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Polyphosphate formation by *Acinetobacter johnsonii* 210A: effect of cellular energy status and phosphate-specific transport system

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Abstract In acetate-limited chemostat cultures of *Acinetobacter johnsonii* 210A at a dilution rate of 0.1 h^{-1} the polyphosphate content of the cells increased from 13% to 24% of the biomass dry weight by glucose (100 mM), which was only oxidized to gluconic acid. At this dilution rate, only about 17% of the energy from glucose oxidation was calculated to be used for polyphosphate synthesis, the remaining 83% being used for biomass formation. Suspensions of non-growing, phosphate-deficient cells had a six- to tenfold increased uptake rate of phosphate and accumulated polyphosphate aerobically up to 53% of the biomass dry weight when supplied with only orthophosphate and Mg^{2+}. The initial polyphosphate synthesis rate was 98 ± 17 nmol phosphate min^{-1} mg $protein^{-1}$. Intracellular poly-β-hydroxybutyrate and lipids served as energy sources for the active uptake of phosphate and its subsequent sequestration to polyphosphate. The H^+-ATPase inhibitor *N,N′*-dicyclohexylcarbodiimide caused low ATP levels and a severe inhibition of polyphosphate formation, suggesting the involvement of polyphosphate kinase in polyphosphate synthesis. It is concluded that, in *A. johnsonii* 210A, (i) polyphosphate is accumulated as the energy supply is in excess of that required for biosynthesis, (ii) not only intracellular poly-β-hydroxybutyrate but also neutral lipids can serve as an energy source for polyphosphate-kinase-mediated polyphosphate formation, (iii) phosphate-deficient cells may accumulate as much polyphosphate as activated sludges and recombinants of *Escherichia coli* designed for polyphosphate accumulation.

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Introduction

Three enzymes have been suggested to be involved in the biosynthesis of polyphosphate: polyphosphate kinase (EC 2.4.7.1; PPK), 1,3-diphosphoglycerate: polyphosphate phosphotransferase (Kulaev 1979) and dolychylpyrophosphate:polyphosphate phosphotransferase (Kulaev 1990). Only the pathway that involves PPK has been unambiquously demonstrated in bacteria (Wood and Clark 1988; Kornberg 1995). PPK catalyses the formation of long-chain polyphosphate, up to 1000 P_i residues, in a reversible reaction: polyP_n + ATP ⇔ polyP_{n+1} + ADP. The enzyme is activated by Mg^{2+} and acts in a processive way (Ahn and Kornberg 1990; Akiyama et al. 1992).

The gene encoding PPK has been cloned, sequenced, knocked out and overproduced in *E. coli* (Akiyama et al. 1992) and *Klebsiella aerogenes* (Kato et al. 1993b). Although the gene *ppk* seems also to be present in *A. johnsonii* 210A (H.Y. Kim, personal communication), crude cell-free extracts of a wide variety of *Acinetobacter* spp. showed only low PPK activities, ranging from 0.7 to 4.7 nmol min^{-1} mg $protein^{-1}$ (T'Seyen et al. 1985). Similar values were reported for *Acinetobacter* strains B8 and P, determined by a spectophotometric assay method, but in extracts from other strains, including *A. johnsonii* 210A, the enzyme did not show any activity at all (Van Groenestijn et al. 1989a). Moreover, the incorporation of the terminal phosphoryl group of ATP into polyphosphate has not been demonstrated in these bacteria. This also holds for bacteria responsible for enhanced biological phosphorus removal in activated sludge (Toerien et al. 1990).

The first aim of this work was to determine the effect of the energy status of phosphate-sufficient, wild-type *A. johnsonii* 210A on its accumulation of polyphosphate during its growth in the chemostat with acetate as carbon source and glucose as auxiliary energy source. Glucose was only oxidized to gluconate by the organism when exogenous pyrroloquinoline quinone (PQQ) was provided (Duine 1991; Van Veen et al. 1993a). *A. johnsonii* 210A contains two phosphate-uptake systems with K_m values of 9 ± 1 μM and 0.7 ± 0.2 μM (Van Veen et al. 1993a). The constitutive, proton-motive-force-driven, low-affinity system mediated the bidirectional transport of phosphate complexed with divalent cations such as $MgHPO_4$ and $CaHPO_4$ (referred to as $MeHPO_4$) (Van Veen et al. 1993b). The binding protein and ATP-dependent, high-affinity transport system mediated the uptake of $H_2PO_4^-$ and HPO_4^{2-} (Van Veen et al. 1994b). The synthesis of this transport system, referred to as the Pst system, was six- to tenfold stimulated by phosphate deficiency (Bonting et al. 1992a; Van Veen et al. 1993a). Therefore the potential of phosphate-deficient cells to accumulate polyphosphate was also studied, using *in vivo* ^{31}P-NMR spectroscopy and non-growing cell suspensions. Phosphate-deficient cells were also used to produce evidence for a PPK-mediated polyphosphate formation.

