Regulation of Intracellular Pyrophosphatase-Activity and Conservation of the Phosphoanhydride-Energy of Inorganic Pyrophosphate in Microbial Metabolism

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The conservation of the phosphoanhydride-energy of inorganic pyrophosphate (PP) in microbial metabolism requires a stringent metabolic control of the intracellular pyrophosphatases (PPases, EC 3.6.1.1). In this article, the rate of intracellular PP-liberation during biosynthesis of cellular constituents is calculated from the specific growth rate and the macromolecular composition of the respective microorganism. This rate is compared with the maximal specific activity of PPase in cell free extracts or purified enzyme preparations in order to investigate the possibility of the limitation of biosynthesis through PPase-activity. The catalytic and regulatory properties of microbial PPases are discussed in respect to the occurrence of PP-dependent transphosphorylases. The evidence showing that certain anaerobic microorganisms including photosynthetic bacteria, can use PP instead of ATP as phosphate donor for transphosphorylation reactions will be discussed.

Introduction

Biosynthesis of macromolecules in living cells is characteristically accompanied by the liberation of inorganic pyrophosphate (PP). Furthermore, PP is a byproduct of numerous reactions leading to the synthesis of monomers required for polymer synthesis. Since the work of Kornberg [1] it is presumed that in growing cells the equilibria are shifted in the direction of biosynthesis through the hydrolytic split of PP to inorganic phosphate (P_i) by the action of specific inorganic pyrophosphatases (PPases, EC 3.6.1.1). The general view up to now was that PPases display such high activities in the cells that the concentration of PP in cytoplasm approaches zero [2].

However, in the last few years several papers were published showing clearly that the intracellular concentration of PP is in a range (0.1 – 1 mM) which is comparable to that of other intermediates of energy metabolism [3–5]. Thus, the question arises whether the concept of PP as a waste product of biosynthesis which has to be removed solely by hydrolytic splitting to P_i is true for all organisms. In this article it will be shown that in some microorganisms the rate of biosynthetic PP-liberation approaches the maximal capacity for PP-hydrolysis. The literature presenting evidence that in certain microorganisms the phosphoanhydride-energy of the PP-molecule is conserved through the action of PP-specific transphosphorylases will be discussed.

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Abbreviations: ATCC, American Type Culture Collection, Rockville, Maryland (USA); DSM, Deutsche Sammlung von Mikroorganismen, Grisebachstr. 8, D-3400 Göttingen.

Calculation of the Rate of PP-Liberation during Biosynthesis of Cell Material

To prove the validity of the assumption that in certain organisms the control of PPase activity *in vivo* can limit the rate of biosynthesis [6, 7], one must calculate the rate of PP-liberation during growth from the growth rate of the respective organism and compare it with the maximal capacity of PP-hydrolysis through the action of PPases.

Although the maximal *in vitro*-PPase activity (assayed under optimal conditions of pH and substrate concentrations) of a given organism must not necessarily reflect the rate of PP-hydrolysis *in vivo*, the ratio between maximal cell-free PPase activity and the calculated rate of PP-liberation provides a useful parameter to prove the possibility of PPase-regulation in the growing bacterial cell.

The total amount of PP liberated during biosynthesis of a given amount of cellular dry mass can be calculated from the composition of the biomass in terms of biopolymers (proteins, nucleic acids, polysaccharides). For bacteria, which are mainly considered here, the data given by Gunsalus and Shuster [8] will be used. Analyses carried out in our

own laboratory show that these data are also valid for the phototrophic bacterium *Rhodospirillum rubrum*.

The calculation of PP-liberation in the course of protein-, nucleic acid- and polysaccharide synthesis is based on the assumption that — according to equations (1) to (3) — for every monomer inserted in a polymer, one molecule PP is liberated:

$$\text{ATP} + \text{amino acid} + \text{tRNA} \rightleftharpoons \text{aminoacyl-tRNA} + \text{AMP} + \text{PP} \quad (1)$$

$$\text{Nucleosidetriphosphate (NTP)} + (\text{polynucleotide})_n \rightleftharpoons (\text{polynucleotide})_{n+1} + \text{PP} \quad (2)$$

$$\text{Glucose-1-phosphate} + \text{NTP} \rightleftharpoons \text{NDP-glucose} + \text{PP}. \quad (3)$$

For the calculation of PP-liberation during lipid synthesis, it is assumed that the average lipid molecule contains 2 fatty acids with a chain length of 16 C-atoms.

