THE BACTERIAL PEP-DEPENDENT PHOSPHOTRANSFERASE SYSTEM MECHANISM OF GLUCONATE PHOSPHORYLATION IN *STREPTOCOCCUS FAECALIS*

P. BERNSMANN, C. A. ALPERT, P. MUSS, J. DEUTSCHER and W. HENGSTENBERG

Ruhr-Universität Bochum, Abteilung für Biologie, Arbeitsgruppe Physiologie der Mikroorganismen, Universitätsstrasse 150, 4630 Bochum-Querenburg, Postfach 10 21 48, Gebaude NDEF-06, FRG

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1. Introduction

The bacterial PEP-dependent phosphotransferase system (PTS) is involved in transport of various carbohydrates such as disaccharides, aldoses, ketoses, alditols via vectorial phosphorylation [1]. PTS-mediated transport so far studied seemed to be restricted to uncharged carbohydrate molecules. Here, we describe PTS-dependent phosphorylation of gluconate in *Streptococcus faecalis*. In contrast, data available for gluconate uptake in *Escherichia coli* are consistent with a proton gradient-driven accumulation [2].

2. Experimental

2.1. *Cell cultivation*

Streptococcus faecalis strain 26487 (Streptococcen Zentrale, Kiel) was grown in 100 l batches at 37°C in a Chemap fermenter. A 100 l medium was composed of the following: 100 g yeast extract (Ohly Hamburg); 200 g tryptone (Difco); 250 g Na_2HPO_4; 1000 g sodium gluconate.

The pH was kept at 7 by addition of 10% NaOH. After 5–6 h the absorbance reached 10–12. The culture was harvested with a Westfalia continuous flow centrifuge. The yield was 1 kg wet cell paste.

2.2. *Cell-free extracts*

Cell breakage was achieved with a Dynomill KDL (Bachofen Basel) in the continuous flow mode as in [3].

2.3. *Protein preparations from* S. faecalis

2.3.1. HPr protein

Details of the preparation are in [4]. The purification procedure is similar as for the *S. aureus* HPr protein [3].

2.3.2. Enzyme I protein

Enzyme I of *S. faecalis* eluted from a DEAE-cellulose column at the same salt concentration as enzyme I from *S. aureus*. Further steps which are not yet published involve $(NH_4)_2SO_4$ fractionation, acid precipitation (pH 5), Sephadex G-100 chromatography at pH 5, hydroxyapatite chromatography. The material obtained this way was ~70% pure.

2.3.3. Gluconate-6-phosphate dehydrogenase

Preparation of this enzyme from *S. faecalis* was as in [5]. We found this enzyme during enzyme I preparation where it was separated from enzyme I by the hydroxyapatite chromatography.

2.3.4. Factor III specific for gluconate from *S. faecalis*

Cell-free extract from 300 g *S. faecalis* (Dynomill) was applied to a DEAE-cellulose column 12 × 20 cm and eluted with a 7 l gradient 0–0.9 M NaCl in standard buffer [3] behind the enzyme I fractions. To obtain homogeneous material the following steps were used: $(NH_4)_2SO_4$ precipitation 45–65% saturation, dialysis against standard buffer, acid precipitation at pH 5: factor III in supernate, Sephadex G-75 chromatography, DE-52 chromatography 0–0.35 M NaCl gradient, size exclusion chromatography with LKB TSK-G 2000 SW. Yield: 4 mg freeze dried protein. The purification procedure has not yet been optimized to give maximal recoveries.

2.3.5. Assay of gluconate specific PTS

A semi-micro cuvette was loaded with the following compounds:

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2.5 μmol $MgCl_2$
2.5 μmol PEP monocyclohexylammonium salt
5 μg enzyme I
0.5 μmol gluconate
50 μg enzyme II membrane protein in 0.1% Triton (pH 9.3)
10 μg gluconate dehydrogenase
10 μg factor III specific for gluconate
50 μg HPr protein
0.25 μmol NADP

The final volume was 0.37 ml, 0.05 M Tris–HCl (pH 7.5). Absorbance was recorded at 366 nm with an Eppendorf filter photometer at 30°C.

2.4. *Membrane preparation*

Membrane fragments obtained by Dynomill cell breakage elute with the washing buffer during DE-23 cellulose chromatography. The turbid fractions were collected. Membranes precipitated at 40–70% $(NH_4)_2SO_4$ saturation. The pellet was washed twice with standard buffer (pH 7.5) by centrifugation at 120 000 × *g*. Pellet (50 mg) was dissolved in 1 ml 0.08 M Tris–glycine (pH 9.3), 0.1% Triton X-100. This solution was used without centrifugation in the assay system described.