Materials and methods

Microorganism and cultivation

Acinetobacter strain 210A was isolated from activated sludge showing enhanced biological phosphorus removal by Deinema et al. (1985), later identified as *Acinetobacter johnsonii* 210A and deposited in the Netherlands Culture Collection of Microorganisms under the access number LMAU A 130 (Bonting et al. 1992b). It was aerobically grown at 25 °C in 250-ml shaking flasks containing 100 ml medium. A culture pre-grown under similar conditions served as inoculum. This batch medium contained (g l^{-1}) sodium butyrate 1.14, NH_4Cl 0.5, $MgSO_4 \cdot 7H_2O$ 0.25, KH_2PO_4 1.5, K_2HPO_4 0.5 and 2 ml trace element solution as described by Van Groenestijn et al. (1987) (initial pH 7.0). This medium is referred to as the high-P_i medium. Growth was measured by estimating the absorbance at 660 nm and the uptake of phosphate was measured by determining the phosphate concentration in the culture fluid at different times. To obtain phosphate-deficient cells, the organism was grown in the above butyrate medium with only 0.011 g KH_2PO_4 as P-source, 0.215 g KCl as K-source and 6.0 g TRIS/HCl, pH 7.2, as buffer per liter. This modified medium is referred to as the low-P_i medium. The organism was unable to use TRIS as carbon and energy source.

Continuous cultures were grown in a 3-l chemostat (Applikon, The Netherlands) at a dilution rate of 0.1 h^{-1}. The dissolved oxygen concentration and pH were monitored by an Applikon Biocontroller 1030 and maintained at 80%–90% air saturation and 7.0, respectively. The temperature was kept at 20 °C. The medium contained (g l^{-1}) NH_4Cl 1, $MgSO_4 \cdot 7H_2O$ 0.6, KH_2PO_4 0.6, $CaCl_2 \cdot 2H_2O$ 0.07, KCl 0.75 and 4 ml trace element solution. The carbon and energy source was sodium acetate $\cdot 3H_2O$ (1.36 g l^{-1}) or sodium butyrate (1.14 g l^{-1}). Glucose was added to the medium up to concentrations of 100 mM. When added to growth media, the concentration of PQQ was 100 nM.

Polyphosphate formation by non-growing suspensions of phosphate-deficient *A. johnsonii* 210A

Cells of 1 l culture grown in the low-P_i medium were harvested by centrifugation (19 000 *g*, 10 min, 5 °C), washed twice with 50 mM TRIS/HCl buffer, pH 7.0, and resuspended in 100 mM TRIS/HCl buffer (containing 100 mM KCl), pH 7.0 (or 100 mM K-PIPES, pH 6.7). After addition of glucose, PQQ, KH_2PO_4 and $MgSO_4$ to final concentrations of 20 mM, 0.01 mM, 10 mM and 5 mM respectively, and 1 ml D_2O/10 ml suspension, polyphosphate synthesis was started by gassing the suspension with 100% O_2, using an air-lift system as described by Van Veen et al. (1994a). ^{31}P-NMR spectroscopy was used to follow the formation of polyphosphate and the consumption of orthophosphate. At intervals, samples were taken directly out of the NMR tube to measure intracellular ATP (Otto et al. 1984). The H^+-ATPase inhibitor *N*,*N*′-dicyclohexylcarbodiimide (DCCD) was applied at a concentration of 7.5 μg mg protein^{-1}.

Glucose dehydrogenase activity of intact cells

The activity of glucose dehydrogenase of freshly harvested cells, grown either as batch cultures or in the chemostat, was measured with a biological oxygen monitor (Yellow Springs Instruments, Ohio, USA) at 20 °C. The cells were washed twice with 50 mM TRIS/HCl buffer (pH 7.0). A 2-ml sample of the suspension was added to 2 ml buffer with or without 4 mM $CaCl_2$. The reaction was started by injection of glucose to a final concentration of 25 mM. Oxygen consumption was measured polarographically with a Clark-type oxygen electrode. Oxygen uptake rates with glucose were corrected for endogenous respiration. When added to the assay mixture for glucose dehydrogenase, PQQ had a final concentration of 2.5 μM.

