substrates (Fig. 4A). For complex II activity rotenone was added together with succinate to prevent reverse electron transfer to complex I. Remarkably, for all of the substrates used, increased protein propionylation resulted in lower OXPHOS capacity (Fig. 4B and C). Specifically, propionylated cells had 30.7%, 53.3% and 58.7% of the control OXPHOS values when assessing OXPHOS from complex I, II and IV, respectively (Fig. 4C). This goes in line with the previous studies which show that propionyl-CoA suppresses activity of OXPHOS complexes (Schwab et al., 2006; Stumpf et al., 1980).
Discussion

Propionic acidemia (PA) is a disorder of which the etiology is incompletely understood. It is caused by the inability of cells to metabolize propionyl-CoA due to PCC deficiency. PCC deficient patients present with features of mitochondrial dysfunction, the cause of which is not known. The results presented in this study demonstrate a direct connection between propionyl-CoA accumulation and the symptomology of the disorder caused by aberrant protein propionylation. We demonstrate that exposing control cells to high propionate concentrations leads to increased protein propionylation, similar to that in the PCC deficient cells. Furthermore, we confirm that increased protein propionylation leads to impaired mitochondrial respiration, which can be the cause of mitochondrial dysfunction observed in patients.

We started out by investigating whether exposing cells to propionate in culture medium would be sufficient to induce protein propionylation to a similar extent as that in PCC deficient cells. As expected, exposing control fibroblasts to propionate at concentration comparable to that in the plasma of PCC deficient patients, induced protein propionylation. Interestingly, the propionylation bands on the western blot were the same in controls exposed to propionate and in PCC deficient patient cells. Furthermore, exposing patient cells to propionate led to increased protein propionylation also at those specific protein bands and there were no new bands visible. These observations indicate that the protein propionylation profile achieved by exposure to propionate in control cells correlates with the propionylation status of PCC deficient cells.

PA patients present with severe disturbances in mitochondrial metabolism (Fenton., 2001; Schwab et al., 2006). Remarkably, in accordance with these findings, we observed that PCC deficient patient cells had lower maximum respiratory capacity than the controls, which points to a defect in oxidative metabolism. Furthermore, mitochondrial respiration driven by OXPHOS complex I substrate pyruvate was reduced in PCC deficient cells confirming OXPHOS deficiency. To link this OXPHOS defect to excessive propionylation, we performed the same analysis in control fibroblasts exposed to propionate. Strikingly, we also observed decreased mitochondrial respiration in these cells confirming that induction of the extent of propionylation with propionate is sufficient to produce abnormalities in the OXPHOS system, as observed in cells from PCC-deficient patients.

Mitochondrial dysfunction in PA patients presents itself mainly in the organs with high energy requirements that are dependent on OXPHOS including liver, skeletal muscle and heart (de Keyzer et al., 2009; Mardach et al., 2005; Schwab et al., 2006). We therefore investigated the effect of increased protein propionylation on mitochondrial function in liver cells. For this we exposed rat hepatoma cells to propionate and measured mitochondrial respiration driven by complex specific substrates. We observed that increased propionylation resulted in decreased OCR with pyruvate and malate (complex I), succinate (complex II) and, TMPD and ascorbate (complex IV) as substrates. As propionate was previously shown to inhibit mitochondrial metabolism in liver (Brass et al., 1986; Matsuishi et al., 1991), our analyses in propionate-free medium imply that propionate suppresses
Figure 4. Propionate exposure causes decreased OXPHOS in the liver cells. (A) Schematic representation of OXPHOS with complex specific substrates and inhibitors that were used. To measure OXPHOS starting from complex I, pyruvate and malate were injected together with digitonin and ADP. To measure the OXPHOS starting from complex II, succinate was injected as the substrate and rotenone as complex I specific inhibitor to prevent reverse electron transfer to complex I. To measure the OXPHOS starting from complex IV, TMPD and ascorbate were injected. (B) OCR profile generated from the Seahorse run to measure state III OCR in permeabilized control cells and cells exposed to propionate. The first injection contained digitonin to permeabilize the cell, complex specific substrates and ADP to induce state III respiration. For complex I activity (pyruvate and malate), rotenone was injected in the end of the run as it specifically inhibits complex I. For complex II activity (succinate and rotenone), antimycin was injected in the end of the run. (C) Quantification of state III respiration in control cells and cells exposed to propionate from the data in 4C (mean ± SD, *** indicates p<0.001). The table represents the percentage of OXPHOS capacity when assessing OXPHOS from different complexes in cells exposed to propionate compared to controls.
mitochondrial metabolism through protein propionylation.

