Acetogenic bacteria can grow by the oxidation of various substrates coupled to the reduction of CO₂ in the Wood-Ljungdahl pathway. Here, we show that growth of the acetogen *Acetobacterium woodii* on 1,2-propanediol (1,2-PD) as the sole carbon and energy source is independent of acetogenesis. Enzymatic measurements and metabolite analysis revealed that 1,2-PD is dehydrated to propionaldehyde, which is further oxidized to propionyl coenzyme A (propionyl-CoA) with concomitant reduction of NAD. NADH is reoxidized by reducing propionaldehyde to propanol. The potential gene cluster coding for the responsible enzymes includes genes coding for shell proteins of bacterial microcompartments. Electron microscopy revealed the presence of microcompartments as well as storage granules in cells grown on 1,2-PD. Gene clusters coding for the 1,2-PD pathway can be found in other acetogens as well, but the distribution shows no relation to the phylogeny of the organisms.

Acetogenic bacteria are a diverse group of anaerobic bacteria able to reduce two molecules of CO₂ to acetate by the Wood-Ljungdahl pathway (WLP) (1–4). Electrons may derive from molecular hydrogen (autotrophic growth) or from organic donors (heterotrophic growth), such as hexoses, pentoses, formate, lactate, alcohols, and methyl group donors (1). This not only provides the cell with organic material for biomass formation, but the pathway is also coupled to energy conservation for ATP supply (2, 5). The energy-conserving reactions remained an enigma for a long time, but recent discoveries in the model acetogen *Acetobacterium woodii* provided insights into the energy metabolism of this group of anaerobic bacteria (6, 7). In *A. woodii*, the reactions for the oxidation of the substrate can in general be regarded as isolated modules separate from the reactions of the WLP for the reoxidation of the electron carriers by the reduction of CO₂. One has to emphasize that all enzymes of the WLP are soluble and located in the cytoplasm (6). With molecular hydrogen as the electron donor, only one enzyme is necessary for its oxidation, providing the reducing equivalents as reduced ferredoxin (Fd) and NADH in a 1:1 stoichiometry (8). Oxidation of organic substrates, such as hexoses, also yields reduced ferredoxin and NADH that are reoxidized in the WLP. The WLP in *A. woodii* does not use both electron carriers in equal amounts; therefore, a membrane-bound Fd:NAD oxidoreductase (presumably the Rnf complex) provides NADH from reduced ferredoxin, thereby translocating sodium ions across the cytoplasmic membrane that are used for subsequent ATP synthesis by a membrane-bound, sodium ion-dependent ATP synthase (7, 9, 10). The reaction is reversible, and the enzyme can drive Fd reduction at the expense of the electrochemical sodium ion potential.

Besides CO₂, acetogenic bacteria can use different alternative electron acceptors, e.g., nitrate (*Moorella thermoaericaea* [11]) or phenylacrylates (*A. woodii* [12]). These acceptors have a more positive redox potential than the CO₂-acetate pair, thus providing an energetic advantage. In *A. woodii*, the reduction of the phenylacrylate caffeate was studied in detail and followed the same scheme as described before. The enzymes for caffeate reduction (again all soluble) can be regarded as isolated from the electron-providing reactions, connected by the energy-conserving, membrane-bound Fd:NAD oxidoreductase (13–16). Acetogenic bacteria are able to use alcohols as growth substrates as well, but the metabolism has not been studied in detail. The alcohols facilitating growth in *A. woodii* include single alcohols, such as ethanol or methanol, but also diols, such as 1,2-propanediol (1,2-PD) (17). This diol is thought to be an important substrate in anoxic environments, especially those inhabited by acetogenic bacteria. 1,2-PD is the product of the anaerobic degradation of fusose and rhhamnose. Both sugars are components of the plant cell walls, bacterial exopolysaccharides, and glycoconjugates of intestinal epithelial cells. 1,2-PD can thus be assumed as an important substrate in soils or the larger intestine of higher animals (18).

As expected from the conversion of other substrates, it was reported that the acetogen *Acetobacterium carbonicicum* degrades diols to the corresponding acid and acetate (17). This points to a metabolism in which the alcohol is oxidized to the corresponding acid and the electrons are channeled to CO₂, thereby generating acetate in the WLP. However, an inspection of the genome sequence of *A. woodii* prompted us to question this scenario for this...
model acetogen. In contrast to the expectation, here we will describe a different pathway for 1,2-PD utilization in *A. woodii*, the encoding genes, and their regulation. The process does not involve reactions of the WLP and is independent of acetogenesis. 1,2-PD degradation may play an important role in habitats of acetogenic bacteria, but it seems to be a physiological ability that can operate independent of acetogenesis.

**MATERIALS AND METHODS**

**Growth of *A. woodii***. *A. woodii* (DSM 1030) was cultivated at 30°C under anaerobic conditions. The medium was prepared as described previously (19, 20). Fructose (20 mM), 1,2-propanediol (15 mM), 3,3-butanediol (20 mM), glycine-betaine (50 mM), ethanol (50 mM), methanol (60 mM), or ethylene glycol (20 mM) was used as the substrate. Growth was followed by measuring the optical density at 600 nm (OD600).