Total lipid extraction

Freeze-dried cells were extracted with chloroform/methanol/water (2:2:1.8, v/v/v) by the method of Bligh and Dyer (1959). The extracted lipids were then washed according to the procedure of Folch et al. (1957). The total lipid content was measured after drying under nitrogen. Fatty acid methyl esters were prepared by a 15-min incubation at 95 °C in boron trifluoride/methanol as described by Morrison and Smith (1964). The fatty acid methyl esters were extracted with hexane. PHB was extracted and quantified according to the method described by Braunegg et al. (1978).

Fatty acid analysis

Fatty acid composition was determined by gas chromatography: with a 50-m-long capillary column model CP-Sil 88, a temperature programme of 160–220 °C, and a flame ionization detector. The instrument used was a CP-9000 gas chromatograph (Chrompack-Packard). The fatty acids were identified with the aid of standards and the relative amounts were determined from the peak areas of the methyl esters with a Chromatopac C-R6A integrator (Shimadzu, Kyoto, Japan). Replicate determinations showed that the standard deviation of the values was 2%–5%. The values given represent the sum of the *cis* and *trans* isomers.

Analytical methods

Dry weight was measured after centrifugation of a 200-ml sample (19 000 *g*, 10 min, 5 °C), washing the pellet once with demineralized water and drying at 100 °C overnight. Protein was determined by the method of Lowry et al. (1951), with bovine serum albumin as the standard. Orthophosphate and the total phosphorus content of the cells were determined by persulfate digestion according to Standard Methods (American Public Health Association 1976). The polyphosphate content of the cells was calculated from the

total phosphorus content minus the biosynthetic amount of phosphorus that equals 1.5% of the dry weight (Meganck and Faup 1988; Bonting et al. 1992b). Acetate, butyrate and gluconic acid were determined by HPLC, with a Chrompack organic acids column (30 × 6.5 mm inner diameter) and were detected with a differential refractometer (LKB 2142) and a UV detector (LKB 2158). The mobile phase was a 5 mM H_2SO_4 solution, with a flow rate of 0.6 ml min^{-1}. The working temperature was 60 °C. Samples (20 µl each) were injected using a Spectra Physics autosampler (SP8775). Glucose was measured spectrophotometrically with the anthrone/sulfuric acid method (Trevelyan and Harrison 1952).

Stoichiometric coefficients

These coefficients were calculated according to the models developed for the stoichiometry and energetics of microbial growth (Roels 1983).

Results

Accumulation of polyphosphate by phosphate-sufficient cells

When *A. johnsonii* 210A was grown in batch cultures on mixtures of acetate or butyrate plus 5 mM glucose and PQQ, the same growth yields and cellular polyphosphate contents were observed as with acetate or butyrate alone in spite of the fact that glucose was completely oxidized to gluconic acid. This is in line with previous work with *A. calcoaceticus* (Van Schie et al. 1987; see also Duine 1991), where it was suggested that growth in batch cultures is not energy-limited.

In acetate-limited continuous cultures of *A. johnsonii* 210A, however, an increase in biomass was observed from 172 mg dry weight l^{-1} to a maximum of 250 mg dry weight l^{-1} when the glucose concentration in the feed was increased (Fig. 1A). The polyphosphate content of the cells increased simultaneously from 13% to 24% of the biomass dry weight (Fig. 1B). Both cell yield and polyphosphate content increased linearly with the amount of gluconic acid produced from glucose. Glucose also increased the biomass and the polyphosphate content when added to chemostat cultures growing with butyrate as limited carbon source (data not shown).

Cells grown on acetate in the chemostat in the absence and presence of glucose were examined for three polymers other than polyphosphate: protein, poly-β-hydroxybutyrate (PHB) and lipids. Cells grown at different glucose concentrations possessed fairly constant protein and lipid contents, namely 52.0 ± 2.5% and 1.5 ± 0.1% of the biomass dry weight respectively. No cytoplasmic PHB accumulation could be detected in cells grown in a wide variety of glucose concentrations.

