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DIFFERENT PATHWAYS FOR FRUCTOSE AND GLUCOSE UTILIZATION IN *RHODOPSEUDOMONAS CAPSULATA* AND DEMONSTRATION OF 1-PHOSPHOFRUCTOKINASE IN PHOTOTROPHIC BACTERIA

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Summary

Rhodopseudomonas capsulata Kb1 utilizes glucose via the Entner—Doudoroff pathway and fructose via the Embden—Meyerhof pathway. In this bacterium, 1-phosphofructokinase (EC 2.7.1.56) is the key enzyme of the Embden—Meyerhof pathway and is induced by fructose but not by glucose. This enzyme could also be demonstrated in various species of phototrophic bacteria after growth in fructose-salts medium.

Hexoses are degraded by three major pathways, Embden—Meyerhof pathway, Entner—Doudoroff pathway and pentose phosphate pathway. The simultaneous presence of two of these pathways, usually the pentose phosphate pathway plus one other, is quite common [1]. On the other hand the breakdown of one hexose by one pathway and a second hexose by another pathway in one organism is also known, e.g. gluconate and glucose in *Escherichia coli* [2]. Previous workers have always found fructose and glucose to be broken down by one pathway in one organism, either Entner—Doudoroff pathway or Embden—Meyerhof pathway. The Embden—Meyerhof pathway can be used for fructose breakdown via fructose-1-phosphate and for glucose breakdown via fructose-6-phosphate as for example in *Aerobacter aerogenes* [3].

We now show that the phototrophic bacterium *Rhodopseudomonas capsulata*, which utilizes either fructose or glucose, degrades these two hexoses by different pathways. Glucose is degraded by the Entner—Doudoroff pathway [4]. Fructose is apparently degraded by the Embden—Meyerhof pathway using

1-phosphofructokinase (EC 2.7.1.56), which we report for the first time in phototrophic bacteria.

R. capsulata Kb1 and the other phototrophic bacteria listed in Table III were obtained from the Deutsche Sammlung von Mikroorganismen (DSM), Göttingen. *R. capsulata* was grown in a mineral medium containing 12 mmoles KH_2PO_4, 10 mmoles NH_4Cl, 2 mmoles $MgSO_4$, 7 mmoles NaCl, 0.35 mmoles $CaCl_2$ and 10 ml trace element solution SL4 [5] in 1000 ml distilled water adjusted to pH 6.9. This medium was supplemented for growth with 10—20 mmoles of either sodium DL-malate, glucose or fructose and 0.5 g yeast extract or 0.1 mg thiamine. Glucose and fructose were filter-sterilized and added aseptically. For phototrophic cultures glass bottles were completely filled, closed with tightly fitting screw caps and incubated at 30 °C with illumination at approximately 2000 lux. Purity of the cultures was checked by use of Difco AC medium and agar plates. For aerobic cultures fluted Erlenmeyer flasks were used and aerated on a rotatory shaker at 150 rev./min and 30 °C. Inocula were prepared in the same manner and with the same substrate as the main culture.

The cells were harvested in the late exponential growth phase, washed twice in 50 mM phosphate buffer (pH 7.6) and resuspended to a turbidity of $E = 100 - 200$ at 650 nm. Cell extracts were prepared from these suspensions by ultrasonication for 2 min with a Braun Sonic 300 (Quigley, Rochester, U.S.A.), centrifugation at 20 000 x *g* for 30 min and 120 000 x *g* for 90 min. The supernatant was filtered through Sephadex G-25 and used immediately for enzyme assays. Enzyme assays were done as described elsewhere [6]. 1-Phosphofructokinase was assayed similarly to 6-phosphofructokinase (EC 2.7.1.11) but with 3.3 mM fructose-1-phosphate instead of 3.3 mM fructose-6-phosphate as substrate. Controls were done by omitting the individual substrates or the extract in the test assays. One unit of enzyme activity is defined as the conversion of 1 μmole substrate per min at 25 °C. Protein was measured after the method of Lowry et al. [7]. All reagents were obtained from Merck, Darmstadt. The enzymes and biochemicals were purchased from C.F. Boehringer Mannheim.

TABLE I

SPECIFIC ACTIVITIES OF ENZYMES OF HEXOSE METABOLISM IN *Rps. CAPSULATA* Kb1

Enzyme	Specific activity (μmoles/g protein per min) after phototrophic or aerobic (values in brackets) growth in salts medium containing		
	Malate	Glucose	Fructose
Hexokinase (EC 2.7.1.1)	107	101	117
Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)	27	88 (96)	46 (51)
6-Phosphogluconate dehydratase (EC 4.2.1.12) + 2-Keto-6-phosphogluconate aldolase	19	148 (149)	77 (89)
1-Phosphofructokinase (EC 2.7.1.56)	2	2 (3)	39 (98)
6-Phosphofructokinase (EC 2.7.1.11)	3	4 (7)	6 (9)
Fructose-bisphosphate aldolase (EC 4.1.2.13)	140	67	163
Hexosediphosphatase (EC 3.1.3.11)	22	8	43

