The PduQ Enzyme Is an Alcohol Dehydrogenase Used to Recycle NAD\(^+\) Internally within the Pdu Microcompartment of *Salmonella enterica*

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Abstract

*Salmonella enterica* uses a bacterial microcompartment (MCP) for coenzyme B\(_{12}\)-dependent 1,2-propanediol (1,2-PD) utilization (Pdu). The Pdu MCP consists of a protein shell that encapsulates enzymes and cofactors required for metabolizing 1,2-PD as a carbon and energy source. Here we show that the PduQ protein of *S. enterica* is an iron-dependent alcohol dehydrogenase used for 1,2-PD catabolism. PduQ is also demonstrated to be a new component of the Pdu MCP. In addition, a series of in vivo and in vitro studies show that a primary function of PduQ is to recycle NADH to NAD\(^+\) internally within the Pdu MCP in order to supply propionaldehyde dehydrogenase (PduP) with its required cofactor (NAD\(^+\)). Genetic tests determined that a *pduQ* deletion mutant grew slower than wild-type *Salmonella* on 1,2-PD and that this phenotype was not complemented by a non-MCP associated Adh2 from *Zymomonas* that catalyzes the same reaction. This suggests that PduQ has a MCP-specific function. We also found that a *pduQ* deletion mutant had no growth defect in a genetic background having a second mutation that prevents MCP formation which further supports a MCP-specific role for PduQ. Moreover, studies with purified Pdu MCPs demonstrated that the PduQ enzyme can convert NADH to NAD\(^+\) to supply the PduP reaction in vitro. Cumulatively, these studies show that the PduQ enzyme is used to recycle NADH to NAD\(^+\) internally within the Pdu MCP. To our knowledge, this is the first report of internal recycling as a mechanism for cofactor homeostasis within a bacterial MCP.

Introduction

Bacterial microcompartments (MCPs) are a functionally diverse group of proteinaceous subcellular organelles used to optimize metabolic pathways that have toxic or volatile intermediates [1,2,3,4,5]. They are polyhedral in shape, 100–150 nm in cross-section, and consist of metabolic enzymes encapsulated within a protein shell. The function of MCP shells is to restrict the outward diffusion of toxic/volatile metabolic intermediates and help channel them to downstream enzymes thereby minimizing toxicity or carbon loss [6,7,8,9,10]. Based on sequence analyses, MCPs are found in 15–20% of bacteria and participate in 7 or more different metabolic processes [1,3,4,5,11]. Different types of MCPs have related protein shells but differ in their encapsulated enzymes. The carboxysome, which is the archetypal MCP, is found in the majority of autotrophic bacteria where it plays a critical role in global carbon fixation [2,5]. Other MCPs are used for the catabolism of 1,2-propanediol (1,2-PD) or ethanolamine, or have unknown functions [4,8,12,13,14]. In the enterica bacteria, 1,2-PD and ethanolamine degradation are linked to pathogenesis [15,16,17,18,19,20]. In addition, MCPs have a number of potential biotechnology applications since they provide a foundation for the design of synthetic protein cages for use as nanoscale intracellular chemical reactors or as drug delivery vehicles [21,22,23].

*Salmonella enterica* produces a MCP for coenzyme B\(_{12}\)-dependent 1,2-PD utilization (Pdu MCP) [12]. 1,2-PD is a major product of the anaerobic degradation of common plant sugars rhamnose and fucose and is thought to be an important carbon and energy source in anoxic environments [24]. The Pdu MCP consists of a protein shell that encapsulates enzymes and cofactors used for metabolizing 1,2-PD [25]. Twenty-four genes for 1,2-PD utilization (pdu) are found in a contiguous cluster (pocR, pduF and pduABB’CDEGHJKLMNOPQSTUVWX) [12,26,27]. The pdu locus encodes enzymes for 1,2-PD degradation (PduCDELPQW) [12,25,28,29,30,31], the conversion of cobinamide and cobalamin to coenzyme B\(_{12}\) (PduOSX) [32,33,34,35,36,37], the reactivation of diol dehydratase (PduGH) [38,39], eight proteins that likely form the shell of Pdu MCP (PduABB’JKNTU) [12,25,30,41,42] and one structural protein (PduM) that might also be a shell component [43]. The pathway of 1,2-PD degradation begins with the conversion of 1,2-PD to propionaldehyde by coenzyme B\(_{12}\)-dependent diol dehydratase (PduCDE) [26,28] (Fig. 1). Propionaldehyde is then converted to propionate by coenzyme A (CoA)-dependent propionaldehyde dehydrogenase (PduP) [29], phospho-
transacylase (PduL) [30] and propionase kinase (PduW) [31], or to 1-propanol probably by a putative propionaldehyde dehydrogenase (PduQ) [12]. The proposed function of the Pdu MCP is to sequester propionaldehyde produced by diol dehydratase (PduCDE) and channel it to propionaldehyde dehydrogenase (PduP) in order to minimize toxicity and DNA damage [6,7,12,41]. Current models for the Pdu MCP propose that its protein shell selectively retains propionaldehyde while allowing the entrance of required enzyme substrates and cofactors as well as the egress of metabolic products [3,44,45]. Metabolite movement into and out of the Pdu MCP is proposed to occur through centrally located pores in the BMC-domain proteins (PduABB’JKTU) that comprise the bulk of its shell [44,45,46,47]. Based on studies with several different systems, it has been shown that the pores in different BMC domain proteins vary in size and chemical properties suggesting that different shell proteins have pores that act as conduits for particular metabolites [44,47,48,49,50,51].