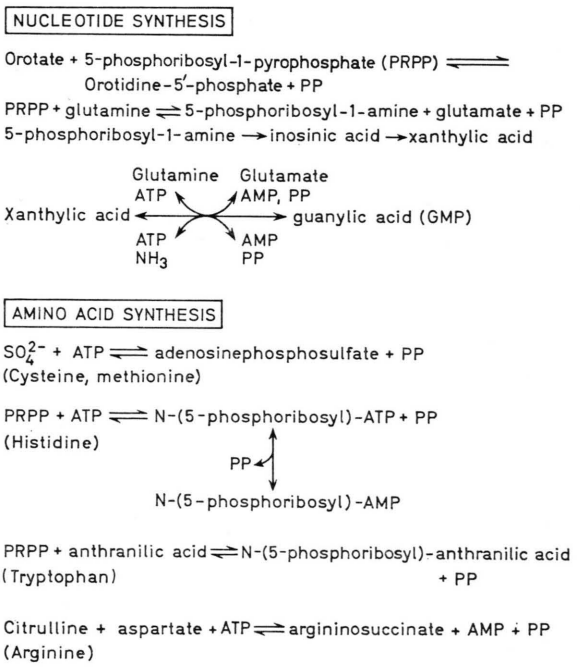
The PP-liberating reactions in the course of lipid-biosynthesis are represented by equations (4) and (5):

$$\text{Fatty acid} + \text{ATP} \rightleftharpoons \text{acyl-AMP} + \text{PP} \quad (4)$$

$$\text{CTP} + \text{cholinephosphate} \rightleftharpoons \text{CDP-choline} + \text{PP}. \quad (5)$$

Based on these reactions, the amount of PP liberated during the biosynthesis of bacterial biomass in a complex medium containing an excess of all monomers (for example, nutrient medium composed of yeast extract and glucose) can be calculated as shown in Table I. The data of Table I show that for each mg of bacterial biomass synthesized in a complex medium, 7.4 μmol of PP are liberated. If the bacterium grows in a minimal medium (for example in a medium containing glucose, ammonium salt, and the usual minerals), the monomers (amino acids, nucleotides) have to be synthesized from the compounds of intermediary metabolism.

There are numerous reactions leading to the liberation of PP. Those which are quantitatively important, are listed in Scheme 1. It is evident that



during the biosynthesis of purine and pyrimidine bases, regularly one molecule PP is liberated. Furthermore, the transformation of IMP to GMP liberates one molecule PP. Thus, for every nucleotide incorporated in the nucleic acid fraction of the cell, an average of 2.25 mol PP is liberated. PP is also produced in the course of amino acid-biosynthesis. Biosynthesis of histidine releases 2 molecules, biosynthesis of arginine, tryptophan, cysteine and methionine releases 1 molecule PP.

Assuming that the frequency of the various amino acids in polypeptide chains is the same, one can expect that about one third of all amino acids synthesized will give rise to the liberation of one molecule PP. The additional PP-liberation in the course of amino acid and purine or pyrimidine biosynthe-

Table I. Liberation of inorganic pyrophosphate (PP) during bacterial growth in complex media (explanation in the text).