At present, treatment of PA patients is based on a low protein diet with sufficient caloric intake, carnitine supplementation and antibiotics. The treatment costs a lot of effort with often disappointing results and the overall prognosis is poor (de Baulny et al., 2005). In some cases liver transplantation is applied, which, however, does not prevent the neurological symptoms (Barshes et al., 2006). Our results suggest that counteracting aberrant protein propionylation in PCC deficient cells could contribute to the treatment of the patients. At the moment, enzymes specifically involved in the regulation of cellular protein propionylation are not known. Sirtuins with strong deacetylase activity (SIRT1-3) were shown to possess depropionylase activity (Feldman et al., 2013). Therefore, administration of sirtuin activators could aid in reducing protein propionylation in PCC deficient cells.

To further investigate the pathological mechanism of propionylation in PCC deficiency it is necessary to determine the subcellular localization of propionylation and to identify propionylated proteins. Because PCC is a mitochondrial enzyme, propionyl-CoA accumulates primarily in the mitochondria and therefore it is likely that protein propionylation is enriched in this organelle. In that case propionylation can directly affect mitochondrial proteins thereby impairing mitochondrial metabolism. Nevertheless, patient and control cells exposed to propionate also clearly have histone propionylation bands visible on the western blot suggesting that propionylation could be acting at the epigenetic level as well.

Overall, the results of our study present new insights with respect to the pathological mechanism of PA involving protein propionylation. More research is necessary to further elucidate the role of propionylation in this disorder. This will give us more understanding of the cause of mitochondrial dysfunction in PA patients. Furthermore, it will also elucidate the role of protein propionylation in the regulation of metabolism in general. Finally, identifying enzymes to counteract protein propionylation could provide an effective treatment for PA patients in the future.

Materials and methods

Propionyl-CoA carboxylase activity

Patient and control cell pellets were resuspended in PBS and sonicated at 40J/W/S. A volume of 10 µl of protein lysate (1mg/ml) was added to 40 µl of the reaction solution (100 mM TRIS/HCl pH 8.0, 200 mM KHCO₃, 10 mM MgCl₂, 10 mM ATP, 1 mM propionyl-CoA) to a final protein concentration of 0.2 mg/ml. After 15 minute incubation at 37°C the reaction was terminated with 10 µl of 2M HCl. The sample was neutralized with 2 M KOH/0.6 M MES buffer after which 30 µl of methanol HPLC grade was added. After centrifugation at 20000 G for 5 minutes at 4°C the supernatant was injected on reversed phase HPLC to analyze the formation of methylmalonyl-CoA.

Propionate exposure

Human dermal fibroblasts and Fao hepatoma cells were routinely cultured in
Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine and 1% (v/v/v) pen/strep/fungizone. For propionate exposure DMEM was replaced with DMEM containing 4 mM propionate. Propionate containing DMEM was prepared with 400 mM propionate stock. Propionate stock was prepared by neutralizing propionic acid (Merck #800605) with NaOH to pH 7.