Our prior studies showed that the PduP propionaldehyde dehydrogenase, which catalyzes the conversion of propionaldehyde+NAD^+→HS-CoA→propionyl-CoA+NADH, is encapsulated within the Pdu MCP [29]. The substrate for PduP (propionaldehyde) is generated internally within the MCP, but how this enzyme is supplied with NAD^+ and HS-CoA is unknown. In this study, we identify the PduQ enzyme as an iron-dependent alcohol dehydrogenase used for 1,2-PD degradation by Salmonella. We also show that PduQ is a new component of the Pdu MCP that plays an important role in regenerating NAD^+ from NADH internally within the MCP, although results suggest other mechanisms such as specific pores may also be used. The findings presented here are the first to show the importance of internal recycling for cofactor homeostasis within a bacterial MCP.

Materials and Methods

Bacterial strains and growth conditions

The bacterial strains used in this study are listed in Table 1. The rich media used were lysogeny broth (LB) also known as Luria-Bertani/Lennox medium (Difco, Detroit, MI) [52] and Terrific Broth (TB) (MP Biomedicals, Solon, OH) [53]. The minimal medium used was no-carbon-E (NCE) medium [54].

Chemicals and reagents

Antibiotics, vitamin B₁₂ (CN-B₁₂, CN-CH₂), DNase I, NAD^+, NADH, NADP^+, and NADPH were from Sigma Chemical Company (St. Louis, MO). Isopropyl β-D-1-thiogalactopyranoside (IPTG) was from Diagnostic Chemicals Limited (Charlotteville PEI, Canada). KOD DNA polymerase was from Novagen (Cambridge, MA). Tag DNA polymerase, restriction enzymes, and T4 DNA ligase were from New England Biolabs (Beverly, MA). Bacterial protein extraction reagent (B-PER II) was from Pierce (Rockford, IL). Other chemicals were from Fisher Scientific (Pittsburgh, PA).

Construction of plasmids and a pduQ-deletion mutant

The pduQ gene was amplified from the genomic DNA of S. enterica by PCR and then cloned into pET-41a vector (Novagen) using NdeI and HindIII restriction sites incorporated into the PCR primers as previously described [55]. The genes for the production of His₅-PduQ and PduQ-His₅ were similarly cloned with the sequence encoding the His₅-tag incorporated into the PCR primers. The resulting plasmids pET-41a-pduQ, pET-41a-His₅-pduQ, as well as the pET-41a-pduQ-His₅ were introduced into

![Figure 1. Model for the Pdu microcompartment and the role of PduQ.](image)

The Pdu MCP consists of a protein shell that encapsulates enzymes and cofactors for metabolizing 1,2-propanediol. The shell is thought to be made from 9 proteins (PduABB’JKMNTU). Encapsulated enzymes include transacylase (PduL) and propionase kinase (PduW). The proposed function of the Pdu MCP is to sequester propionaldehyde and channel it to downstream enzymes in order to prevent toxicity and DNA damage.

Table 1. Bacterial strains used in this study.

| Species and strain | Genotype | Source                  |
|--------------------|----------|-------------------------|
| E. coli            |          |                         |
| BE237              | BL21(DE3)RIL/pET-41a | Lab collection         |
| BE272              | BL21(DE3)RIL/pTA925-pduP | Lab collection        |
| BE1421             | BL21(DE3)RIL/pET-41a-His₅-pduP | Lab collection       |
| BE1422             | BL21(DE3)RIL/pET-41a-pduP | Lab collection        |
| BE1500             | BL21(DE3)RIL/pET-41a-pduQ | This work             |
| BE1501             | BL21(DE3)RIL/pET-41a-His₅-pduQ | This work            |
| BE1502             | BL21(DE3)RIL/pET-41a-pduQ-His₅ | This work           |
| S. enterica serovar Typhimurium LT2 |          |                         |
| BE287              | LT2/pLAC22 | Lab collection          |
| BE182              | ΔpduA6562 | Lab collection          |
| BE192              | ΔpduQ660  | Lab collection          |
| BE324              | ΔpduA6562, ΔpduQ660 | Lab collection      |
| BE903              | ΔpduQ668::frt | This work           |
| BE919              | ΔpduQ688::frt/pLAC22 | This work           |
| BE942              | ΔpduQ688::frt/pLAC22-pduQ | This work        |
| BE1651             | ΔpduQ688::frt/pLAC22-ZmadhB | This work       |
| BE1764             | LT2/pLAC22-ZmadhB | This work             |

