Metabolism of glucose via a modified Entner-Doudoroff pathway in the thermoacidophilic archaebacterium *Thermoplasma acidophilum*

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It has been found that the thermoacidophilic archaebacterium, *Thermoplasma acidophilum*, can metabolise glucose via a modified Entner-Doudoroff pathway involving non-phosphorylated intermediates. Pyruvate and glyceraldehyde are the first products, the glyceraldehyde then being further metabolised to a second molecule of pyruvate via 2-phosphoglycerate. Intermediates of this pathway have been identified by enzymic analysis or by thin-layer chromatography and the individual enzymes involved have been assayed and their kinetic parameters determined. Comparisons are made with the pathways of glucose metabolism in other archaebacteria.

Archaebacteria (Thermoplasma) *Entner-Doudoroff pathway* *Glucose metabolism*

1. INTRODUCTION

It has been proposed that archaebacteria, eubacteria and eukaryotes constitute three primary lines of evolutionary descent, with the archaebacteria being comprised of extreme halophiles, thermoacidophiles and methanogens [1]. Within the thermoacidophiles there are three orders, *Sulpholobus, Thermoplasma* and *Thermoproteus*, although it is thought that *Thermoplasma* is phylogenetically distinct from the other two thermophiles [2]. Indeed, it has been suggested that *Sulpholobus* and *Thermoproteus* may constitute a separate kingdom, the Eocyta [3].

We have compared organisms of the proposed evolutionary kingdoms using the diversity of the citric acid cycle enzymes as an indicator of taxonomic relationships [4–6]. On this basis, *Sulpholobus* and *Thermoplasma* appear very similar to each other and distinct from the halophiles and methanogens [6]. Our studies are now extended to a comparison of the pathways of glucose metabolism. It has been found that in certain halobacteria glucose is catabolised via a modified Entner-Doudoroff pathway in which phosphorylation occurs after the formation of 2-keto-3-deoxygluconate [7]. In contrast, *Sulpholobus* metabolises glucose via an Entner-Doudoroff pathway in which no phosphorylated intermediates are present [8]. Given this diversity of glucose metabolism between archaebacterial genera, we now report the pathways in *Thermoplasma*, finding the conversion of glucose to pyruvate and glyceraldehyde as in *Sulpholobus*, followed by a further oxidation of the glyceraldehyde involving phosphorylated intermediates.

2. EXPERIMENTAL

All chemicals used were of analytical grade. *Thermoplasma acidophilum* (DSM 1728) was grown at 59°C (pH 2.0) as in [9] and cell extracts were prepared as in [5]. The extracts were dialysed vs 100 mM Tris-HCl (pH 8.0) before use. Unless otherwise stated, enzyme activities were assayed

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spectrophotometrically at 55°C in 100 mM Tris-HCl (pH 8.0). Glucose dehydrogenase was assayed as in [10] in 0.4 mM $NADP^+$ and 50 mM glucose. Gluconate dehydratase was assayed in 10 mM $MgCl_2$ and 50 mM potassium gluconate, and the production of 2-keto-3-deoxygluconate was measured as in [11]. The combined activities of gluconate dehydratase and 2-keto-3-deoxygluconate aldolase were assayed in 10 mM $MgCl_2$ and 50 mM potassium gluconate; pyruvate formation was determined with 0.1 mM NADH and pig heart lactate dehydrogenase (2.5 μkat) [12]. Glyceraldehyde dehydrogenase was assayed in 0.2 mM $NADP^+$ and 5 mM DL-glyceraldehyde as in [13]. Glycerate kinase was assayed at 40°C in a coupled assay containing 10 mM $MgCl_2$, 10 mM KCl, 0.2 mM NADH, 5 mM ATP, 1 mM ADP, 5 mM DL-glycerate, 5 mM EGTA, yeast enolase (50 nkat), rabbit muscle pyruvate kinase (2.5 μkat) and pig heart lactate dehydrogenase (1.5 μkat); the decrease in A_{340} was measured with time against a control in the absence of DL-glycerate. Enolase was assayed in 10 mM $MgCl_2$ and 1 mM D-2-phosphoglyceric acid as in [14] and pyruvate kinase was measured according to [15] in 10 mM $MgCl_2$, 10 mM KCl, 5 mM ADP, 0.2 mM NADH, 10 mM phospho*enol*pyruvate and pig heart lactate dehydrogenase (2.5 μkat).

Chromatographic detection of pyruvate and glyceraldehyde was performed on thin-layer silica gel plates after reaction of the metabolites with 2,4-dinitrophenylhydrazine and extraction with ethylacetate. 2-Keto-3-deoxygluconate was detected chromatographically as described in [11].

3. RESULTS

3.1. *Products of glucose metabolism*

Dialysed cell-free extracts of *T. acidophilum* were incubated at 55°C with glucose and $NADP^+$, and the products were identified either enzymically or by thin-layer chromatography on silica gel as described in section 2. Pyruvate and glyceraldehyde were produced, with one molecule of $NADP^+$ reduced per pyruvate formed. Similar results were found when NAD^+ was substituted for $NADP^+$. Pyruvate and glyceraldehyde were also formed from D-gluconate in the absence of $NAD(P)^+$ and the production of 2-keto-3-deoxygluconate was detected as well. The glyceraldehyde was further