**Preparation of cell suspensions.** The medium and all buffers were prepared using the anaerobic techniques described (21, 22). All preparation steps were performed under strictly anoxic conditions at room temperature in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) filled with 95% to 98% N2 and 2% to 5% H2 as described previously (20). *A. woodii* (DSM 1030) was grown until late exponential phase, harvested by centrifugation (10,000 × g; 10 min), and washed two times with imidazole buffer (50 mM imidazole-HCl, 20 mM MgSO4, 20 mM KCl, 20 mM NaCl, 4 mM dithioerythritol [DTE], 1 mM liter−1 resazurin, pH 7.0). Cells were resuspended in imidazole buffer and transferred to Hungate tubes. The protein concentration of the cell suspension was determined as described previously (23). To remove the remaining H2 from the cell suspension from the anaerobic chamber, the gas phase of the cell suspension was changed to N2, and the cells were stored on ice until use. For the experiments, the cells were suspended in the same buffer to a concentration of 1 mg ml−1 in 115-ml glass bottles. The bottles contained a final volume of 10 ml buffer under an N2 atmosphere or an N2 and CO2 (80:20 [vol/vol]) atmosphere at 0.5 × 105 Pa overpressure and were incubated at 30°C in a shaking water bath. Samples for substrate/product determination were taken with a syringe, cells were removed by centrifugation (15,000 × g; 2 min), and the supernatant was stored at −20°C until further analysis.

**Determination of 1,2-PD, propionaldehyde, 1-propanol, and propionate.** Remaining suspended particles were removed from the supernatant of the samples taken from the cell suspension experiments by a second centrifugation step (15,000 × g; 5 min). A total of 400 μl of the resulting supernatant was mixed with 500 μl 13.6 M acetone and 50 μl 2 M phosphoric acid. A total of 50 μl of 200 mM ethanol was added as the internal standard. The samples were analyzed by gas chromatography on a Clarus 580 GC (PerkinElmer, Waltham, MA) with an ELITE-200 capillary column (30 m by 0.25 mm; PerkinElmer, Waltham, MA) with the following temperature profile: 60°C for 1 min followed by a temperature gradient to 160°C with 10°C min−1. Helium was used as the carrier gas with a flow rate of 40 cm min−1. The sample volume was 50 μl, injected with an autosampler (sample temperature of 60°C, injection temperature of 250°C, split of 1:50). 1,2-PD, propionaldehyde, 1-propanol, and propionate were analyzed with a flame ionization detector at 250°C. The peak areas were proportional to the concentration of each substance and calibrated with standard curves. A total of 10 mM ethanol was used as the internal standard in all cases.

**Determination of acetate.** Production of acetate was measured enzymatically in a coupled assay with acetyl coenzyme A (acetyl-CoA) synthetase, citrate synthase, and malate dehydrogenase according to the manufacturer’s instructions (R-Biopharm AG, Darmstadt, Germany).

**Preparation of cell extract.** *A. woodii* was cultivated and harvested as described for the preparation of the cell suspensions. All steps were performed under strictly anoxic conditions. Cells were washed in buffer A (25 mM Tris-HCl, 420 mM saccharose, 2 mM DTE, 4 μM resazurin, pH 7.0), resuspended in 20 ml buffer A with 100 μg lysozyme, and incubated for 60 min at 37°C. Then the cells were sedimented by centrifugation (10,000 × g; 10 min) and resuspended in 3 ml lysis buffer (25 mM Tris-HCl, 20 mM MgSO4, 2 mM DTE, 4 μM resazurin, pH 7.5). Cells were disrupted by a single passage through a French press (SLM Aminco; SLM Instruments, USA) with 100 MPa. Cell debris and whole cells were removed by a centrifugation step (3,500 × g; 20 min), and the supernatant was stored at 4°C.

**Measurement of enzymatic activities.** Measurements of the enzymatic activities were carried out in 1 ml buffer in anaerobic 1.8-ml cuvettes (Glaserättebau Ochs, Bovenden-Lengern, Germany) sealed with a rubber stopper and a gas phase of 100% N2. All measurements were performed at 30°C in a UV/Vis spectrophotometer (type 8453; Agilent Technologies, USA).

**Propanol dehydrogenase activity.** This activity was measured by the propionaldehyde-dependent oxidation of NADH at 340 nm (ε = 6.2 mM−1 cm−1) in buffer containing 50 mM KCl, 2 mM DTE, and 35 mM KPO4 (pH 8.0). A total of 0.5 mM NAD and 20 mM propionaldehyde were added to the standard assay.

**CoA-dependent aldehyde dehydrogenase activity.** This activity was measured by the propionaldehyde-dependent reduction of NAD at 340 nm (ε = 6.2 mM−1 cm−1) in buffer containing 50 mM KCl, 2 mM DTE, and 35 mM KPO4 (pH 8.0). A total of 250 μM CoA, 2 mM NAD, and 20 mM propionaldehyde were added to the standard assay.