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Enzyme assays

Enzyme assays were performed under anaerobic conditions as described [32]. All reactions were initiated by addition of NADH or NAD\(^+\) to assay mixtures except where stated otherwise. Propionaldehyde reduction assays contained 100 mM Na\(_2\)HPO\(_4\)-NaH\(_2\)PO\(_4\) (pH 7.0), 0.4 mM NADH, 150 mM propionaldehyde and the amount of enzyme indicated in the text. 1-propanol oxidation assays contained 100 mM Tris-HCl (pH 9.0), 2 mM NAD\(^+\), 800 mM 1-propanol and enzyme as indicated. The interconversion between propionaldehyde and 1-propanol was monitored spectrophotometrically by measuring the absorbance change at 340 nm due to consumption or formation of NADH and quantified using \(\Delta A_{340} = 6.22 \text{ mM}^{-1} \text{ cm}^{-1}\). PduP activities in anaerobically purified Pdu MCPs were assayed in 100 mM Na\(_3\)HPO\(_4\)-NaH\(_2\)PO\(_4\) (pH 7.0), 200 mM 1,2-PD, 10 mM propionaldehyde, 40 \mu M NAD\(^+\), and 100 \mu M HS-CoA by monitoring the formation of thioster bonds at 232 nm with \(\Delta A_{232} = 4.5 \text{ mM}^{-1} \text{ cm}^{-1}\).

Metal analysis

The content of Fe in the purified PduQ was determined by a high-resolution double-focusi ng inductively coupled plasma mass spectrometry (ICP-MS) using a Finnigan Element I (Thermo Scientific) operated at medium resolution (\(m/\Delta m = 4,000\)) as described in a previous report [60]. Prior to metal analysis samples were applied to a Sephadex\textsuperscript{TM} G-25 PD-10 desalting column (GE Healthcare, Piscatway, NJ) pre-equilibrated with MilliQ water (resistivity >18 m\(\Omega\)) and eluted with MilliQ water inside an anaerobic chamber. Eluates were collected in metal-free tubes (Labcon, Petaluma, CA). As a control, water was used in place of purified PduQ and collected in the same volume range from a parallel PD-10 column. Ga was used as an internal standard to quantify the elemental concentrations of interest.

Spectroscopic analyses

Metal analysis was carried out as described previously [43]. Pdu MCPs were isolated as described except that the growth media additionally contained 50 \mu M ferric citrate [43]. The tandem mass spectroscopy was performed as previously described [32].

Hi-tag protein affinity pull-down assays

The soluble cell lysates containing recombinant Hi-tagged baits were applied to the pre-equilibrated Ni-NTA column, and the column was washed with 50 mM potassium phosphate, pH 8.5, 300 mM NaCl, 1 mM DTT, 0.5 mM AEBSF, and 100 mM imidazole. A soluble cell lysate containing a native recombinant protein prey was loaded onto the column which was again washed with the buffer described above. The His-tagged bait and any bound proteins were eluted with 50 mM potassium phosphate, pH 8.5, 300 mM NaCl, 1 mM DTT, 0.5 mM AEBSF, and 300 mM imidazole and the eluate was analyzed by SDS-PAGE.
PduQ Recycles NAD⁺ within the Pdu Microcompartment

Expression and purification of PduQ-His₆ protein

Based on its similarity to alcohol dehydrogenases, PduQ was previously proposed to be a 1-propanol dehydrogenase involved in 1,2-PD degradation by Salmonella [12]. However, the function of PduQ has not been investigated experimentally. To further characterize this enzyme, E. coli strain BE1502 was constructed to produce high levels of C-terminally His₆-tagged PduQ protein via a T7 expression system. Strain BE1502 produced relatively large amounts of protein near the expected molecular mass of PduQ-His₆ (40.3 kDa) (Fig. S2). PduQ-His₆ was purified under aerobic and anaerobic conditions by Ni-NTA affinity chromatography. Based on SDS-PAGE anaerobically purified both aerobic and anaerobic conditions by Ni-NTA affinity chromatography. Subtracting the background (0.1 and 0.4 mol min⁻¹ mg⁻¹) of the expression strain BE1502 had 0.7 mol min⁻¹ mg⁻¹ propionaldehyde reductase activity, and control extracts from the PduQ-His₆ expression strain (BE1502) exhibited a T7 expression system. Strain BE1502 produced relatively large amounts of protein near the expected molecular mass of PduQ-His₆ (40.3 kDa) (Fig. S2). PduQ-His₆ was purified under aerobic and anaerobic conditions by Ni-NTA affinity chromatography. Subtracting the background (0.1 and 0.4 mol min⁻¹ mg⁻¹) of the expression strain BE1502 had 0.7 mol min⁻¹ mg⁻¹ propionaldehyde reductase activity, and control extracts from the PduQ-His₆ expression strain (BE1502) exhibited a non-native recombinant PduQ enzyme with similar enzymatic activities.