Polymer	% of biomass [a]	Monomer units		PP-liberation	
		Mol. weight	μmol per 100 mg biomass	Molecules per monomer	μmol per 100 mg biomass
Protein	60	110	545	1	545
RNA, DNA	20	300	67	1	67
Polysaccharide	10	166	60	1	60
Lipid	10	30 [b]	333	0.2 [b]	66
				Sum:	740

[a] Data of Gunsalus and Shuster (ref. 8).
[b] (CH_2-CH_2) as monomer units (15 per lipid molecule).

sis will increase the amount of PP liberated from 7.4 to 10 μmol per mg biomass. Since there is a fixed relationship between biomass and PP-liberation, the rate v_p of PP-production in the course of biosynthesis will be directly proportional to the growth rate μ of the cell population, *i. e.* $v_p = k \cdot \mu$. The constant k is 7.4 μmol PP/mg biomass for bacterial growth in complex medium, or 10.0 μmol PP/mg biomass for growth in minimal medium. Using these constants, the rates of PP-liberation were calculated for various bacteria whose PPases have been studied in our own or another laboratory (see Table II). The data of Table II allow the distinction of two groups of organisms. In *Rhodopseudomonas capsulata, Thiobacillus ferrooxidans, Desulfovibrio desulfuricans* and *Bacillus subtilis*, the ratio between the maximal PPase-activity (measured in cell free extracts) and the rate of PP-liberation is considerably greater than 10. In contrast, this ratio attains a value of about 10 or less in *Azotobacter vinelandii, Rhodospirillum rubrum* and *Leuconostoc mesenteroides*. *Escherichia coli* K12 occupies an intermediate position.

According to Atkinson [9] the enzyme concentrations in the living cell are balanced in such a way that the steady state levels of the corresponding substrates will be of the order of the Michaelis constant of the enzyme. If this is also the case for PPases, the rate of PP-hydrolysis in the organisms of the first group would not be significantly changed even if the *in vivo* PPase-activity would be reduced to 10% of the *in vitro*-rate. Contrary to this, in an organism of the second group, PPase-activity could – under certain physiological conditions – control the rate of biosynthesis. This is clearly shown by experiments with a temperature-sensitive mutant of *Escherichia coli* having its lesion in the gene coding for PPase: Above the critical temperature causing partial inactivation of PPase-activity, the DNA-synthesis is severely restricted [6].

These latter results, as well as the calculations of this article, do not confirm the statement of Josse and Wong [2] that the PPase-activity of *Escherichia coli* K12 is at least 1000-fold greater than the rate of PP-liberation. This statement of Josse and Wong [2] must be in error. According to these authors, the PPase represents 0.2% of the cellular protein of *Escherichia coli* K12. Assuming an average molecular mass of 100 000 dalton for proteins and a dry mass of 10^{-13} g per cell, *Escherichia coli* K12 contains 3.6×10^5 protein molecules or 700 PPase molecules per cell.

The crystallized PPase has a specific activity of 1333 mmol PP/min·g protein, corresponding to a turnover number of 1.6×10^5 (based on a molecular mass of 120 000 dalton) [2]. With 700 PPase molecules, every *E. coli* cell has a capacity to split $700 \times 1.6 \times 10^5 = 1.12 \times 10^8$ molecules PP/min. In

Table II. PPase activity and rate of biosynthetic PP-liberation in microorganisms.

Organism	Growth rate [h^{-1}]	Temperature [°C]	PPase-activity [μmol/h·mg protein]	PP-liberation [μmol/h·mg protein]	PPase / PP-liberation	Reference
Rhodospeudomonas capsulata (ATCC 23782)	0.37	34	480	6.1	79	unpublished [c]
Thiobacillus ferrooxidans	0.10	30	120	1.65	73	13
Desulfovibrio desulfuricans	0.055	30	1140	19.8 [a]	57	15
Bacillus subtilis	0.50	37	276	8.25	33	16
E. coli K12 (DSM 498)	1.40 [b]	37	240	17.2 [b]	14	unpublished [c]
Azotobacter vinelandii (DSM 86)	0.35	30	57	5.8	10	unpublished [c]
Rhodospirillum rubrum S1	0.18	34	24	3.0	8	14
Leuconostoc mesenteroides (DSM 20193)	0.53 [b]	30	6.6	6.5 [b]	1	unpublished [c]

