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SHORT COMMUNICATIONS

Activities of 6-Phosphofructokinases and Inorganic Pyrophosphatase in Aerobic Methylotrophic Bacteria

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The classical radioisotopic and enzymological studies of J.R. Quayle led to the discovery of the ribulose-monophosphate (RuMP) cycle of formaldehyde assimilation in methanotrophs and methylobacteria [1, 2]. The key reactions of the RuMP cycle catalyzed, by 3-hexulosephosphate synthase (HPS) and phospho-3-hexuloisomerase, form fructose-6-phosphate from formaldehyde and ribulose-5-phosphate. It was believed that further phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate is carried out by ATP-dependent 6-phosphofructokinase (ATP-PFK), although its low activity (2–8 mU/mg protein) in methanotrophs questioned the significance of the glycolytic pathway of phosphosugar transformation in the RuMP cycle. The discovery of the activity of pyrophosphate-dependent 6-phosphofructokinase (PP_i-PFK) and a high intracellular concentration of inorganic pyrophosphate in three species of methanotrophs employing different pathways of C_1 compound assimilation stimulated the study of the role of PP_i and reappraisal of the concepts of the organization and regulation of the RuMP cycle in aerobic methylotrophic bacteria of various taxonomic positions [3]. Along these lines, in this work, the activities of PP_i- and ATP-dependent 6-phosphofructokinases and pyrophosphatase (PP_i-ase) were determined in a broad spectrum of aerobic methanotrophs and methylobacteria employing various pathways of C_1 assimilation.

The work used strains of obligate methanotrophs and methylobacteria from the collection of the Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences. Cells were harvested in the early stationary phase, as described earlier [3, 4]. A 12-h *Escherichia coli* K12 culture grown in liquid medium with glucose was also used. Cells were separated from the medium by centrifugation, washed with 50 mM phosphate buffer (pH 7), and destroyed with an IBPM-press in a frozen state. To obtain cell-free extracts, cell homogenates were centrifuged (12000 *g*, 20 min) and dialyzed against the same buffer. The activities of PP_i-PFK (EC 2.7.1.90), ATP-PFK (EC 2.7.1.11), and inorganic pyrophosphatase (EC 3.6.1.1) were determined as described earlier [4]. The data presented are the means of three measurements.

The results of determination of the activities of PP_i-PFK, ATP-PFK, and PP_i-ase in obligate and facultative methylobacteria are shown in the table. Since the detected activity of ATP-PFK could in fact be the activity of PP_i-PFK at the expense of PP_i admixtures, a preparation of yeast inorganic pyrophosphatase (0.2 U/ml) was introduced into the incubation mixture for ATP-PFK determination in further experiments. Under these conditions, the ATP-PFK activity was not revealed.

The highest PP_i-PFK activity was revealed in type I methanotrophs, which assimilate formaldehyde via the RuMP cycle. In type II methanotrophs with the serine pathway, the activity of PP_i-PFK was lower than in type I methanotrophs. The reversibility of this reaction and the necessity of the synthesis of hexose phosphates from triose phosphates in type II methanotrophs suggests the involvement of PP_i-PFK in the gluconeogenesis of these bacteria. The lowest values of the PP_i-PFK activity were recorded in type X methanotrophs of the genera *Methylococcus* and *Methylocaldum*, which simultaneously employ three pathways of C_1 assimilation. The activity of PP_i-ase in cell-free extracts of methanotrophs was considerably lower than in cell-free extracts of most of the methylobacteria studied and *E. coli* K12.

Among the strains of facultative methylobacteria employing the fructose-bisphosphate variant of the RuMP cycle, the activity of PP_i-PFK, along with low activities of PP_i-ase and ATP-PFK, was noted only in *Amycolatopsis methanolica*, as was already shown earlier [4, 5]. Although the *pfk* gene has been revealed in the genome of the facultative methylotroph *Methylibium petroleiphilum*, there are no data on the PP_i-PFK activity in this bacterium [6]. In most of the methylobacteria studied, PP_i-PFK and ATP-PFK were not revealed, the PP_i-ase activity being high. Only *Bacillus*

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Activities of PP_i-dependent and ATP-dependent 6-phosphofructokinases and inorganic pyrophosphatase in aerobic methylotrophic bacteria