In vitro catalytic activities of the PduQ-His₆ enzyme

PduQ-His₆ purified under strictly anaerobic conditions was tested for propionaldehyde reductase and 1-propanol dehydrogenase activities after each purification step (Table 2). The whole-cell extract from the PduQ-His₆ expression strain (BE1502) exhibited about 24.2-fold higher propionaldehyde reductase activity (2.9 ± 0.2 mol min⁻¹ mg⁻¹) than did extracts from the control strain carrying the expression plasmid without insert (BE237) (0.12 ± 0.03 mol min⁻¹ mg⁻¹). Purification of the PduQ-His₆ protein by anaerobic Ni-NTA chromatography increased the propionaldehyde reductase specific activity approximately 19.1-fold to 55.5 ± 4.2 mol min⁻¹ mg⁻¹. Assays showed that the PduQ-His₆ protein also catalyzed the reverse reaction, the oxidation of 1-propanol to propionaldehyde. Whole cell extracts of the expression strain BE1502 had 0.7 ± 0.1 mol min⁻¹ mg⁻¹ 1-propanol dehydrogenase activity, and control extracts from BE237 had 0.42 ± 0.08 mol min⁻¹ mg⁻¹ activity. Purification of the PduQ-His₆ protein by anaerobic Ni-NTA chromatography increased the 1-propanol dehydrogenase specific activity about 7.3-fold to 5.1 ± 0.7 mol min⁻¹ mg⁻¹. The differences in the activity increases of purified PduQ with different assay methods (propionaldehyde reductase and 1-propanol dehydrogenase) can be explained by differences in propionaldehyde reductase and 1-propanol dehydrogenase background activities present in crude cell extracts. Subtracting the background (0.1 and 0.4 mol min⁻¹ mg⁻¹) of both propionaldehyde reductase and 1-propanol dehydrogenase activities compared to the anaerobically purified enzyme (when assays were conducted immediately after purification). Further studies showed that PduQ lost measurable activity after several hours of air exposure (2 h for aerobically purified enzyme and 5 h for anaerobically purified PduQ). These results indicated that PduQ is oxygen sensitive.

Aerobic purification substantially inactivates PduQ-His₆

PduQ-His₆ was also purified under aerobic conditions. Aerobically purified PduQ-His₆ lost about 80% of both propionaldehyde reductase and 1-propanol dehydrogenase activities compared to the anaerobically purified enzyme (when assays were conducted immediately after purification). Further studies showed that PduQ lost measurable activity after several hours of air exposure (2 h for aerobically purified enzyme and 5 h for anaerobically purified PduQ). These results indicated that PduQ is oxygen sensitive.
PduQ reaction requirements, linearity and pH optima

To determine the PduQ reaction requirements, key assay components were individually omitted. For propionaldehyde reduction, there was no detectable activity in the absence of NADH, propionaldehyde, or anaerobically purified PduQ-His6. In the 1-propanol dehydrogenase assays, no activity was measurable in the absence of NADH, 1-propanol, or anaerobically purified PduQ-His6. The effects of PduQ-His6 concentration on its enzymatic activities were also determined. Propionaldehyde reductase was proportional to PduQ-His6 concentration from 10 to 95.5 nM when 0.4 mM NADH and 150 mM propionaldehyde were used as substrates. Linear regression yielded an $R^2$ value of 0.9991. 1-propanol dehydrogenase activity was linear from 0.16 to 1.4 mM PduQ-His6, with an $R^2$ value of 0.9994 when the assay mixture contains 2 mM NADH and 800 mM 1-propanol. Subsequent characterization of PduQ was done working within the linear ranges of these assays. The pH optima for PduQ-His6 were determined in 100 mM Na$_2$HPO$_4$-NaH$_2$PO$_4$ (pH 6.0–7.5), 100 mM Tris-HCl (pH 7.5–9.0) and 100 mM Glycine-$\text{NaOH}$ (pH 9.0–10.0). The anaerobically purified PduQ-His6 exhibited maximal activity for propionaldehyde reduction at pH 7.0 while the maximal 1-propanol dehydrogenase activity was achieved at pH 9.0 (Fig. S3), which is consistent with the pH preference of several other alcohol dehydrogenases [61].