[a] Rate of PP-liberation during SO_4^{2-}-reduction included. [b] Growth in complex medium (nutrient broth). [c] PPase-activity of cell free extracts was measured at the growth temperature of the organism in reaction mixtures containing 40 mM Tris-HCl (pH 9.0), 5 mM $MgSO_4$ and 1 mM Na_4PP according to ref. 14. The PPases in the cell free extracts were stabilized by 0.1 mM $CoCl_2$ (*Rps. caps., Lc. mes.*) or 10 mM $MgSO_4$ (*E. coli, A. vinelandii*).

our laboratory, *E. coli* K12 grows at a growth rate of $\mu = 1.4\ h^{-1} = 0.023\ min^{-1}$ in a complex medium at 37 °C. Thus, the rate of PP-liberation during growth at 37 °C is $\mu \cdot k = 0.023 \times 7.4$ μmol PP/mg biomass·min

or 1.7×10^{-17} mol PP/min $\times 10^{-13}$ g biomass

or 10.2×10^{6} molecules PP/min $\times 10^{-13}$ g biomass.

This calculation based on the kinetic data of crystallized *E. coli* K12-PPase [2] clearly shows that the capacity for PP-hydrolysis is only about 1 order of magnitude higher than the rate of PP-liberation. This value corresponds to the ratio calculated on the basis of maximal PPase-activity in crude extracts (see Table II).

Catalytic and Regulatory Properties of Microbial PPases

The calculations made in the foregoing section show that in some microorganisms, PPase-activity can limit the rate of biosynthesis of cellular constituents. It is reasonable, therefore, to consider the catalytic properties of those PPases which are likely to be pacemakers of biosynthesis.

The true substrate of the constitutive PP-specific PPases (EC 3.6.1.1) is not the free $P_2O_7^{4-}$-ion (or the protonated derivative of that), but rather a complex between $P_2O_7^{4-}$ and a bivalent metal ion [10, 11]. For the majority of the microbial PPases studied thus far, Mg^{2+} is the most active cosubstrate; other bivalent metal cations (Mn^{2+}, Co^{2+}, Zn^{2+}) being less active [12–14].

There are some exceptions of this rule. The PPases from *Desulfovibrio desulfuricans* [15], *Bacillus subtilis* [16] and *Rhodopseudomonas capsulata* [17] are most active with metals of the transition group (Mn^{2+}, Co^{2+}). It is interesting to note, however, that all these PPases belong to the low-molecular weight group (see Table III) whose activity is probably not subject to metabolic control *in vivo* (see Table II). It seems, therefore, that the Mg^{2+}-activated microbial PPases are the most likely candidates for metabolic control through changes in the Mg^{2+}/PP-ratio. To understand the significance of the Mg^{2+}/PP-ratio for PPase-control, one has to consider the multiple effects of Mg^{2+} on the Mg^{2+}-activated PPases. Firstly, Mg^{2+} "activates" these enzymes as a consequence of the fact that it is part of the substrate. Secondly, it has been shown that uncomplexed, free Mg^{2+} facilitates the binding of the substrate $MgP_2O_7^{2-}$ to the catalytic site of the PPase from *Rhodospirillum rubrum* [18] and yeast [19], thereby increasing the reaction rate in a way reminiscent of allosteric activation. Finally, Mg^{2+} activates certain microbial PPases by decreasing the concentration of the uncomplexed free $P_2O_7^{4-}$-ion which is a potent inhibitor of the PPases from *Rhodospirillum rubrum* ($K_i = 5$ μM) [14], *Alcaligenes eutrophus* ($K_i = 50$ μM) [20], and *Azotobacter vinelandii* ($K_i = 85$ μM) [20].

The strong inhibition of these PPases by uncomplexed $P_2O_7^{4-}$ is reflected in the sigmoidal shape of the Mg^{2+}-saturation curve measured in reaction mixtures with a constant total PP-concentration. Although the inhibition by $P_2O_7^{4-}$ seems to be

Table III. Properties of microbial PPases.