**Hydrogenase, formate dehydrogenase, and Rnf activity.** The activities were measured as described previously (8, 24, 25).

**General molecular methods.** PCR and other standard molecular methods were performed as described previously (26). For isolation of total RNA, cells were grown on 1,2-PD to early exponential growth phase, and RNA was extracted using the NucleoSpin RNA II kit (Machery-Nagel, Düren, Germany). RNA was transcribed into cDNA using Moloney murine leukemia virus (MMLV) reverse transcriptase and random primer (both Promega, Mannheim, Germany), which was then used as the template for a PCR. cDNA and chromosomal DNA were used as negative and positive controls, respectively. Used primers (see Fig. S6 in the supplemental material) yielded PCR products bridging the intergenic regions of the genes of interest.

**Bioinformatic DNA sequence analysis.** All DNA and protein sequences were retrieved from the National Center for Biotechnology Information database. Pairwise alignments of protein sequences were performed using ClustalW2 (27, 28). Homology searches were performed using BLASTp with default settings (http://blast.ncbi.nlm.nih.gov/blast.cgi). Protein sequences were analyzed for conserved domains and functional sites by InterProScan 5 (28, 29).

**Analytical methods.** Protein concentration was measured according to Bradford (30) (except for whole cells as described above). Proteins were separated in 12% polyacrylamide gels and stained with Coomassie brilliant blue G250. For immunological detection of PduB (encoded by Awo_c25900), the protein was produced with a His6 tag in Escherichia coli DH5α, purified by affinity chromatography according to the manufacturer’s instructions (New England BioLabs GmbH, Frankfurt/Main, Germany), and used for immunization of a rabbit (Davids Biotechnologie, Regensburg, Germany). Sample preparation for Western blot analysis was performed as described previously (15). A total of 20 μg of each cell extract was separated by SDS-PAGE. Proteins were transferred to a nitrocellulose membrane (Protran BA 83; GE Healthcare, United Kingdom) followed by immunoblotting with a 1:10,000 dilution of the rabbit antiserum. Primary antibody detection was performed with a goat anti-rabbit IgG (H+L)—horseradish peroxidase (HRP) conjugate (Bio-Rad, USA; dilution of 1:10,000).

**Electron microscopy.** Cells were grown to the stationary growth phase, harvested and fixed with 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.25) for 2 h at room temperature, and washed 3 times for 10 min in the same buffer, followed by an incubation in 1% OsO4 in 0.1 M Na-cacodylate buffer (pH 7.25) for 1 h at room temperature. After washing steps in distilled water (dH2O) for 10 min and 3 washing steps in acetate buffer (pH 5.2), pellets were embedded in 3% agar, incubated with 1% uranyl acetate overnight, and washed 3 times in dH2O for 5 min.
followed by dehydrogenation by a graded ethanol series. Samples were embedded in low-viscosity resin, Premix Hard kit (Agar Scientific, Essex, United Kingdom). Ultrathin sections were inspected with a transmission electron microscope (EM208S; FEI, USA) at 80 kV. Images were recorded on a slow-scan 2 k × 2 k charge-coupled-device (CCD) camera (Gatan GmbH).

RESULTS

The pdu gene cluster of *A. woodii* and properties of the deduced gene products. The genome of *A. woodii* harbors a gene cluster of 20 genes (Awo_c25930 to Awo_c25740) whose products are similar to the well-characterized Pdu proteins of *Salmonella enterica* (Fig. 1). We analyzed the function of the putative proteins by pairwise alignment of each protein sequence with the sequence from *S. enterica* using ClustalW2, using global BLAST searches, and by identification of conserved domains or functional residues using InterProScan 5. The identities of the pairwise alignments are given in Fig. 1. The *pdu* cluster of *A. woodii* codes for 10 proteins with putative enzymatic function in 1,2-PD degradation, 3 proteins with unknown function, 5 putative organelle shell proteins, and two proteins with a putative regulatory function. In *S. enterica*, 1,2-PD is dehydrated in the first step by a B12-dependent 1,2-PD dehydratase to propionaldehyde followed by a disproportionation to propanol and propionate (31). In *A. woodii*, the genes *pduCDE* (Awo_c25890 to Awo_c25870) code for three subunits with similarity to the *S. enterica* dehydratase enzyme. The products of the subsequent genes *pduGH* (Awo_c25860 and Awo_c25850) are similar to the proteins responsible for activation of the dehydratase in *S. enterica*. The gene *pduS* (Awo_c25760) codes for a cob(III)alamin and cob(II)alamin reductase that is needed for the synthesis and recycling of the B12 cofactor of the dehydratase (32). PduP (Awo_c25770) is similar to a CoA-dependent aldehyde dehydrogenase that could potentially oxidize propionaldehyde to propionyl-CoA followed by a phosphorylation to propionyl-phosphate catalyzed by the product of *pduL* (Awo_c25830). In *S. enterica*, propionyl-phosphate is converted to propionate by a propionate kinase (PduW), yielding 1 mol of ATP (31, 33). The electrons from the oxidation of propionaldehyde are disposed by the reduction of another propionaldehyde to propanol by an alcohol dehydrogenase (PduQ) (34). Homologs of the genes encoding PduW and PduQ are missing in the gene cluster of *A. woodii*, as is a homolog of the gene encoding PduF, a propanediol facilitator responsible for 1,2-PD uptake. The *pduA*, *pduB*, *pduK*, *pduN*, and *pduT* genes of *A. woodii* potentially encode bacterial microcompartment (BMC) shell proteins. In addition, 3 genes are present in the cluster of *A. woodii* whose products cannot be assigned to a function in 1,2-PD degradation. The product of *Awo_c25820* is similar to the protein EutT that is proposed as a chaperone for the assembly of the BMC in ethanamine degradation in different enterobacteria. The *Awo_c25810* gene codes for a soluble flavoprotein and *Awo_c25750* codes for a protein with 8 predicted transmembrane helices. In both cases, it is not possible to predict a function from the sequence. At the beginning of the *pdu* cluster in *A. woodii* are two genes coding for a putative histidine kinase and a response regulator. The sequence of the kinase (*Awo_c25930*) does not show any predicted transmembrane helices and therefore seems to be a soluble protein. The kinase consists of an N-terminal PocR domain, a middle part belonging to the histidine kinase superfamily, and a C terminus exhibiting the ATPase domain. The response regulator (*Awo_c25920*) has an N-terminal phosphorylation site of the Rec superfamily and a C-terminal helix-turn-helix AraC domain. The gene cluster suggests the potential of *A. woodii* to oxidize 1,2-PD to propionyl-CoA and also suggests the presence of a BMC associated with this metabolism. However, whether or not 1,2-PD is disproportionate as in *S. enterica* or converted to acetate and propionate as described for *A. carbinolicum* could not be delineated from the genomic data. Therefore, the 1,2-PD metabolism in *A. woodii* was analyzed experimentally.