NAD(H)/NADP(H) preference of PduQ-His6

To determine the cofactor specificity, anaerobically purified PduQ-His6 was assayed for both 1-propanol dehydrogenase and propionaldehyde reductase activities in the presence of either NADH or NADPH at 0.4 mM, or NAD$^+$ or NADP$^+$ at 2 mM. Results showed that anaerobically purified PduQ-His6 preferred NAD$^+$/NADH as co-substrates. The relative activities with NADP$^+$ and NADPH were 11% (1-propanol dehydrogenase) and 13% (propionaldehyde reductase), respectively.

Characterization of Fe ion in PduQ-His6

ICP-MS was used to determine the iron content of PduQ-His6 (Fig. S4). The anaerobically purified PduQ-His6 protein contained 1.01±0.04 Fe-atom per monomeric unit while the aerobically purified PduQ-His6 protein contained 0.18±0.02 Fe-atom per monomeric unit, indicating that iron was lost in the presence of oxygen. In addition, the PduQ catalytic activities were almost totally inhibited by 1 mM EDTA. In conjunction with the finding that aerobically purified PduQ-His6 has about 20% relative activity compared to anaerobically purified enzyme (above), we infer that oxygen-labile iron is required for catalysis by PduQ.

Kinetic analysis of PduQ-His6 activities

Steady-state kinetic studies for propionaldehyde reduction and 1-propanol oxidation were performed using anaerobically purified PduQ-His6 with varied concentrations of one substrate and a fixed excess concentration of the other substrate. Kinetic parameters were obtained by non-linear curve fitting to the Michaelis-Menten equation $v = V_{\text{max}} [S]/(K_m + [S])$ using GraphPad Prism 5 Software (GraphPad Software, San Diego, CA). For propionaldehyde reduction, the $K_m$ values for NADH and propionaldehyde were 45.3±5.3 μM and 16.0±2.0 mM, respectively (Table 3). The enzyme $V_{\text{max}}$ was 77.7±3.0 or 81.3±4.5 μmol min$^{-1}$ mg$^{-1}$ when propionaldehyde or NADH was held at 150 mM and 400 μM, respectively, and the other substrate was varied. For 1-propanol oxidation, non-linear regression indicated a $K_m$ of 267.5±22.5 μM and 95.8±9.2 mM for NAD$^+$ and 1-propanol, respectively (Table 3). The enzyme $V_{\text{max}}$ was 9.2±0.7 or 8.4±0.8 μmol min$^{-1}$ mg$^{-1}$ when the 1-propanol or NAD$^+$ was held at fixed concentrations of 800 mM and 2 mM, respectively, and the other substrate was varied.

PduQ is a component of Pdu MCP

To determine the cellular location of PduQ, MCPs purified from wild-type S. enterica and pduQ deletion mutant BE903, were analyzed by SDS-PAGE, Western blot, MALDI-TOF MS-MS and enzymatic assays. A band near 39 kDa in wild-type MCPs (Fig. 2A lane 2) was absent in MCPs purified from the pduQ deletion mutant (Fig. 2A lane 3). Digestion of this band with trypsin followed by MALDI-TOF MS-MS identified three sequences: MNTFSLQTR (Mowse Score = 96), R-FNAGVR-Α (760.41) and R-IPAMQVAALAVTLR-Τ (1584.86), and all of them matched the PduQ protein. The Mowse Score was 96 indicating a high likelihood of a correct identification. In addition, Western blots with antiserum against PduQ peptide (51-64) detected a band near 39 kDa in wild-type MCPs (Fig. 2B lane 1), while no band was detected from the ΔpduQ MCPs (Fig. 2B lane 2). Moreover, the NADH-dependent reduction of propionaldehyde occurred at rates of 2.48±0.19 or 0.24±0.03 μmol min$^{-1}$ mg$^{-1}$ in anaerobically purified wild-type MCPs and crude cell extracts, respectively, showing the PduQ was enriched in the purified MCPs. As expected no activity was detected in ΔpduQ MCPs. We also found that the activity of PduQ in the purified MCP correlated well with the activity of the purified enzyme. The activity of PduQ in the purified MCP was 2.48±0.19 μmol min$^{-1}$ mg$^{-1}$. The specific activity of the purified enzyme was 55.5±2.2. This implies that PduQ is 4.5% of the total MCP protein. Our prior studies indicated that the band just below PduQ (PduO) is 3.6% of the total MCP protein [25] and PduQ band has similar intensity to PduO in the purified Pdu MCP on SDS-PAGE (Fig. 2A lane 2). PduP was previously estimated to make up about 8% of the total MCP protein [25]. This suggests an approximate stoichiometry of 2 PduP/PduQ. The $V_{\text{max}}$ values for PduP and PduQ were previously determined to be 85 and 77 U in

### Table 3. Kinetic parameters for propionaldehyde reduction and 1-propanol oxidation by anaerobically purified PduQ-His6.