Organism	Properties of PPase				Reference
	Mol. weight	Requirement for stability	Substrate	Inhibitor	
Desulfovibrio desulfuricans	38 000 [a]	Reducing agent	CoPP	—	15
Rhodopseudomonas capsulata	60 000 [b]	Co^{2+}	MgPP, CoPP, MnPP	—	17
Bacillus subtilis	68 000 [a]	Mn^{2+}, Co^{2+}	CoPP, MnPP	—	16
Rhodospirillum rubrum	100 000 [b]	Zn^{2+}	MgPP	free $P_2O_7^{4-}$	18
Acotobacter vinelandii (DSM 86)	100 000 [b]	Mg^{2+}	MgPP	free $P_2O_7^{4-}$	unpublished [c]
E. coli K12	120 000 [a]	—	MgPP	free $P_2O_7^{4-}$, ADP	2

[a] Sucrose density gradient or analytical ultracentrifugation. [b] Gelfiltration through Sephadex G200. [c] The *A. vinelandii*-PPase was partially purified from a cell free extract in 40 mM Tris-HCl (pH 8) containing 100 mM $MgSO_4$ by a procedure employing a heat treatment of the extract (75 °C, 10 min), removal of nucleic acids by protamine sulfate (0.1 mg/mg protein) precipitation and collection of the protein fraction precipitating between 60 and 80% saturation with $(NH_4)_2SO_4$. This fraction had a spec. activity of 25 units/mg protein and was filtered through a Sephadex G200-column together with marker proteins (J.-H. Klemme and R. Lüderitz, unpublished).

competitive, the kinetic analysis of these saturation curves is complicated by the fact that, in addition to the decrease of the inhibitor ($P_2O_7^{4-}$)-concentration due to the increase in total Mg^{2+}, the reaction rate increases also due to the activating effect of free Mg^{2+}-ions. Thus, the Hill-coefficients of such saturation curves are rather complex functions of two cooperating processes. It can be calculated that the Hill-coefficient n_H of the Mg^{2+}-saturation curve (at a constant total PP-concentration) never exceeds a value of 2 if the activating effect of Mg^{2+} is only due to the complex-formation with the competitive inhibitor $P_2O_7^{4-}$. Accordingly, the Hill-coefficient of a metal-saturation curve can be a useful diagnostic index in the case of PPases.

The response of such Mg-activated PPases to Mg^{2+} is governed by three parameters: (i) The pH of the reaction mixture (because the stability constant of the MgPP-complex depends on pH). (ii) The ratio between the K_m-value for the substrate, MgPP, and the K_i-value for the inhibitor, $P_2O_7^{4-}$, and (iii) the K_a-value of the activator, free Mg^{2+}.

Information concerning the existence of other allosteric ligands of microbial PPases is still scarce. The pronounced sensitivity of the unpurified *Rhodospirillum rubrum* enzyme to ATP and NADH [14] is lost during purification, and the reported inhibition of this enzyme by phosphorylated C_3-compounds [18] is too weak to be of metabolic importance. It seems, therefore, that the ratio between the concentration of Mg^{2+}-ions available to the binding sites of the PPase and the concentration of total cytoplasmic PP controls the activity of this enzyme.

The total Mg^{2+}-concentration in *Escherichia coli* is about 20 mM (*cf.* ref. 21). Since bacterial PPases generally have a rather high affinity to their substrate with K_m-values ranging from 5 μM (*E. coli* PPase [10]) to about 0.1 mM (PPases of *Alcaligenes eutrophus* and *Azotobacter vinelandii* [20]), and since the cytoplasmic concentration of PP was found to be in the range of 0.1 – 1 mM in microorganisms [3-5], it would seem at the first glance that PPases function at their maximal capacity in all microorganisms. However, one should remember in this connection that most of the cellular Mg^{2+} is not available to enzymatic binding sites because the ribosome fraction of the cell binds up to 85% of the total Mg^{2+} (*cf.* refs 22, 23). Thus, the concentration of the cytoplasmic Mg^{2+} is probably not higher than 4 – 5 mM. If one considers that the adenylate pool of the bacterial cell (4 – 5 mM [24]) contains two Mg^{2+}-chelating compounds, ADP and ATP, it becomes evident that the concentration of the cytoplasmic free Mg^{2+} is dependent on the relative concentrations of AMP, ADP and ATP, *i. e.* dependent on the "Energy Charge" of the adenylate pool [25]. It has been calculated that, with a cytoplasmic Mg^{2+}-concentration of 4 mM and an adenylate pool of 5 mM, the concentration of uncomplexed free Mg^{2+} is only about 0.3 mM at the physiological value (0.8) of the energy charge [26]. It may be concluded, therefore, that in organisms with a "regulated" PPase, the rate of PP-hydrolysis is controlled by the energy charge of the adenylate system through chelation of the activating Mg^{2+}-ion.