Washed cells of *A. johnsonii* 210A, grown in the absence of glucose and PQQ, appeared to be unable to oxidize glucose unless PQQ was provided (Table 1). Reconstitution of glucose dehydrogenase did not only require PQQ, but was also almost threefold improved by 2 mM $CaCl_2$. Ca^{2+} could be replaced by the supernatant from the chemostat culture, probably because of the presence of Ca^{2+} and Mg^{2+}. When the organism was grown in the presence of glucose and PQQ, somewhat similar oxidation rates for glucose were observed to those detained with cultures grown without glucose and PQQ (Table 1), indicating that glucose plus PQQ did not further induce the synthesis of the apo-enzyme. Glucose and the coenzyme PQQ were also separately examined for their ability to stimulate apo-enzyme synthesis, in the presence and absence of the protein synthesis inhibitor chloramphenicol. Both glucose and PQQ did not further induce the synthesis of the apo-enzyme (data not shown).

The activity of washed cells was 35%–50% lower than that of fresh unwashed samples taken from the chemostat (Table 1). Unwashed cells possessed a maximum specific activity of glucose dehydrogenase that could account for the observed formation of gluconic acid in the chemostat. In fact there was a slight overcapacity of glucose dehydrogenase. The highest specific activity, calculated from the production of gluconic acid in the chemostat at a dilution rate of 0.1 h^{-1}, amounted to 368 µmol glucose min^{-1} g dry $weight^{-1}$. The apparent K_m value for glucose, as measured with whole cells, was 1.8 mM, a value similar to that found in other *Acinetobacter* species (Dokter et al. 1987).

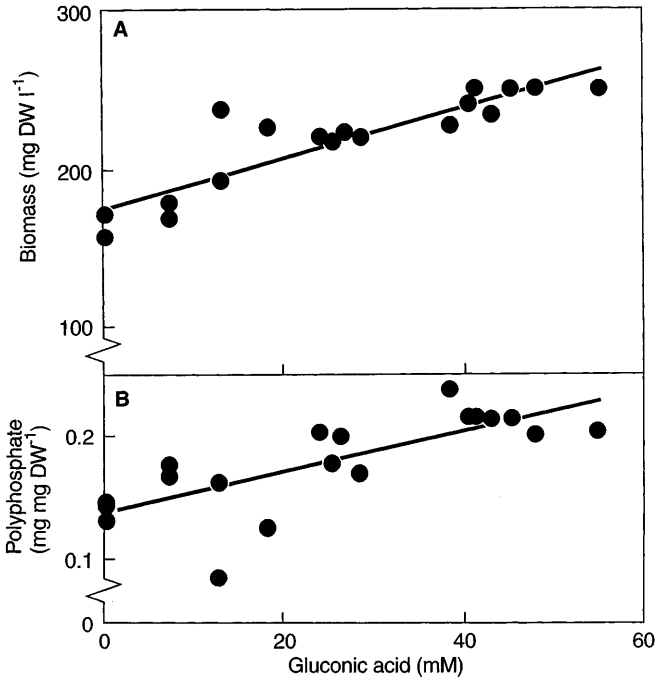

Fig. 1 **A** Relation between biomass formation and gluconic acid production from glucose in continuous cultures of *Acinetobacter johnsonii* 210A growing on acetate. *DW* dry weight. **B** Relation between cellular polyphosphate content and gluconic acid production from glucose in continuous cultures of *Acinetobacter johnsonii* 210A growing on acetate

Table 1 Dependence of *Acinetobacter johnsonii* 210A glucose dehydrogenase activity (nmole of glucose per minute and per milligram dry weight) on pyrroloquinoline quinone (*PQQ*) and on pretreatment of the cells. The bacteria were grown in continuous culture under the conditions described in Materials and methods. The PQQ was added to cell suspensions in the biological oxygen monitor

Growth	Pretreatment	Glucose dehydrogenase activity (nmol Glc min^{-1} mg^{-1})	
		−PQQ	+PQQ
Acetate	Washed	0	60
	Washed + Ca^{2+}	0	164
	Washed + supernatant	0	177
Acetate + PQQ + 30 mM glucose	Washed	156	156
Acetate + PQQ + 30 mM glucose	Not washed	240[a]	–

[a] Endogenous respiration was assumed to be identical with that of washed cells grown in the presence of PQQ and 30 mM glucose