The specific activities of important enzymes of the Embden—Meyerhof pathway and Entner—Doudoroff pathway were measured in cells of *R. capsulata* Kb1 after phototrophic or aerobic growth in malate-, glucose- or fructose-salts medium (Table I). In fructose-grown cells 1-phosphofructokinase was strongly induced (about 20-fold). Hexose diphosphatase had a higher specific activity in fructose-grown cells than in glucose-grown cells, which may be explained by the role it plays in the formation of hexose-6-phosphates in fructose-grown cells. In glucose-grown cells the levels of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydratase and 2-keto-3-deoxy-6-phosphogluconate aldolase were about 2-fold increased compared to fructose-grown cells, while the level of fructose-bisphosphate aldolase was lower. This ratio of specific activities is consistent with the fact that glucose is utilized by the Entner-Doudoroff pathway, while fructose is apparently degraded by the Embden-Meyerhof pathway. Hexokinase and 6-phosphofructokinase were constitutively present in all extracts, the latter only at low specific activities, indicating that this enzyme is of no real importance for sugar utilization in this organism.

TABLE II

INDUCTION OF 1-PHOSPHOFRUCTOKINASE BY FRUCTOSE IN GLUCOSE-GROWN CELLS OF *Rps. CAPSULATA* Kb1

The cells were grown in a glucose-salts medium (anaerobic, light or aerobic, dark) harvested in the late exponential phase, washed with salts medium and resuspended in a fructose (20 mM)-salts medium without and with 200 μg chloramphenicol/ml. For induction the suspensions were incubated for 8 h either anaerobically in the light or aerobically in the dark.

Growth conditions before induction	Specific activity of 1-phosphofructokinase (μmoles/g protein per min)		
	without induction	after 8 h induction by 20 mM fructose under:	
		growth conditions	growth conditions + chloramphenicol
Anaerobic, light	2	25	2
Aerobic, dark	3	33	4

1-Phosphofructokinase seems to be the key enzyme of Embden—Meyerhof pathway, induced only in fructose-grown cells. The induction of this enzyme was demonstrated by incubating glucose-grown cells for 8 h with fructose under growth conditions (Table II). The presence of chloramphenicol prevented the induction of 1-phosphofructokinase. The enzyme was induced to some extent under anaerobic dark conditions, when inoculated with cells previously grown anaerobically in the light. Under these induction conditions no growth occurred, but presumably a fermentation of fructose can take place similar to that found in *Rhodospirillum rubrum* [8]. 1-Phosphofructokinase was first demonstrated in *Aerobacter aerogenes* [9] and has since been found in the following heterotrophic organisms: *E. coli*, Clostridia *Bacteroides symbiosus*, *Arthrobacter pyridinolis* and *Butyrivibrio fibrisolvens*. The experiments reported in this paper were stimulated by the failure to detect 6-phosphofruc-

TABLE III

SPECIFIC ACTIVITY OF 1-PHOSPHOFRUCTOKINASE IN DIFFERENT PHOTOTROPHIC BACTERIA

Organism	Specific activity (μmoles/g protein per min) after phototrophic growth in salts medium containing:		
	Thiosulfate or malate	Glucose	Fructose
Thiocystis violacea 2711* DSM No. 207	No growth	No growth	63
Thiocapsa roseopersicina 6311* DSM No. 219	1	No growth	77
Rhodospirillum rubrum S1 DSM No. 467	1	No growth	18
Rhodopseudomonas spheroides 17023 DSM No. 158	2	2	47
Rhodopseudomonas spheroides 1760-1 DSM No. 159	2	2	39
Rhodospeudomonas capsulata Kb1 DSM No. 155	2	2	39

*The mineral medium was prepared as described by Pfennig [13].

tokinase in significant amounts in phototrophic bacteria, although *R. rubrum* was shown to degrade fructose by the Embden—Meyerhof pathway [10]. Therefore, several species of phototrophic bacteria were assayed for 1-phosphofructokinase activity (Table III). This enzyme was found in various strains of the Rhodospirillaceae and Chromatiaceae, when grown in fructose-salts medium. This is the first time that 1-phosphofructokinase has been shown in autotrophic organisms.

The pattern of fructose utilization by the Embden—Meyerhof pathway and glucose utilization by the Entner—Doudoroff pathway in *Rps. capsulata* is apparently shared by *Rps. spheroides*. This bacterium contains 1-phosphofructokinase in fructose-grown cells (Table III) and all the enzymes of the Entner—Doudoroff pathway in glucose-grown cells [11]. The involvement of 1-phosphofructokinase for fructose utilization is consistent with the fact, that in *Rps. spheroides* and *R. rubrum* fructose is transported into the cell by a *P*-enolpyruvate transferase system which phosphorylates fructose in 1-position [12]. Conclusive evidence for different pathways of fructose and glucose utilization may be provided by radiorespirometric techniques.

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