Although there is considerable variation in the size of bacterial PPases (see Table III), it would be premature yet to correlate the structural and the regulatory properties of the enzymes. One point should be emphasized, however. Organisms with a large excess of PPase tend to contain a low molecular weight enzyme which is not activated by Mg^{2+} (but rather by Co^{2+} or Mn^{2+}) and not inhibited by free $P_2O_7^{4-}$ (PPases of *Desulfovibrio desulfuricans*, *Bacillus subtilis* and *Rhodopseudomonas capsulata*). On the other hand, PPases of the other group of organisms, *i. e.* PPases of the regulated type, tend to be of high molecular weight ($\geqq$ 100 000 dalton), are strongly inhibited by free $P_2O_7^{4-}$ and, in some cases at least, activated by free Mg^{2+} (PPases of *Escherichia coli*, *Rhodospirillum rubrum* and *Rhodopseudomonas gelatinosa*).

Non-Hydrolytic Split of PP by PP-Dependent Transphosphorylases

Considering the high standard free energy of hydrolysis of the PP molecule of $\Delta G^0 = -8.0$ Kcal/mol [27], it seems possible that the maintenance of a low cytoplasmatic PP-concentration can be accomplished by enzymes transferring one of the phosphate-groups of the PP-molecule to an organic acceptor according to Eqn (6):

$$PP + R-OH \rightleftharpoons R-O-P + P_i \,. \qquad (6)$$

The occurrence of such transphosphorylation reactions would conserve at least a part of the phosphoanhydride-energy of the PP-bond and would be reflected in a higher molar growth yield (g biomass/mol substrate) of the respective organism. It has been discussed already in 1966 by Wood *et al.* [27] if the

conservation of the phosphoanhydride-energy of PP could explain the unusually high molar growth yields of propionic acid bacteria [28].

This discussion was based on the fact that the reaction catalyzed by the enzyme phosphoenolpyruvate-carboxytransphosphorylase (EC 4.1.1.38) [29] could readily proceed in the direction of PEP-synthesis [30] according to Eqn (7):

$$PP + \text{oxaloacetate} \rightleftharpoons PEP + CO_2 + P_i . \quad (7)$$

In the last years, three other PP-dependent transphosphorylases were found in propionic acid bacteria which possibly contribute to the conservation of the energy of PP, namely:

(i) Pyruvate, phosphate dikinase [31]
(Pyruvate + P_i + ATP $\rightleftharpoons$ PEP + PP + AMP)
(ii) Pyrophosphate, L-serine phosphotransferase [32]
(PP + serine $\rightleftharpoons$ O-phosphoserine + P_i), and
(iii) Pyrophosphate, D-fructose-6-phosphate-1-phosphotransferase [33]
(Fructose-6-P + PP $\rightleftharpoons$ fructose-1,6-bisphosphate + P_i).