Accumulation of polyphosphate by suspensions of phosphate-deficient cells

Orthophosphate added to suspensions of phosphate-deficient cells rapidly disappeared from the external medium, and polyphosphate was formed (Fig. 2). No other major signals in the ^{31}P-NMR spectrum were observed, indicating that orthophosphate was most likely largely converted into polyphosphate. Based on the results of five independent experiments, an initial polyphosphate formation rate of 98 ± 17 nmol phosphate min^{-1} mg $protein^{-1}$ was calculated. No intracellular accumulation of orthophosphate occurred. However, if suspensions of phosphate-deficient cells were incubated without added Mg^{2+} (the activator of PPK), measured over the first 10 min of the incubation period the rate of polyphosphate synthesis was three- to sixfold reduced and a significant intracellular orthophosphate accumulation up to 20–40 mM occurred (Fig. 3). The initially high uptake rate during the first 2 min of the incubation period rapidly declined, but 2 mM Mg^{2+}, restored polyphosphate synthesis and the intracellular orthophosphate accumulation disappeared along with the extracellular orthophosphate. As with phosphate-sufficient cells, only long-chain polyphosphates of about 700 phosphate groups were synthesized by phosphate-deficient cells. The polyphosphates were localized in the cytoplasm of the cells, and not in the periplasm (Bonting 1993). In the experiments reported in the Figs. 2 and 3, glucose did not affect polyphosphate formation. This is because of the enormous endogenous respiration as a result of the presence of substantial amounts of intracellular carbon polymers (Bonting et al. 1992a; Van Veen et al. 1993a).

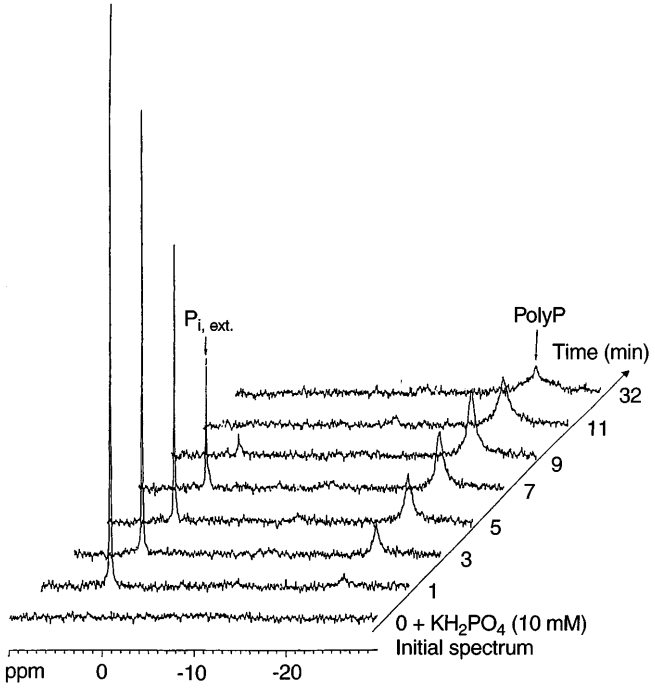

Fig. 2 Polyphosphate formation by phosphate-deficient cells of *A. johnsonii* 210A at 25 °C in TRIS/HCl buffer, pH 7.0, containing 100 mM KCl and 5 mM $MgSO_4$, as measured by in vivo ^{31}P-NMR. The reaction was started by injecting KH_2PO_4 to a final concentration of 10 mM. *PolyP* polyphosphate

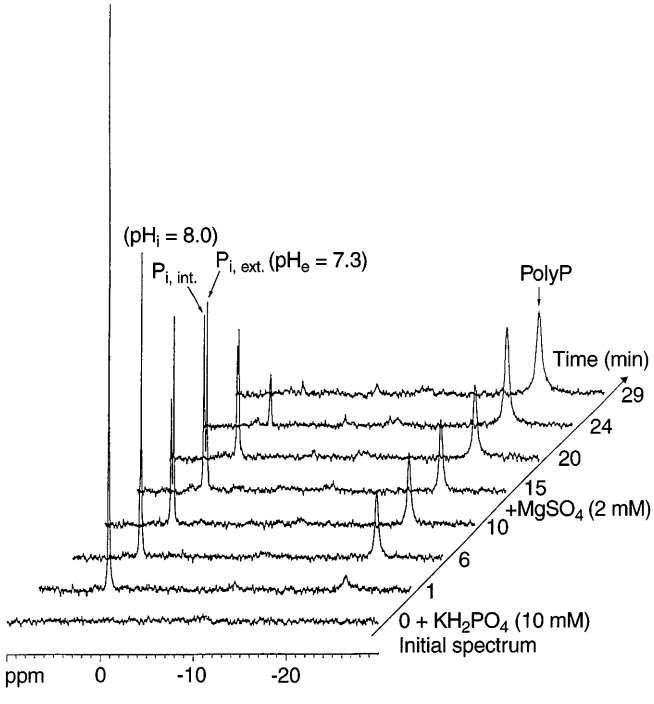

Fig. 3 Polyphosphate formation by phosphate-deficient cells of *A. johnsonii* 210A at 25 °C in TRIS/HCl buffer, pH 7.0, containing 100 mM KCl, initially in the absence of added Mg^{2+}, as measured by in vivo ^{31}P-NMR. After an incubation period of 15 min, $MgSO_4$ was added to a final concentration of 2 mM. pH_e external pH, pH_i internal pH, P_i, *int*. intracellular phosphate; P_i, *ext*. extracellular phosphate, *PolyP* polyphosphate