Growth kinetics and product pattern during growth of *A. woodii* on 1,2-PD. As predicted from the genome sequence, *A. woodii* can grow with 1,2-PD as the sole carbon and energy source (Fig. 2). With 15 mM 1,2-PD, the culture reached an average final optical density ± standard error of the mean (SEM) of 0.5 ± 0.02 (n = 3 experiments), with a growth rate of 0.28 h⁻¹. Substrate concentrations up to 45 mM 1,2-PD had no effect on the growth rate or final optical density. With 5 mM 1,2-PD, the final optical density decreased to 0.4 ± 0.01 (n = 3 experiments).

Interestingly, acetate could not be detected in samples from the growing cultures, arguing against the involvement of the WLP in the 1,2-PD metabolism. The independence of 1,2-PD degradation...
from acetogenesis is further emphasized by growth experiments done in the absence of bicarbonate and CO$_2$. These cultures reached the same final optical density as cultures with CO$_2$, demonstrating that 1,2-PD is used as the carbon and energy source and the conversion is independent of an external electron acceptor.

Analysis of the substrate and product pool (Fig. 2) showed that the substrate 1,2-PD is consumed completely in growing cultures and propionate and propanol are formed as products in equal amounts. The consumption of 14.5 mM 1,2-PD resulted in the production of 6 mM each product ($n=3$ experiments). The results are in agreement with a stoichiometry of the fermentation of 1,2-propanediol → 0.5 propanol + 0.5 propionate.

The consumption of 1,2-PD was linear, with a rate of 0.9 mM 1,2-PD h$^{-1}$ ($n=3$ experiments). Propionaldehyde was produced with a constant rate of 0.2 mM h$^{-1}$ during the whole growth experiment, while propionate showed a change in the rate from 0.1 mM h$^{-1}$ in the exponential growth phase to 0.3 mM h$^{-1}$ in the stationary growth phase. By combining the production rate of propionate and propionaldehyde (0.3 mM product h$^{-1}$ during the exponential growth phase), the experiment showed that the substrate is consumed much faster (0.9 mM 1,2-PD h$^{-1}$) than the products are formed. Apparently, the product flows predominantly into the biomass in the exponential growth phase. After approximately 7.5 h, the growth rate decreased and the rate of propionate production increased, arguing that most substrate is now converted to product. The intermediate propionaldehyde was detected only in small amounts (<0.5 mM) in the medium.

**Conversion of 1,2-PD by resting cells.** To analyze the 1,2-PD metabolism in nongrowing (resting) cells, _A. woodii_ was grown on 15 mM 1,2-PD and cells were harvested in the late exponential growth phase and washed two times with the reaction buffer. The protein concentration was adjusted to 1 mg ml$^{-1}$. 1,2-PD was added to the cell suspension at time point zero to a concentration of 15 mM. In contrast to growing cells, 1,2-PD was rapidly degraded within the first 20 min (Fig. 3). The initial rate of 1,2-PD consumption was 2.5 μmol min$^{-1}$ mg$^{-1}$. After 20 min, 1,2-PD was not detectable any more. At the same time, propionaldehyde appeared in the medium with a maximum of 8 mM after 10 min. In the following time period, propionaldehyde decreased and the products propanol and propionate were formed. The production rate of propanol and propionate were much lower (~0.3 μmol min$^{-1}$ mg$^{-1}$) than the consumption rate of 1,2-PD. From 14.5 mM 1,2-PD, 8.3 mM propanol and 6.3 mM propionate were produced ($n=3$ experiments).