Table 1

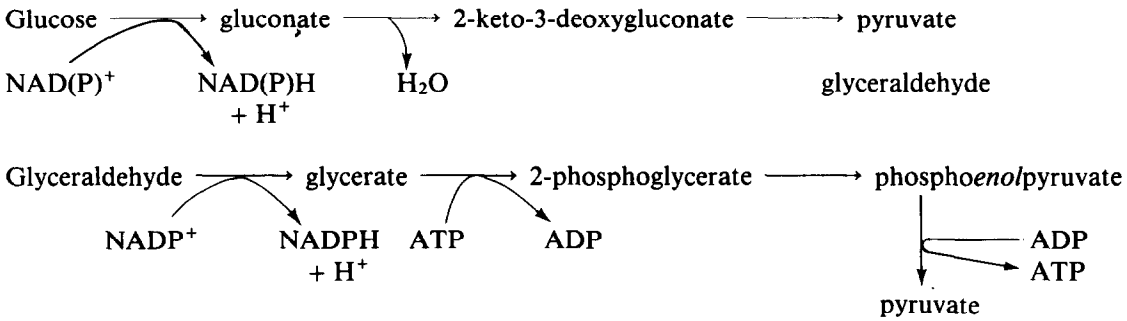
Enzymic activities in cell-free extracts of *T. acidophilum*

Enzyme	Temperature of assay (°C)	Specific activity (nmol/min per mg)	K_m (mM)		
D-Glucose dehydrogenase	55	211	D-glucose	10.0	(± 0.9)
			$NADP^+$	0.045	(± 0.005)
			NAD^+	>30.0	
D-Gluconate dehydratase	55	2	D-gluconate	5.4	(± 1.4)
D-Gluconate dehydratase and KDG-aldolase	55	4	–		
DL-Glyceraldehyde dehydrogenase	55	64	DL-glyceraldehyde	0.23	(± 0.02)
			$NADP^+$	0.0037	(± 0.0005)
DL-Glycerate kinase	40	32	DL-glycerate	0.11	(± 0.01)
			ATP	1.1	(± 0.06)
Enolase	55	162	2-phosphoglycerate	0.062	(± 0.004)
Pyruvate kinase	55	66	phospho*enol*pyruvate[a]	0.48	(± 0.02)
			ADP	0.1	(± 0.01)

[a] $S_{0.5}$ value

metabolised to a second molecule of pyruvate in the presence of $NADP^+$, ADP and ATP. Again, NADPH and pyruvate were produced stoichiometrically. Similarly, glycerate, D-2-phosphoglycerate and phospho*enol*pyruvate were converted to pyruvate in the presence of ADP and ATP.

Glyceraldehyde-3-phosphate could not be detected on incubation of dialysed cell-free extracts with glucose and ATP. Neither was pyruvate formed from glyceraldehyde-3-phosphate or D-3-phosphoglyceric acid and the appropriate cofactors.



3.2. *Enzyme activities*

The data suggest the presence in *Thermoplasma* of an Entner-Doudoroff pathway involving non-phosphorylated intermediates, as found in *Sulpholobus* [8], followed by the conversion of the glyceraldehyde to pyruvate using phosphorylated metabolites. In support of this we have found all the enzymic activities associated with the proposed pathway (table 1). Consistent with the data in section 3.1, the glucose dehydrogenase could use either NAD^+ or $NADP^+$ although the K_m for NAD^+ was >500-times that for $NADP^+$. The D-gluconate dehydratase was found to be unstable in the dialysed extracts and this may account for the low measured enzymic activity. DL-Glyceraldehyde was NADP-dependent, no activity being found with NAD^+. DL-Glycerate kinase was inhibited by Ca^{2+} and therefore had to be assayed in EGTA. The pyruvate kinase activity exhibited a sigmoidal dependence on the concentration of phospho*enol*pyruvate but not on that of ADP.

The activities of the following glycolytic enzymes could not be detected: phosphofructokinase, fructose-1,6-bisphosphatase, fructose-1,6-bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate mutase. Glucose-6-phosphate dehydrogenase, a key enzyme of the pentose phosphate cycle, also appeared to be absent.

4. DISCUSSION

The data reported are consistent with the following scheme for glucose metabolism in *T. acidophilum*:

The conversion of glucose to glyceraldehyde and pyruvate is precisely that found in the thermoacidophilic archaebacterium *Sulpholobus* [8] and differs from the Entner-Doudoroff pathways in eubacteria and the modified form in the halophilic archaebacteria [7]. Taken with the comparisons of the citric acid cycle enzymes [6], the observations reported here and in [8] are consistent with the possible phylogenetic closeness of *Sulpholobus* and *Thermoplasma*. De Rosa et al. [8] did not report the further metabolism of glyceraldehyde and so, at the moment, no comparison can be made with that part of our suggested pathway in *Thermoplasma*.

We have been unable to detect several key glycolytic enzymes, although on the basis of respiratory activities, Searcy and Whatley [16] conclude that this and the pentose phosphate pathway are present in *T. acidophilum*. At present, we cannot resolve the apparently contradictory data on the absence or presence of glycolysis; however, their observed respiratory activities on D-

gluconate and DL-glyceraldehyde, the absence of 2-keto-3-deoxy-6-phosphogluconate aldolase activity and the conversion of glucose to gluconolactone (an intermediate in the glucose dehydrogenase catalysed reaction) are all consistent with the presence of the suggested, modified Entner-Doudoroff pathway.

This metabolic scheme has so far been reported only in the thermoacidophilic archaebacteria, *Sulpholobus* and *Thermoplasma*. In view of the modified Entner-Doudoroff pathway in the extreme halophiles [7] it would now appear prudent to extend these investigations to the turnover of hexoses in the third order of archaebacteria, the methanogens. Only then can a proper comparison be made with those pathways in eubacterial and eukaryotic species.

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