To investigate whether ATP was involved in the synthesis of polyphosphate, cells were pre-incubated with the H^+-ATPase inhibitor DCCD. After a pre-incubation of 45 min a relatively high intracellular ATP level and a significant polyphosphate formation were observed (Fig. 4). Incubation of the cells with the ATPase inhibitor for 135 min resulted in a significantly reduced cellular ATP concentration and polyphosphate synthesis. When the pre-incubation period was prolonged to 225 min, the cells showed very low ATP levels and were no longer able to synthesize polyphosphate (Fig. 4). Non-growing suspensions of phosphate-sufficient cells were also examined for polyphosphate formation using ^{31}P-NMR and, in this case, glucose was provided as an extracellular energy source. No clear-cut increase in the polyphosphate signal was observed during an incubation period of 30 min.

In none of the polyphosphate synthesis experiments were the signals of short-chain polyphosphates, such as pyro-, tri- and tetraphosphate, or resonances of terminal and penultimate phosphate groups of longer polyphosphate chains observed, pointing to a direct synthesis of highly polymerized polyphosphates via a processive mechanism.

In several polyphosphate synthesis experiments a number of phosphorylated compounds, such as acetylphosphate, phospho*enol*pyruvate, ADP and glucose-6-phosphate, were examined by ^{31}P-NMR for their ability to serve as precursors of polyphosphate in cell-free extracts. None of them gave a polyphosphate signal in the NMR spectrum.

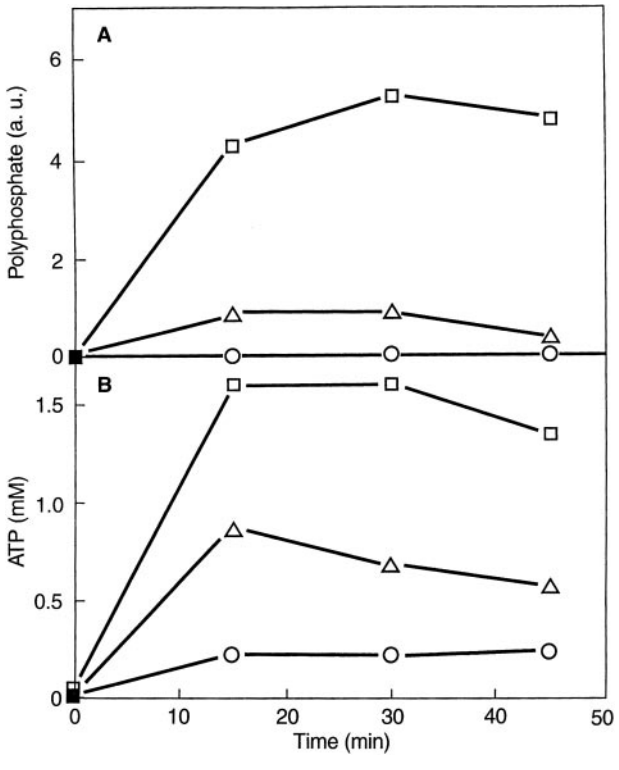

Fig. 4A, B Inhibition of polyphosphate formation in *A. johnsonii* 210A by *N,N'*-dicyclohexylcarbodiimide (DCCD). Time course of polyphosphate formation (**A**) and intracellular ATP levels (**B**) in 100 mM K-PIPES buffer (pH 6.7) containing 10 mM $MgSO_4$, 50 mM glucose and 0.02 mM pyrroloquinoline quinone. Pre-incubation with DCCD was for 45 min (□), 135 min (△) and 225 min (○). *a.u.* arbitrary units

Accumulation of carbon polymers by phosphate-limited cells

On re-examining the composition of carbon polymers in phosphate-deficient *A. johnsonii* 210A, it was found that, in addition to the earlier detected PHB (Bonting et al. 1992a), the cells also contained about 20% lipids on a dry-weight basis. Palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$) and oleic acid ($C_{18:1}$) were the predominant fatty acids of these lipids, in an approximate molar ratio of 1:1:2. Glycerol was easily detected in the aqueous phase of the lipid hydrolysate (not shown). Other minor fatty acids were lauric acid, β-hydroxylauric acid, myristic acid and stearic acid. Cells grown on the high-P_i medium contained only about 1.5% lipids on a dry-weight basis, with a $C_{16:0}/C_{16:1}/C_{18:1}$ molar ratio of about 2:1:1, similar to the situation in the *Acinetobacter* strains P, Q and R (Deinema et al. 1980).