The results demonstrate that 1,2-PD is dehydrated in the first step to propionaldehyde that is further disproportionated to propanol and propionate. The rate-limiting step is the conversion of propionaldehyde to propanol and propionate. It was surprising that propionaldehyde appeared as intermediate in high concentrations in the medium outside the cells. With growing cells, only small amounts could be detected, as expected for this toxic intermediate.

**Characterization of the enzymatic activities involved in 1,2-PD utilization.** The experiments with growing and resting cells demonstrated that 1,2-PD is converted to propionate and propanol in _A. woodii_, and acetogenesis is apparently not involved. The genes of the _pdu_ cluster of _A. woodii_ code for putative enzymes of the oxidative part, namely, the conversion of 1,2-PD to propionate. A gene coding an enzyme for the reduction of propionaldehyde to propanol is missing in the cluster. To elucidate the detailed reactions of 1,2-PD utilization in _A. woodii_, we measured the respective enzymatic activities in crude extract of 1,2-PD-grown cells.

1,2-PD is dehydrated to propionaldehyde that is oxidized to propionyl-CoA in _S. enterica_ by a CoA-dependent aldehyde dehydrogenase (35, 36). This activity was also present in 1,2-PD-grown cells of _A. woodii_ (Fig. 4A). NAD was reduced with propionaldehyde with an activity of 3 U mg$^{-1}$. The activity was strictly dependent on the presence of CoA with an apparent _K_m_ value of 120 μM (see Fig. S1A in the supplemental material). The apparent _K_m_ values of NADH and propionaldehyde were determined to be 0.4 mM and 8 mM, respectively (see Fig. S1B and C). Propionaldehyde was the best substrate for NAD reduction, but acetaldehyde could substitute for propionaldehyde with 30% of the activity (see Fig. S2A in the supplemental material). Only very low activity was detected with formaldehyde. NADP served as an alternative electron acceptor, with around 20% of the activity (see Fig. S2B).
Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) were reduced with only 1% of the activity. Ferredoxin was not reduced.

The dehydration of 1,2-PD was measured indirectly by the same assay used for the CoA-dependent aldehyde dehydrogenase. Therefore, 1,2-PD instead of propionaldehyde was used as the substrate, and formation of NADH was followed. The CoA-dependent aldehyde dehydrogenase activity can be measured in this assay only if 1,2-PD is converted to propionaldehyde in the first step. With 1,2-PD as the substrate, NAD reduction was observed with a rate of 0.4 U mg⁻¹. Other diols, such as 2,3-butanediol or ethylene glycol, were also oxidized but, compared to 1,2-PD, with only less than 10% of the activity (see Fig. S3 in the supplemental material).

In S. enterica, NADH generated from propionaldehyde oxidation is reoxidized by the reduction of the same amount of propionaldehyde to propanol. Despite the lack of a gene for an alcohol dehydrogenase (ADH) in the pdu cluster of A. woodii, the experiments with whole cells proved the production of propanol in A. woodii as well. We therefore tried to measure ADH activity in crude extract. With propionaldehyde as the substrate, NADH oxidation could indeed be seen with a specific activity of 0.6 U mg⁻¹. Other diols, such as 2,3-butanediol or ethylene glycol, were also oxidized but, compared to 1,2-PD, with only less than 10% of the activity (see Fig. S3 in the supplemental material).

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The apparent Kₘ values for NADH and propionaldehyde were determined to be 0.17 mM and 2.8 mM, respectively (see Fig. S5 in the supplemental material). Again, 1,2-PD could be used as the substrate as well, and NADH oxidation with 1,2-PD proceeded with a rate of 0.4 U mg⁻¹. In vivo, NADH generated by the oxidation of propionaldehyde to propionyl-CoA should actually be reoxidized by the reduction of propionaldehyde to propanol, and thus the level of NADH should stay constant. That this was apparently not the case may result from an inhibition of propionaldehyde oxidation by the lack of CoA in the assay, which is required for the oxidation of propionaldehyde to propionyl-CoA (see above). Vice versa, propionaldehyde oxidation could be measured initially because NAD is used in large excess and NADH is not present at the beginning.

Regulation of the pdu gene cluster and enzymes of the Wood-Ljungdahl pathway. The genes encoding 1,2-PD-degrading enzymes are clustered with those potentially encoding BMCs. The entire cluster is preceded by genes encoding a two-component system, including a protein with high similarity to the 1,2-PD-specific transcription regulator PocR (31, 37, 38). We asked whether expression of BMC genes is coregulated with genes encoding the 1,2-PD-degrading enzymes. Therefore, mRNA was isolated from cells grown on 1,2-PD and transcribed into cDNA. To test for the presence of mRNA containing the transcripts of several genes, PCR was performed with primers that result in products bridging the intergenic regions. Product is obtained only if the two genes are transcribed on the same mRNA. Primers that bridge pduA/B, pduB/C, pduH/K, and pduK/L all yielded fragments of the expected size, indicating that pduA/B, pduB/C, pduH/K, and pduK/L are one mRNA, respectively (see Fig. S6 in the supplemental material).