**Dulbecco’s Modified Eagle Medium (DMEM)**

**Seahorse mitochondrial respiratory flux analysis**

Control and PCC deficient fibroblasts were routinely cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine and 1% (v/v/v) pen/strep/fungizone. On the day prior to the respiratory analysis, the cells were plated at 30,000 cells/well in Seahorse 96 well culture plates followed by overnight incubation. 30 minutes before the run, the medium was replaced with DMEM (Sigma-Aldrich #5030) supplemented with 5 mM glucose and 2mM glutamine. The DMEM did not contain sodium bicarbonate, pen/strep/fungizone and serum. To analyze mitochondrial respiration, oxygen consumption rate (OCR) was analyzed following injections of oligomycin (1.5 µM final concentration), FCCP (1 µM), antimycin (2.5 µM) and rotenone (1.25 µM).

**Analysis of OXPHOS in permeabilized cells**

Control fibroblasts and Fao cells were exposed to 4 mM propionate DMEM for three weeks and five days, respectively. On the day prior to the respiratory analysis, the cells were plated at 30,000 cells/well in Seahorse 96 well culture plates followed by overnight incubation in regular DMEM. Shortly before placing the plates into the Seahorse Analyzer, culture medium was replaced with MAS buffer (pH, 7.4, 220 mM mannitol, 70 mM sucrose, 10mM KH$_2$PO$_4$, 5mM MgCl$_2$, 2 mM HEPES, 1 mM EGTA and 0.6% BSA-fatty acid free). OCR was analyzed following a single injection of pyruvate/malate/ADP/digitonin (Complex I), succinate/rotenone/ADP/digitonin (Complex II), TMPD/ascorbate/ADP/digitonin (Complex IV), dissolved in MAS buffer without BSA at pH 7.4. Final digitonin concentration was 30 µg/ml for Fao cells and 100 µg/ml for fibroblasts. Final substrate concentrations were: pyruvate (5 mM), malate (2.5 mM), succinate (10 mM), ADP (1 mM). After injection of substrate, oligomycin was injected at 1.5 µM final concentration followed by injection of antimycin (2.5 µM) and rotenone (1.25 µM).

**SDS–PAGE and Western blotting**

Cells were harvested by trypsinization, followed by lysis in PBS with 0.1% triton containing deacetylase inhibitors (1 µM trichostatin A and 10 mM nicotinamide) by sonication at 40J/W/s on ice. Samples were ran on NuPAGE 4-12% gradient gels (Invitrogen), followed by transfer of proteins to nitrocellulose. For protein propionylation analysis propionyl-lysine antibody (#201 LOT: TAB0317P001, PTM Biolabs) was used. Antibody for β-actin (#A5441 monoclonal, Sigma-Aldrich) was used as loading control. IR-dye based secondary antibodies (LI-COR) were used to detect antibody signals using the Odyssey scanner (LI-COR).
**Propionylcarnitine measurement**

Fao cells were exposed to 4 mM propionate in DMEM for five days. To wash out propionate, the medium was replaced with regular DMEM and the cells were harvested on the next day. For propionylcarnitine measurement we used cells exposed for five days to propionate, cells after the propionate wash out step and control cells that were cultured in regular DMEM. The cells were harvested by trypsinization and cell pellets were resuspended in 0.5 ml MQ to 1 mg of protein homogenate. Internal standard (50 pmol $^2$H$_3$ propionyl-carnitine) was added to the homogenate, followed by 500 µl of acetonitrile. The samples were vortex-mixed and centrifuged at 14000 rpm 4°C for 10 minutes. The supernatant was transferred to a glass vial and the solvent was evaporated at 42°C under a stream of nitrogen. A 100 µl volume of propylation reagent, a 4:1 (v/v) mixture of propan-2-ol and acetylchloride, was added to the residue, vortex-mixed and incubated for 10 minutes at 65°C. The propylation reagent was evaporated at 42°C under a stream of nitrogen and the residue was taken up in 100 µl of acetonitrile. Propionylcarnitine was quantified by Electrospray Ionization Tandem Mass Spectrometry (ESI-MS/MS) as described in (Vreken et al., 1999).
Protein propionylation and mitochondrial dysfunction in PCC deficiency

References