Figure 5 shows that both PHB and lipids were oxidized during the uptake of extracellular phosphate and its subsequent sequestration to polyphosphate by phosphate-deficient cells. The cellular polyphosphate content at the end of the experiment amounted to 53% of the biomass dry weight at the most. The two carbon polymers found in phosphate-deficient cells of *A. johnsonii* 210A fulfil the three criteria to be considered as energy reserves: (1) they are accumulated when growth is lim-

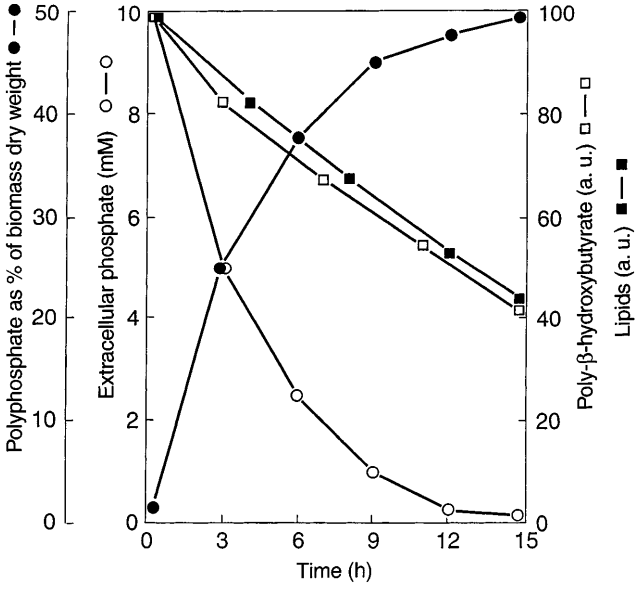

Fig. 5 Polyphosphate formation by phosphate-deficient cells of *A. johnsonii* 210A driven by oxidation of poly-β-hydroxybutyrate and lipids. Incubation conditions were as described in Fig. 2 and the incubation contained 1.9 g biomass dry weight/l. *a.u.* arbitrary units

ited by a nutrient (in this case phosphate) in the presence of excess carbon and energy source, (2) they are utilized when there is insufficient exogenous carbon and energy source and (3) their catabolism yields energy and CO_2.

Discussion

Oxidation of glucose by a conventional PQQ-dependent glucose dehydrogenase (Duine 1991) in an acetate-limited continuous culture of *A. johnsonii* 210A at a dilution rate (D) of 0.1 h^{-1} resulted in an increase in cell yield of about 45% and an increase in cellular polyphosphate content by about 85%. Without glucose, 42% of the acetate provided was assimilated (Fig. 6). Of the assimilated acetate, 21% will be converted to carbon dioxide (Gommers et al. 1988). Glucose oxidation to gluconic acid provides the cells with additional energy and can replace a part of the acetate used for dissimilation. Consequently more acetate was left to be assimilated when glucose was supplied (Fig. 6). At the maximum glucose oxidation rate (at $D = 0.1\ h^{-1}$), about 62% of the acetate was assimilated. Theoretically acetate can be assimilated completely, with glucose serving as the sole source of energy. However, *A. johnsonii* 210A could oxidize 55 mM glucose at the most, which is 63% of the value required for complete assimilation of acetate. The experiments with glucose addition have clearly shown that polyphosphate accumulation by this organism is to some extent proportional to the energy supply and the results form an extension of previous results obtained under sulfur-limited conditions (Van Groenestijn et al. 1989b). However, by using the models for the energetics of microbial growth described by Roels (1983), we calculated that only about 17% of the energy from glucose oxidation was spent on polyphosphate formation and as much as 83% on biomass formation, and the cells only showed a polyphosphate content of about 25% on a dry-weight basis. Only by using phosphate-deficient cells, which possessed a six- to tenfold increased uptake rate of orthophosphate (Bonting et al. 1992a; Van Veen et al. 1993a), could the polyphosphate content of the cells be increased to about 50%. This may also be partly the result of a derepressed synthesis of PPK since, in *Klebsiella aerogenes* and possibly other gram-negative bacteria as well, including *Acinetobacter* spp, the synthesis of PPK is derepressed by phosphate starvation (Harold 1966; Bonting 1993).