3. Results

3.1. *Dependence of gluconate phosphorylation on PTS proteins*

Table 1 shows rates of gluconate-6-P formation with respect to PTS components and PEP. It is obvious from the above results, that the carbohydrate gluconate is substrate of a PEP-dependent phosphotransferase system. Preliminary experiments using crude extracts of *S. faecalis* cells grown in the presence of gluconate showed a more efficient PEP-dependent phosphorylation of gluconate compared to ATP-dependent phosphorylation. In the system using purified PTS proteins no phosphorylation with ATP was observed (table 1).

Table 1
PEP dependent gluconate phosphorylation

Assay composition	nmol NADPH formed/min
Complete system	1.5
–Enzyme I	0.3[a]
–HPr	–
–Factor III specific for gluconate	–
–membranes	–
–PEP	–
–PEP, +ATP	–

[a] The gluconate-6-P-dehydrogenase was contaminated with small amounts of enzyme I (cf. section 2)

3.2. *Inducibility of the gluconate PTS in* S. faecalis

Experiments to find gluconate phosphorylation activity were not successful if the cells were grown in complex media without gluconate or with glucose. Only cells grown in the presence of gluconate possess specific PTS components.

3.3. *Metabolism of gluconate by* S. faecalis

The PEP-dependent phosphorylation of gluconate to gluconate-6-phosphate and the presence of the 6-phosphogluconate-dehydrogenase in *S. faecalis* which converts gluconate-6-P to ribulose-5-P and CO_2 [5] are indicative arguments for further metabolism of ribulose-5-P via the pentose-phosphate pathway. This suggestion is supported by the amount of acid produced by the metabolism of gluconate by whole cells suspensions of *S. faecalis* as determined with a pH stat. Gluconate only results in an acid production of ~66% of the acid produced by glucose.

This is reasonably explained by the stoichiometry

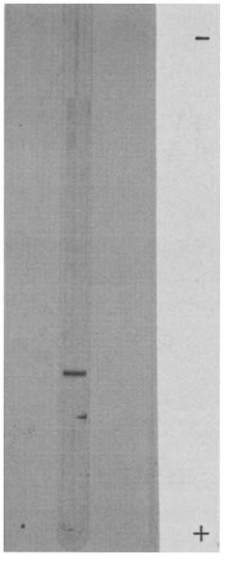

Fig.1. Acrylamide gel electrophoresis of homogeneous factor III specific for gluconate. Conditions: 7.5% Acrylamide; 0.4 M Tris–glycine (pH 9.3); stained with Coomassie blue.

of the pentose-phosphate cycle where 3 mol gluconate are converted to 2 mol glucose, which are metabolized by glycolysis to give 4 mol lactic acid.

The K_m-value for gluconate consumption of whole cell suspensions has been determined from the pH-stat progress curves to be 2×10^{-5} M.

3.4. *Some properties of the factor III component specific for gluconate*

As described for other PEP-dependent sugar phosphorylation systems the gluconate PTS possesses an inducible sugar specific soluble component. This protein was purified to homogeneity (fig.1). The native M_r-value was determined on a calibrated molecular sieve column to be 50 000. The M_r of the polypeptide chain on SDS gels was estimated at 12 000. This would suggest that the protein has a subunit structure. Further research will be done to compare this factor III type protein with the analogous protein of *S. aureus* specific for galactosides [6].

3.5. *The membrane component*

Membrane fragments obtained after cell breakage with glass beads were eluted in the wash fluid from the DE-23 column. The enzyme II activity was stable at 0.1% Triton X-100. Further attempts will be made to isolate this protein.

4. Discussion

The above results describe the existence of a PEP-dependent inducible gluconate-specific PTS which connects the uptake of gluconate to this step. PTS mutants of *S. faecalis* are the appropriate way to finally prove this. However, in all PT systems studied so far accumulation and phosphorylation of the sugar are strictly coupled. Since gluconate is a negatively-charged substrate, questions arise how electroneutrality is maintained during gluconate uptake. In *E. coli* alkalinization of the medium during gluconate uptake was observed [7]. One explanation is symport of 1 mol gluconate with 1 mol H^+.

To decide whether *S. faecalis* achieves electroneutrality during gluconate uptake or whether it transports gluconate as anion the isolation of a mutant which does not further metabolize 6-phosphogluconate is necessary. A strain without 6-P-gluconate dehydrogenase activity should meet these requirements; we shall attempt to isolate it.

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