We next analyzed the synthesis of the 1,2-PD-degrading enzymes by measuring the activities of key enzymes in cells grown on different substrates. The propanol dehydrogenase (measured in the direction of propionaldehyde reduction) as well as the CoA-dependent propionaldehyde dehydrogenase activities were present in cell extract from cells grown on 1,2-PD as described above. In cell extracts from cells grown on H₂ and CO₂ or fructose, propanol dehydrogenase was present only with 1.5% (7 mM mg⁻¹) of the specific activity, showing that this enzyme seems to be specific for 1,2-PD metabolism. CoA-dependent propionaldehyde dehydrogenase activity was determined in cell extracts from H₂- and CO₂-grown cells with a specific activity of 0.3 U mg⁻¹ and in cell extracts from fructose-grown cells of 0.7 U mg⁻¹, corresponding to 11 and 23% of the activities measured in cells grown on 1,2-PD. Next the cellular levels of a putative BMC shell protein were analyzed with antibodies generated against heterologously produced PduB. PduB was most present in cells grown on 1,2-PD, as expected (Fig. 5). However, other diols, such as 2,3-butanediol or ethylene glycol, as well as ethanol, induced PduB synthesis. Small amounts of PduB were detected in cells grown on fructose and on H₂ and CO₂, whereas it was not detectable in cells grown on methanol.

To test the presence of the WLP during 1,2-PD metabolism, the activities of key enzymes of the WLP were determined. Formate dehydrogenase, hydrogenase, CO-dehydrogenase, and the Fd:NAD oxidoreductase activities were reduced by a factor of around 3 on cells grown on 1,2-PD compared to those on cells...
H₂ and CO₂ as the substrate (39). In both cases, caffeate and CO₂ used as electron acceptor by 0.01 U mg⁻¹ the substrates as indicated, and hydrogenase (H₂ase), formate dehydrogenase (FDH), carbon monoxide dehydrogenase (CODH), and Fd:NAD oxidoreductase.

Alternative electron acceptors for 1,2-PD metabolism. A. woodii is able to use phenylacrylates, like caffeate, as an alternative electron acceptor. The more positive redox potential of caffeate compared to that of CO₂ provides an energetic advantage. Caffeate is used as electron acceptor by A. woodii either with fructose or with H₂ and CO₂ as the substrate (39). In both cases, caffeate and CO₂ are reduced simultaneously. To test if caffeate can serve as an electron acceptor during 1,2-PD metabolism, A. woodii was grown with 20 mM 1,2-PD in the presence of 5 mM caffeate, but caffeate was not reduced. This experiment emphasizes the independence of 1,2-PD metabolism of external electron acceptors. Neither CO₂ nor caffeate are reduced, and the oxidation of propionaldehyde to propionyl-CoA is tightly coupled to the reduction of propionaldehyde to propionaldehyde acceptor. The maximal optical density was reached with 5 mM 1,2-PD and could not be increased by increasing the amount of substrate. When using 15 mM 1,2-PD, apparently around 3 mM 1,2-PD was not converted into products and therefore most likely into biomass. The electron micrographs of cells grown on 1,2-PD also showed the presence of large inclusions resembling storage granules. This

Presence of microcompartments. The pdu operon of A. woodii includes genes coding for putative BMC proteins that are produced during growth on diols (Fig. 5). Electron micrographs proved the presence of polyhedral bodies in the cytoplasm of A. woodii (Fig. 7A). These organelles were absent on cells grown on lactate (Fig. 7B), a substrate where PduB could not be detected in the cell extracts, as shown before. The organelles had a size of around 150 nm and are present in multiple copies per cell. The cells grown on 1,2-PD also showed large inclusions, resembling storage granules, with a diameter above 400 nm.

DISCUSSION

Acetogenic bacteria are classified as acetogens for their ability to produce acetate from two molecules of CO₂ by the reactions of the Wood-Ljungdahl pathway (reductive acetyl-CoA pathway) (1–4). The electrons for this reduction can be provided, for example, by molecular hydrogen or the oxidation of organic substrates, such as hexoses, that are converted to 3 mol of acetate (homoacetogenesis). 1,2-PD was also reported as a substrate promoting growth of some acetogenic bacteria, e.g., A. carbinolicum (17). The products were the corresponding acid, derived from the oxidation of the alcohol, and acetate, derived from the reduction of CO₂ (17). In the present work, we presented evidence that a closely related acetogen, A. woodii, is able to grow on 1,2-PD as the sole substrate independent of acetogenesis.