Although we were unable to detect PPK activity spectrophotometrically in crude cell-free extracts of *A. johnsonii* 210A, the indirect evidence for a PPK-mediated polyphosphate synthesis is as follows: (i) only highly polymerized polyphosphate, mediated via a processive mechanism, was formed, (ii) polyphosphate synthesis was inhibited by DCCD, indicating the involvement of ATP, (iii) Mg^{2+} strongly stimulated polyphosphate formation and this divalent cation is the known specific activator of PPK, (iv) no other precursors of polyphosphate could be detected by ^{31}P-NMR during polyphosphate formation, (v) a number of central phosphorylated intermediates could not serve as precursors of polyphosphate and we were not able to detect a membrane-bound proton-translocating enzyme that catalysed polyphosphate formation.

The polyphosphate content of polyphosphate-accumulating bacteria is strongly dependent on growth conditions and may vary from 1% to 25% of their dry weight (Harold 1966; Toerien et al. 1990; Meganck and Faup 1988; Appeldoorn et al. 1992; Bonting 1993). Activated sludges from full-scale treatment systems showing enhanced biological phosphorus removal possess polyphosphate contents ranging from 5% to about 20% of their dry weight (Meganck and Faup 1988; Toerien et al. 1990). The highest polyphosphate contents, at least 50% on a dry weight basis, have been encountered in (i) activated sludges from laboratory-scale treatment systems (Wentzel et al. 1988, 1989; Appeldoorn et al. 1992; Smolders 1995) designed for biological phosphorus removal, (ii) *Microlunatis phosphovorus* (Nakamura et al. 1995) and the unidentified bacterium of Ubukata and Takii (1994), and (iii) in recombinants of *E. coli* containing increased dosages of the genes encoding PPK and the Pst transport system (Kato et al. 1993a,b; Hardoyo et al. 1994). Since phosphate-deficient cells of *A. johnsonii* 210A may accumulate polyphosphate to about 50% of their dry weight, these cells clearly belong to the latter group of polyphosphate-accumulating bacteria. The possible usefulness of *Acinetobacter* spp. and the above recombinants of *E. coli* in wastewater treatment systems has been discussed previously (Kortstee et al. 1999).

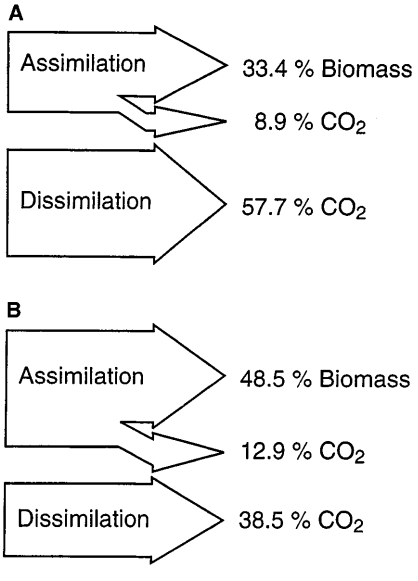

Fig. 6A, B Schematic representation of the influence of glucose as an auxiliary energy source on the assimilatory and dissimilatory flows of acetate in *A. johnsonii* 210A under substrate-limiting growth conditions. **A** Without glucose, **B** with maximum glucose oxidation

In *A. johnsonii* 210A the constitutive $MeHPO_4$ carrier and the ATP- and binding-protein-dependent phosphate-uptake system enable the organism to acquire phosphate efficiently from its habitat through uptake of the predominant phosphate species $MeHPO_4$, $H_2PO_4^-$ and HPO_4^{2-} (Van Veen et al. 1993a,b; Van Veen et al. 1994b). The ATP-dependent transport system has to be inactivated to a certain extent when phosphate-deficient cells encounter phosphate-rich environments to prevent unnaturally high intracellular phosphate concentrations. Inactivation of the ATP-dependent phosphate-uptake system may occur in high intracellular phosphate concentrations (Van Veen et al. 1994a). The rapid levelling-off of the uptake of phosphate by cells with a strongly reduced capacity to synthesize polyphosphate as a result of lack of Mg^{2+}, the activator of PPK (Fig. 3), was therefore not surprising.

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