| Reaction | Variable substrate | $K_m$ (μM) | $V_{\text{max}}$ (μmol min$^{-1}$ mg$^{-1}$) | $k_{\text{cat}}$ (s$^{-1}$) | $k_{\text{cat}}/K_m$ (μM$^{-1}$ s$^{-1}$) |
|----------|-------------------|------------|-----------------------------------|-----------------|-------------------------------------|
| Propionaldehyde reduction$^b$ | NADH | 45.3±5.3 | 77.7±3.0 | 52.2 | 1.15 |
| | propionaldehyde | (16.0±2.0 )×10$^2$ | 81.3±4.5 | 54.6 | 3.41×10$^{-3}$ |
| 1-propanol oxidation$^c$ | NAD$^+$ | 267.5±22.3 | 9.2±0.7 | 6.18 | 2.31×10$^{-2}$ |
| | 1-propanol | (95.8±9.2 )×10$^2$ | 8.4±0.8 | 5.64 | 5.89×10$^{-5}$ |

$^b$The values of $K_m$ and $V_{\text{max}}$ were from non-linear regression using GraphPad Prism 5. The results shown are based on two independent experiments in which assays were done in triplicate.

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The growth defect of a $A_{pduQ}$ mutant can be complemented by PduQ but not by a homologous non-MCP associated Adh enzyme

Complementation tests showed that production of PduQ from pLAC22 fully corrected the growth defect of the $A_{pduQ}$ mutant at CN-B$_{12}$ levels ranging from 20–150 nM. This confirmed that the observed growth defect was due to the $pduQ$ deletion, but not due to polarity or an unknown mutation. In contrast, the non-MCP associated Adh2 of *Z. mobilis* ($Zm$Adh2) was unable to correct the growth defect of a $pduQ$ deletion mutant even though its enzymatic activity was similar (Fig. 4). In the growth curves shown, 10 µM IPTG was used for induction of both PduQ and $Zm$Adh2 which resulted in 0.52±0.04 and 0.63±0.03 µmol min$^{-1}$ mg$^{-1}$ propionaldehyde reductase activity, respectively. Furthermore, $Zm$Adh2 production was unable to correct the growth phenotype of a $A_{pduQ}$ deletion mutant when induced with IPTG at concentrations up to 500 µM. Further controls showed that expression of $Zm$Adh2 from pLAC22 had little effect on the growth of LT2 on 1,2-PD or was slightly stimulatory. The finding that $Zm$Adh2 was unable to correct the growth rate defect of a $pduQ$ deletion mutant is contrary to the idea that the role of PduQ is simply to provide additional capacity for NADH oxidation. Moreover, this finding suggests that PduQ has a MCP specific function since the salient difference between PduQ and $Zm$Adh2 is that PduQ localizes to the MCP while $Zm$Adh2 is cytoplasmic. For the growth curves shown in Fig. 5 cultures were grown with 75 nM CN-B$_{12}$ with 3 or more repetitions in triplicate. At 75 nM CN-B$_{12}$ level the $A_{pduQ}$ mutant had a clear growth defect (this phenotype is reduced at lower CN-B$_{12}$ concentrations) and the possibility of propionaldehyde toxicity is minimized [6].

Preventing MCP formation genetically is epistatic to the growth defect of a $pduQ$ mutation

We tested the effects of a $pduQ$ deletion on growth of *Salmonella* on 1,2-PD in a background containing a $pduAB$ deletion which was previously shown to prevent formation of the Pdu MCP [6,41]. At lower levels of CN-B$_{12}$, the $A_{pduQ}$ mutant grew slower than wild-type *Salmonella* and the $A_{pduAB}$ mutant grew faster as was previously reported [6,41] (Fig. 5A). More importantly, the $A_{pduAB}$ strain and $A_{pduAB}$ $A_{pduQ}$ strain grew similarly. This indicates that growth defect of the $pduQ$ deletion mutant depends on an intact MCP suggesting that the PduQ enzyme has a specific role in optimizing the function of the Pdu MCP. Furthermore, the finding that the $A_{pduAB}$ $A_{pduQ}$ mutant grew faster than wild-type indicates that the electron transport chain has sufficient capacity to support wild-type rates of 1,2-PD degradation in the absence of PduQ under the conditions used.