When growing on 1,2-PD, A. woodii produced almost equal amounts of propionate and propanol as end products, but no acetate was formed. Growth was not dependent on the presence of CO₂ or bicarbonate as the carbon source or the external electron acceptor. The maximal optical density was reached with 5 mM 1,2-PD and could not be increased by increasing the amount of substrate. When using 15 mM 1,2-PD, apparently around 3 mM (corresponding to 20% of the substrate) 1,2-PD was not converted into products and therefore most likely into biomass. The electron micrographs of cells grown on 1,2-PD also showed the presence of large inclusions resembling storage granules. This

![Cellular levels of PduB in cells grown on different substrates. A. woodii was grown on the substrates as indicated and harvested in the late exponential growth phase. Cell extracts were separated on a 12% SDS-PAGE gel. The presence of PduB was determined immunologically with antibodies raised against heterologously produced PduB.](http://jb.asm.org/)

![Enzymatic activities of key enzymes of the WLP and 1,2-PD metabolism in cells grown on 1,2-PD, fructose, or H₂ and CO₂. (A) A. woodii was grown on the substrates as indicated, and hydrogenase (H₂ase), formate dehydrogenase (FDH), carbon monoxide dehydrogenase (CODH), and Fd:NAD oxidoreductase as key enzymes for the WLP were measured in the cell extract. (B) Propanol dehydrogenase and propionaldehyde dehydrogenase were measured in the cell extract as key enzymes of the 1,2-PD metabolism. The specific activities in cell extract from cells grown on fructose were set to 1, and the other activities are relative to this value. Actual activities in cell extract from fructose-grown cells were 62 ± 2 U mg⁻¹ (H₂ase), 2 ± 0.1 U mg⁻¹ (FDH), 3.4 ± 0.2 U mg⁻¹ (CODH), 0.15 ± 0.01 U mg⁻¹ (Fd:NAD oxidoreductase), 7 ± 2 mU mg⁻¹ (propanol dehydrogenase), and 0.64 ± 0.12 U mg⁻¹ (propionaldehyde dehydrogenase).](http://jb.asm.org/)

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could be another sink of the substrate when using increased 1,2-PD concentrations that did not result in further growth. We could not clearly identify genes in the genome of *A. woodii* for the synthesis of poly-B-hydroxyalcanoates, but the genome contains genes for the synthesis and degradation of glycogen (Awo_c03510 to Awo_c03450).

The degradation of 1,2-PD in *A. woodii* proceeds via an initial dehydration of 1,2-PD to propionaldehyde followed by a disproportionation into propanol and propionate. Propionaldehyde is oxidized with NAD as the electron acceptor in a CoA-dependent reaction, yielding propionyl-CoA. The generated NADH is recycled by the reduction of propionaldehyde to propanol in an NADH-dependent reaction. This pathway resembles the 1,2-PD metabolism of the enterobacterium *S. enterica*, where propanol and propionyl-CoA are formed in a similar manner (31, 34, 36, 40). A phosphate propanoyltransferase followed by a propionate kinase leads to propionate from propanoyl-CoA (41). The last reaction leads to the phosphorylation of ADP, yielding one ATP by substrate-level phosphorylation. The intermediate propionaldehyde has damaging effects on DNA and inhibits respiratory processes (42). During growth of *A. woodii*, propionaldehyde could be detected only in small amounts outside the cells. However, when resting cells were used, a concentration of up to 10 mM of the intermediate was produced quickly, followed by a slower conversion to propanol and propionate. In *S. enterica*, the toxic intermediate is trapped inside the BMCs by catalyzing the respective enzymatic reactions inside these proteinaceous microcompartments (42). The *pdu* cluster of *A. woodii* contains genes coding for BMC shell proteins as well. We showed in this study that the genes for these shell proteins are cotranscribed with the enzymes of the 1,2-PD metabolism, and the shell protein PduB could be detected in cell extracts. Electron microscopic images provided evidence for the presence of BMCs in the cytoplasm of *A. woodii* grown on 1,2-PD. Similar compartments were recently described in the acetogen *Acetonema longum* (43). Deletion of genes coding for BMC proteins in *S. enterica* led to the production of propionaldehyde during growth (42). The production of propionaldehyde in resting cells of *A. woodii* could be originated from a loss of BMCs by the washing procedure of the cells prior to the experiment. The washing buffers did not contain 1,2-PD, which could result in not completely closed BMCs or the complete disassembly by so-far-uncharacterized regulation mechanisms subjected to the presence of the substrate or intermediates of the pathway. Noteworthy, a fraction of the propionaldehyde (around 5 mM from 15 mM 1,2-PD) was retained by the cells, which is consistent with the assumption that a small fraction of the BMCs might have survived the procedure.

The conversion of 1,2-PD by resting cells led to a fast production of propionaldehyde followed by a slower disproportionation to propanol and propionate. In growing cultures, 1,2-PD also decreased faster than propanol and propionate were formed, arguing that the rate-limiting step is the formation of propionate and propanol. The enzyme activities in crude extract do not explain the kinetics observed with whole cells. In crude extract, the dehydration of 1,2-PD with 0.4 U mg$^{-1}$ is slower than the oxidation/reduction of propionaldehyde. An explanation could be the velocity of the subsequent steps from propionyl-CoA to propionate, the

**FIG 7** Electron microscopic images of *A. woodii* grown on 1,2-PD (A) or lactate (B). Cells were grown on the respective substrate to the late exponential growth phase. Cells grown on 1,2-PD showed structures with sizes around 100 to 200 nm, resembling microcompartments (m), and much larger structures that could be storage compartments (s).
transport of the products out of the cells, or the involvement of the BMC in these reactions influencing the turnover.