Interestingly, it was an organism taxonomically rather distinct from propionic acid bacteria, the anaerobic amoeba *Entamoeba histolytica*, in which a similar set of PP-dependent transphosphorylases has been discovered. There seems to be no doubt that in the glucose-fermenting amoeba, the energy of pyrophosphate is conserved in at least two catabolic reactions. Firstly, this organism apparently lacks pyruvate kinase (EC 2.7.1.40) and the enzyme pyruvate, phosphate dikinase (EC 2.7.9.1) was shown to be responsible for the glycolytic flux [34]. Secondly, the activity of the classical ATP-dependent 6-phosphofructokinase (EC 2.7.1.11) is insufficient to account for the rate of glycolysis in this organism [5]. Instead of the ATP-dependent phosphofructokinase, *Entamoebia histolytica* contains a PP-dependent enzyme with a rather high specific activity (0.45 units/mg protein) [5]. In addition, the amoeba lacks the conventional ATP-dependent acetate kinase (EC 2.7.2.1) and contains a PP-dependent kinase whose activity would also be sufficient to account for the glycolytic production of acetate from glucose [35]. The intracellular concentration of PP in this anaerobic microorganism (0.18 mM) is 13 times greater than the K_m of the PP-dependent phosphofructokinase [5]. It is very likely, therefore, that the PP-dependent transphosphorylases can effectively compete with the hydrolytic PPase for their common substrate. Unfortunately, nothing is known about the regulatory properties of the PPase from *Entamoeba histolytica* or the propionic acid bacteria.

The evidence that in propionic acid bacteria the PP-dependent transphosphorylases are functioning in the direction of utilization of PP as a phosphate donor is based on molar growth yield experiments. Glucose is fermented by these organisms according to Eqn (8):

$$1\ \text{Glucose} \rightarrow 1.33\ \text{propionate} + 0.67\ \text{acetate} + 0.67\ CO_2 . \quad (8)$$

The maximal ATP-yield of this fermentation can be calculated as follows. The net ATP-gain of the glycolytic conversion of glucose to pyruvate is 2 mol/mol glucose. 1 mol ATP is produced in the course of acetate formation from acetylphosphate through the action of acetate kinase (EC 2.7.2.1). In addition, the formation of propionic acid *via* C_4-dicarboxylic acids is accompanied by an electron transport from NADH to fumarate. The free energy change in this cytochrome-linked electron transport chain is large enough to allow the formation of 2 mol ATP per 2 e^-, *i.e.* the formation of 1 mol propionate from pyruvate is coupled to the formation of 2 additional mol ATP [36]. Thus, the total net ATP-gain of the propionic acid fermentation according to Eqn (8) is 2 (glycolysis) + 2.66 (propionate formation) + 0.67 (acetate formation) = 5.33/mol glucose. Using the Y_{ATP}-value of Bauchop and Elsden [28] (Y_{ATP} = g biomass/mol ATP = 10), the maximal molar growth yield for propionic acid bacteria should be about 53 g/mol glucose. However, in a complex medium with pH-control, the molar growth yield of *Propionibacterium freudenreichii* can be as high 82 g/mol glucose [36]. The most reasonable explanation for this very high growth yield is the assumption of a Y_{ATP}-value considerably greater than 10. For *Propionibacterium freudenreichii*, a value of 15.3 can be calculated from the data given before. Although it must be admitted that the reason for unusually high Y_{ATP}-values must not necessarily be the conservation of the phosphoanhydride-energy of the PP-molecule [37], it is tempting to ascribe the very efficient use of the free energy of glucose fermentation by propionic acid bacteria to the function of PP-dependent transphosphorylases. A systematic search for these enzymes in other groups of microorganisms with unusually high molar growth yields (for example, the rumen bacteria *Selenomonas ruminantium* [37] and *Bacteroides amylophilus* [37]) would

provide further evidence for the validity of this concept. It is of interest to note in this connection that the PP-dependent pyruvate, phosphate dikinase (EC 2.7.9.1) has been shown to be present in *Bacteroides symbiosus*, an obligate anaerobe which lacks pyruvate kinase [38].

Considering the fact that in *Rhodospirillum rubrum* and two other photosynthetic bacteria, *Rhodopseudomonas viridis* and *Rhodopseudomonas palustris*, various energy-dependent reactions (transhydrogenation, succinate-linked NAD-reduction, cytochrome reduction) occur which can be driven by PP [39–43], the existence of a regulated PPase in two of these bacteria [17] makes it likely that the use of PP as a phosphate donor for transphosphorylation reactions is not limited to certain amoebae, propionic acid and rumen bacteria, but more widespread in the microbial world than hitherto recognized.

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