We note that the tests shown in Fig. 5A were done at 40 nM CN-B$_{12}$. At this cofactor concentration, $A_{pduQ}$ has a clear phenotype and the chances of propionaldehyde toxicity (which is more severe in the $A_{pduAB}$ background [6,41]) are minimized. Nonetheless, we also compared the growth of the $A_{pduAB}$ and the $A_{pduAB}$ $A_{pduQ}$ mutants on 1,2-PD minimal medium supplement-
ed with saturating concentrations of CN-B₁₂ (150 nM). At higher levels of CN-B₁₂, the epistatic effect of \( \text{D}_{\text{pduAB}} \) on \( \text{D}_{\text{pduQ}} \) was partially obscured by a period of growth arrest (from about hour 17 to hour 27) resulting from accumulation of propionaldehyde to toxic levels as was previously reported [6,41] (Fig. 5B). Even so, it can be seen that the growth of the \( \text{D}_{\text{pduAB}} \text{D}_{\text{pduQ}} \) strain was similar to that of the \( \text{D}_{\text{pduAB}} \) strain until the onset of propionaldehyde toxicity.

The PduQ enzyme can recycle NAD\(^{+}\) for PduP in purified MCPs

To determine whether PduQ can recycle NAD\(^{+}\) for the PduP reaction in vitro, we measured the PduP activity in anaerobically purified MCPs from wild-type \textit{Salmonella} and a \( \text{pduQ} \) deletion mutant. The method used to purify the Pdu MCPs was previously shown to yield intact MCPs [43]. Electron microscopy of both whole cells and purified MCPs indicated that \( \text{pduQ} \) deletion mutant had no major effect on MCP morphology (Fig. S5 and S6). In addition, the yield of MCPs and the protein ratios were similar for both wild-type and \( \text{D}_{\text{pdu}} \) MCP indicating that MCPs lacking PduQ were intact and stable. The PduP assay used measured the formation of propionyl-CoA by monitoring absorbance at 232 nm. Results showed that the concentration of propionyl-CoA produced by wild-type MCP reached 62.3 \( \mu \text{M} \) in 8 min which was higher than the concentration of NAD\(^{+}\) added to the assay (40 \( \mu \text{M} \) ) (Fig. 6). Since the PduP reaction requires 1 molecule of NAD\(^{+}\) per molecule of propionyl-CoA formed, recycling of NADH to NAD\(^{+}\) by PduQ is indicated. In contrast, the concentration of propionyl-CoA produced by MCPs purified from a \( \text{pduQ} \) deletion mutant was 27.6 \( \mu \text{M} \) in the same time period (Fig. 6) which is substantially less than the amount of NAD\(^{+}\) added to the assay (40 \( \mu \text{M} \) ). Results also showed the initial PduP reaction rates were similar in wild-type and \( \text{ApduQ} \) MCPs indicating both contained similar amounts of PduP. In addition, controls showed that there was no measurable PduP activity without added HS-CoA, NAD\(^{+}\) or propionaldehyde. Thus, these results show that purified MCPs recycle NAD\(^{+}\)/NADH such that PduP converts NAD\(^{+}\) to NADH and PduQ converts NADH back to NAD\(^{+}\) which is consistent with internal recycling by PduQ.

Binding interactions between PduQ and PduP

To examine the potential binding interactions between PduQ and PduP \textit{in vitro}, His-tag pull-down assays were performed. Crude cell extracts containing His-tagged bait or potential target proteins were sequentially passed over a Ni-NTA affinity column and then the column was washed and eluted with buffers containing low and high imidazole concentrations, respectively. In this test, binding is indicated for proteins that co-elute with the His-tagged bait at high imidazole concentrations while proteins that do not bind the His-tagged bait pass through the column at low imidazole concentrations. SDS-PAGE demonstrated that native recombinant PduQ co-eluted with N- or C-terminally His-tagged PduP at high imidazole concentrations, and in a reciprocal experiment native recombinant PduP co-eluted with C-terminally His-tagged PduQ (Fig. 7). Control experiments showed that native recombinant PduQ and native recombinant PduP did not bind to the Ni-NTA column. Thus, these results indicate that PduQ and PduP bind to one another under the conditions used. The binding of PduQ and PduP is consistent with roles in regenerating NADH/NAD\(^{+}\) for one another (Fig. 1). In addition, prior studies localized PduP to the interior of the Pdu MCP [29]; hence, these results suggest PduQ is also a MCP lumen enzyme. We also note that native recombinant PduP did not co-elute with PduQ having a His-tag on its N-terminus (Fig. 7 lane 7). This suggests that the N-
terminus of PduQ may be involved in PduQ-PduP binding and that the N-terminal His-tag interfered with this interaction.