Upstream of the pdu cluster in *A. woodii* are two genes coding for a putative histidine kinase and a response regulator. Upstream of the pdu cluster of *S. enterica* is only a single gene coding for the positive regulator PocR, regulating the expression of the pdu operon in response to 1,2-PD (31, 37, 38). The putative histidine kinase of *A. woodii* has an N-terminal PocR domain, similar to PocR of *S. enterica*, but lacks the C-terminal AraC helix-turn-helix domain for the binding of DNA found in PocR. The PocR domain is assumed in general to be responsible for the binding and sensing of simple hydrocarbon derivatives (44). The AraC domain is encoded in the gene for the response regulator in *A. woodii* that also has an N-terminal phosphorylation site. It seems that in contrast to a single regulator in *S. enterica*, a two-component system is responsible for sensing of a hydrocarbon derivative and the regulation of gene expression in *A. woodii*. The regulation pattern observed in this study argues either for an unspecific sensing of alcohols with a chain length of at least two carbon atoms or the sensing of the aldehyde intermediates of the degradation of each alcohol. Ethanol, ethylene glycol, 1,2-PD, and 2,3-butanediol stimulated the synthesis of the protein PduB encoded within the pdu cluster but not methanol. Small amounts of PduB are present in cells grown on fructose or on H₂ and CO₂, which could result from the fact that *A. woodii* is known to produce small amounts of ethanol on these substrates (45). The observed regulation is in agreement with the measured enzymatic activities where acetaldehyde, the oxidation product of ethanol, could substitute for propionaldehyde, albeit with lower activities. However, cells grown on 1,2-PD showed no ethanol-dependent alcohol dehydrogenase activity. This indicates that the enzymes of the pdu system are responsible for the oxidation of 1,2-PD but not for oxidation of ethanol, and the expression of the pdu genes on this substrate could have the function of an aldehyde detoxification system.

Growth on 1,2-PD in a B₁₂-dependent manner as described for *S. enterica* has been described for different genera, including *Klebsiella*, *Shigella*, *Yersinia*, *Listeria*, *Lactobacillus*, and *Lactococcus* (40, 46). None of these genera includes acetogenic bacteria. Analysis of the genomes of all sequenced acetogens revealed the presence of a pdu cluster similar to the one described in this work in *Acetomema longum*, *Clostridium carboxidivorans*, *Clostridium scatologenes*, and *Eubacterium limosum*. The presence of the pdu cluster is dispersed within acetogenic bacteria with no connection to the phylogeny of the organisms, and single genera include members with and without the pdu cluster, a characteristic of the distribution of the acetogenic metabolism itself. The acetogens with a pdu cluster were isolated from sediments, sheep rumen, and termite guts (47–51). The source of 1,2-PD in all these environments could be the degradation of plant material.

We finally want to address the thermodynamics of the observed disproportionation of 1,2-PD used by *A. woodii* in comparison to the use of CO₂ as the electron acceptor. The oxidation of 1 mol of 1,2-PD to propionate leads to 1 mol of NADH and 1 mol of ATP (Fig. 8). Since NADH is recycled by the reduction of propionaldehyde to propionate, no ATP is consumed, resulting in an ATP gain of 0.5 mol ATP per mol of 1,2-PD. As an alternative, CO₂ could be used as the electron acceptor. In *A. woodii*, 2 NADH, 1 ferredoxin, and 1 H₂ (equivalent to 0.5 NADH and 0.5 ferredoxin) are necessary for the reduction of 2 CO₂ to acetate (6, 8, 24). The endergonic reduction of ferredoxin from NADH is catalyzed by the membrane-bound Rnf complex energized by a sodium ion gradient generated by ATP hydrolysis by the ATP synthase (7, 25). This reverse electron flow consumes ATP and thus the conversion of 1,2-PD to propionate with CO₂ as the electron acceptor would result in a theoretical ATP gain of 0.75 mol ATP per mol of 1,2-PD. Thus, the observed pathway of 1,2-PD disproportionation has theoretically a slightly lower ATP gain than the use of CO₂ as the electron acceptor, but this energetic disadvantage might be compensated by the fact that only one enzyme is needed for the reduction of propionaldehyde to propanol, but a much larger number of enzymes (the complete WLP) would be needed for reduction of CO₂ to acetate. The disadvantages could result on the one hand from the costs for biosynthesis of the enzymes but also from the kinetics, e.g., that the use of one enzyme might be an advantage compared to the kinetics of the whole reaction series of the WLP. This hypothesis is in accordance with the downregulation of the enzymes of the WLP when *A. woodii* grows on 1,2-PD. In summary, the observed pathway, the genetic organization, the regulation, as well as the phylogenetic distribution all point to an independence of acetogenesis and 1,2-PD metabolism that could be an
adaptation and an ecological advantage in anaerobic habitats where 1,2-PD is present as the substrate.

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