Methylotroph	Carbon source	C_1 assimilation pathway	Activity (mU/mg protein)		
			PP_i-PFK	ATP-PFK	PP_i-ase
Obligate methanotrophs					
Methylomonas methanica 12	CH_4	RuMP (FBP + KDPG)	750	0	40
Methylomonas methanica 68 VKM	CH_4	RuMP (FBP + KDPG)	480	0	14
Methylobacter chroococcum 90	CH_4	RuMP (FBP + KDPG)	240	0	15
Methylosinus trichosporium OB3b	CH_4	Serine	110	0	60
Methylosinus trichosporium 44	CH_4	Serine	200	0	40
*Methylocystis echinoide*s 2	CH_4	Serine	210	0	25
Methylococcus capsulatus Bath	CH_4	RuMP + RuBP + serine	86	0	20
Methylococcus capsulatus 874	CH_4	RuMP + RuBP + serine	60	0	10
Methylocaldum szegedienze O-12	CH_4	RuMP + RuBP + serine	10	0	ND
Methylobacteria					
Amycolatopsis methanolica	CH_3OH	RuMP (FBP)	35	6	17
Mycobacterium methylovorum 915	CH_3OH	RuMP (FBP)	0	24	1500
Pseudomonas esterophilus 27	Methyl acetate	RuMP (FBP)	0	152	1700
Arthrobacter sp. 2B2	CH_3OH	RuMP (FBP)	0	0	120
Mycobacterium vaccae	CH_3OH	RuMP (FBP)	0	0	320
Ancylobacter aquaticus Z 238	CH_3OH	RuMP (FBP)	0	0	870
Bacillus methanolicus M40	CH_3OH	RuMP (FBP)	0	200	2600
Bacillus methanolicus M11	CH_3OH	RuMP (FBP)	0	329	1500
Bacillus methanolicus PM6	CH_3NH_2	RuMP (FBP)	0	0	336
Arthrobacter globiformis	CH_3NH_2	RuMP (FBP)	0	0	90
Arthrobacter globiformis	Glucose	RuMP (FBP)	0	0	320
Acidomonas methanolica	CH_3OH	RuMP (FBP)	0	26	396
Methylophilus methylotrophus "Ch"	CH_3OH	RuMP (KDPG)	0	0	530
Methylophilus methylotrophus "V"	CH_3OH	RuMP (KDPG)	0	0	314
Methylophilus methylotrophus	CH_3OH	RuMP (KDPG)	0	0	443
Methylophaga marina VKM	CH_3OH	RuMP (KDPG)	0	0	660
Xanthobacter autotrophicum 32P	CH_3OH	RuBP	0	0	280
Xanthobacter autotrophicum 25P	CH_3OH	RuBP	0	0	200
Methylopila capsulata IM1	CH_3OH	Serine (icl^-)	0	0	270
Aminobacter aminovorans	CH_3NH_2	Serine (icl^+)	0	0	212
Hyphomicrobium zavarzinii ZV	CH_3OH	Serine (icl^+)	0	0	50
Escherichia coli K12	Glucose		0	225	1200

Note: RuBP, ribulose-1,5-bisphosphate; FBP, fructose-1,6-bisphosphate; KDPG, 2-keto-3-deoxy-6-phosphogluconate; ND, not determined.

methanolicus M40 and M11 and *Pseudomonas esterophilus* sp. 27RD cells grown on C_1 compounds exhibited high activities of ATP-PFK (150–350 mU/mg) and PP_i-ase (1.5–2.5 U/mg).

Thus, we found high PP_i-PFK activity accompanied by low PP_i-ase activity and absence of ATP-PFK in type I, II, and X methanotrophs, employing the RuMP and serine pathways of C_1 metabolism, respectively, or both of these pathways in combination with the Calvin cycle. On the contrary, in methylobacteria of various taxonomic positions, irrespective of the C_1 assimilation pathways employed, the PP_i-ase activity was high and the PP_i-PFK activity was absent, with the exception of the facultative methylotroph *A. methanolica.* Consequently, all methanotrophs, despite the pheno- and genotypic differences, exhibit a relationship between methane oxygenation and the presence of PP_i-PFK.

It was recently discovered that, in the *Mc. capsulatus* Bath genome [7], the *pfk* gene is in close proximity to the *hpp* gene of the membrane PP_i-ase, which has a rather high homology (60%) with the H^+-translocating PP_i-ase of *Rhodospirillum rubrum* [8]. Our preliminary data indicate localization of these genes in a single operon. Coordinated expression of these genes suggests an important role of PP_i in the metabolism of methanotrophs. However, it still remains to be clarified whether PP_i synthesis takes place on the membranes (ensuring PP_i-PFK functioning in the forward reaction) or, on the contrary, the energy of the PP_i formed during the reverse reaction can be transformed into the energy of the electrochemical potential with the involvement of H^+-PP_i-ase.

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