Discussion

Prior studies indicated that a 1-propanol dehydrogenase was involved in 1,2-PD degradation and that the role of this enzyme was to regenerate NAD(P)⁺ from NAD(P)H [12,24]. Previous work also showed that a gene in the pdu operon (pduQ) encoded an enzyme with homology to known Adh enzymes [12]. The results presented in this report establish experimentally that the PduQ enzyme of S. enterica is an iron-dependent Adh used for 1,2-PD degradation. Purified PduQ catalyzed the conversion of propionaldehyde to 1-propanol and the reverse reaction with specific activities of 55.5±6.4 and 5.1±0.7 μmol min⁻¹ mg⁻¹, respectively. These rates are similar to those measured for other catabolic Adh enzymes [61]. We also showed that a pduQ deletion mutant was impaired for growth on 1,2-PD which provided in vivo evidence for its role in 1,2-PD degradation. In addition, in vitro studies showed that PduQ prefers NADH to NADPH as a co-substrate which is consistent with its role in regenerating NAD⁺ for the second step of 1,2-PD degradation (catalyzed by the PduP enzyme) which preferentially uses NAD⁺ [29]. Thus, results presented here in conjunction with previous work indicate that PduQ is a 1-propanol dehydrogenase used to regenerate NAD⁺ from NADH during 1,2-PD metabolism.

Our prior work showed that S. enterica uses a MCP for B₁₂-dependent 1,2-PD degradation [12]. Subsequent proteomics studies identified many of the protein components of the Pdu MCP, but missed PduQ [25]. In this study, enzyme assays, SDS-PAGE, Western blot (Fig. 2) and MS-MS demonstrated that PduQ is a component of purified Pdu MCPs. The enzymatic activity of PduQ was enriched 10-fold in purified MCPs compared to crude cell extracts which is similar to results seen for two other MCP enzymes, PduCDE and PduP [25,29]. The finding that PduQ co-purified with the Pdu MCP through a treatment involving detergent extraction and differential centrifugation indicates a tight association between PduQ and the Pdu MCP. In addition, His-tag pull-downs indicated that PduQ binds PduP which is known to localize to the lumen of the Pdu MCP. This suggests that PduQ is also a lumen enzyme. This idea is further supported by the fact that PduQ has a putative C-terminal targeting sequence of...
whether PduQ produces NAD+ is needed to provide extra NADH-oxidation capacity. However, studies with purified MCPs that showed PduQ can convert NADH to NAD+ suggested that PduQ has a MCP-specific function. Moreover, we also conducted in vitro studies described above indicate that the PduQ enzyme in 1,2-PD degradation is to regenerate NAD+ from NADH facilitating recycling to NAD+ via the electron transport chain and eliminating the need for PduQ which plays the primary role in oxidizing NADH internally within the MCP. In addition, results showed that the growth defect of a pduQ mutant could be fully corrected by production of PduQ from a plasmid but not by production of the non-MCP associated iron-dependent Adh2 from Z. mobilis even though Adh2 has similar catalytic properties and was produced with similar specific activity [56] (Fig. 4). This demonstrates that NADH can be converted to NAD+ at a substantial rate independently of PduQ during growth of Salmonella on 1,2-PD. It also suggests that NADH/NAD+ homeostasis within the Pdu MCP is mediated by both internal recycling and specific pores. Importantly, this model could apply to a variety of enzymatic cofactors required by diverse MCPs.

Supporting Information

Figure S1 Sequence alignment by Clustal X2. EcFucO, 1,2-propanediol dehydrogenase from Escherichia coli (GI 16130706); KpDhaT, 1,3-propanediol dehydrogenase from Klebsiella pneumoniae (GI 940440); SeEutG, ethanol dehydrogenase from Salmonella enterica (GI 687647); ZmAdh2, Adh2 from Zymomonas mobilis (GI 5653492); ScPduQ, 1-propanol dehydrogenase from S. enterica (GI 5069460). Boxed: Glycine-rich motif involved in NAD+ binding. Arrows: histidine residues that coordinate iron. Triangle: histidine residue essential for catalysis and thought to interact with the substrate.

Figure S2 SDS-PAGE analysis of the anaerobic purification of PduQ-His6 from E. coli BE1052. Lane 1, protein standards; lane 2, 10 μg whole-cell extract; lane 3, 10 μg soluble fraction; lane 4, 2 μg PduQ-His6 following Ni affinity chromatography. The gel was a Bio-Rad 10–20% gradient ready gel stained with Coomassie.

Figure S3 pH dependence of propionaldehyde reduction and 1-propanol oxidation activities catalyzed by PduQ-His6 of S. enterica. The maximal activities for propionaldehyde reduction and 1-propanol oxidation were achieved at pH 7.0 and 9.0, respectively. The buffers used were 100 mM Na2HPO4-NaH2PO4 at pH 6.0–7.5; 100 mM Tris-HCl at pH 7.5–9.0 and 100 mM Glycine-NaOH at pH 9.0–10.0.
Figure S5  Identification of Fe in purified PduQ-His6p, using ICP-MS (cps: counts per second).
(TIF)

Figure S5  Electron microscopy of wild-type Salmonella enterica and a pduQ deletion mutant. Triangles point to microcompartments. A number of sections were examined and the cells shown are representative.
(TIF)

Figure S6  Electron microscopy of MCPs purified from wild-type Salmonella enterica and a pduQ deletion mutant. The image shown is a representative negative stain.
(TIF)

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