# DISTRIBUTION OF 1-PHOSPHOFRUCTOKINASE AND PEP:FRUCTOSE PHOSPHOTRANSFERASE ACTIVITY IN CLOSTRIDIA

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## 1. Introduction

Many clostridia grow readily on fructose as source of energy and carbon. Since facultative anaerobes metabolize fructose using the PEP:fructose phosphotransferase system and 1-phosphofructokinase [1–3] it seemed desirable to assay extracts of various clostridia for these enzyme activities. The presence of 1-phosphofructokinase in *Clostridium pasteurianum* has been reported by Kotzé [4] and the PEP-dependent formation of fructose-1-phosphate was shown in fructose-grown *Cl. thermocellum* [5].

## 2. Materials and methods

The procedures for growing *Cl. butyricum* (ATCC 19 398), *Cl. pasteurianum* (ATCC 6013), *Cl. roseum* (ATCC 17 797) and *Cl. rubrum* (ATCC 14 949) have been described previously [6]. *Cl. kluyveri* (ATCC 8 527) was grown according to [7] and *Cl. formicoaceticum* (ATCC 27 078) according to [8].

Cells were harvested after 16–20 hr of growth by centrifugation at 4°C, washed twice with 50 mM Tris–HCl buffer, pH 8.0, and stored at −20°C. Cell-free extracts were prepared as described [6] and their protein content was determined according to Lowry et al. [9]. Protein of sonicated cell suspensions was estimated by the method of Schmidt et al. [10].

The assays for 1-phosphofructokinase, 6-phosphofructokinase and the PEP:fructose phosphotransferase system are given in tables 1 and 3. One enzyme unit catalyzes the conversion of 1 μmole of substrate per min under the conditions defined.

## 3. Results and discussion

Cell-free extracts of the clostridial species depicted in table 1 were assayed for 1- and 6-phosphofructo-

Table 1
Specific activity of 1-phosphofructokinase and 6-phosphofructokinase in cell-free extracts of various clostridia

| Microorganism | Specific activity | |
|---|---|---|
| | 1-Phosphofructokinase (U/g protein) | 6-Phosphofructokinase (U/g protein) |
| *Cl. pasteurianum* | 350.0 | 71.0 |
| *Cl. roseum* | 355.0 | 89.0 |
| *Cl. rubrum* | 360.0 | 160.0 |
| *Cl. butyricum* | 307.0 | 146.0 |
| *Cl. formicoaceticum* | 27.0 | 62.0 |
| *Cl. tetanomorphum* | 0 | 49.0 |
| *Cl. kluyveri* | 0 | 0 |

*Cl. tetanomorphum* was grown on glucose and *Cl. kluyveri* on ethanol plus acetate. All other clostridia were grown on fructose.
For determination of enzyme activity the following components were incubated in a final volume of 2 ml at 30°C for 10 min; 50 mM Tris–HCl buffer, pH 8.0; 5 mM $MgCl_2$; 2 mM ATP; 5 mM fructose-1-P or fructose-6-P; 5 mM phosphoenolpyruvate; 20 μg pyruvate kinase and cell-free extract (1–2 mg of protein). The reaction was stopped by heating the mixture in a boiling water bath for 2 min. After centrifugation, an aliquot of the clear supernatant was analyzed for pyruvate formed. Controls were run without fructose-1-P or fructose-6-P. Since the assays were conducted under aerobic conditions in the absence of reducing agents pyruvate:ferredoxin oxidoreductase was inactive and the breakdown of pyruvate during the incubation at 30°C was negligible.


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kinase (1-PFK and 6-PFK) activity. *Cl. kluyveri* is nonsaccharolytic [7]; neither 1-PFK nor 6-PFK could be detected in this microorganism. *Cl. tetanomorphum* grows on glucose but not on fructose [11]. In accordance with this it contained 6-PFK but lacked 1-PFK. With fructose as growth substrate the level of 1-PFK in the typical saccharolytic clostridia was significantly higher than the one of 6-PFK. *Cl. formicoaceticum* which carries out a homoacetate fermentation showed comparatively low 1-PFK activity.

The effect of the growth substrate on the level of 1-PFK and 6-PFK in *Cl. pasteurianum* is shown in table 2. Substrates which necessitated gluconeogenesis during growth (pyruvate, gluconate) repressed 6-PFK activity. 1-PFK was less affected. Surprisingly, 1-PFK activity exceeded 6-PFK activity with all growth substrates tested. Patni and Alexander [5] found that *Cl. thermocellum* contained significant amounts of 1-PFK only when the cells were grown with fructose. The same has been reported for *Aerobacter aerogenes* [12] and *Escherichia coli* [3]. Glucose-grown cells of *Bacteroides symbiosus,* however, also contained 1-PFK levels comparable of those of fructose-grown cells [13].

Sonicated cell suspensions of the clostridial species were tested for the presence of the PEP:fructose phosphotransferase system. It can be seen from table 3 that with the exception of *Cl. formicoaceticum* the formation of fructose-1-phosphate from PEP and fructose could be demonstrated. It, therefore, can be concluded that the saccharolytic clostridia investigated employ the transferase system in conjunction with 1-phosphofructokinase to channel fructose into the Embden–Meyerhof pathway. In *Cl. formicoaceticum,* a reaction leading to the formation of fructose-1-phosphate could not be found, and it remains to be elucidated what system is employed by this microorganism to transport and phosphorylate fructose. The same is true for *Cl. thermoaceticum* which also contains 1-PFK but lacks a detectable phosphotransferase system (M. Gottwald, unpublished results).

Table 2
Phosphofructokinase activity of cell-free extracts of *Cl. pasteurianum* grown on different substrates

| Growth substrate | Specific activity | |
|---|---|---|
| | 1-Phosphofructokinase (U/g protein) | 6-Phosphofructokinase (U/g protein) |
| Pyruvate | 82.0 | 9.0 |
| Gluconate | 93.0 | 0 |
| Glucose | 111.0 | 36.0 |
| Sucrose | 333.0 | 45.0 |
| Fructose | 350.0 | 71.0 |

Assay as described in table 1.

Table 3
PEP-dependent conversion of fructose to fructose-1-phosphate by sonicates of various clostridia

| Microorganism | nmole F-1-P/min/mg protein |
|---|---|
| *Cl. pasteurianum* | 57.2 |
| *Cl. roseum* | 5.5 |
| *Cl. rubrum* | 42.4 |
| *Cl. butyricum* | 21.2 |
| *Cl. formicoaceticum* | ND* |
| *Cl. tetanomorphum* | ND |
| *Cl. kluyveri* | ND |

* ND, not detectable.
1 ml of the reaction mixture contained: 50 mM Tris–HCl buffer, pH 7.6; 10 mM PEP; 20 mM fructose; 10 mM $MgCl_2$; 10 mM mercaptoethanol; sonicate containing 4–18 mg of protein. The mixture was incubated at 37°C for 0, 20 and 40 min, and the reaction was stopped by heating it in a boiling water bath for 5 min. Controls were run without fructose or PEP. After centrifugation, an aliquot of the clear supernatant was analyzed for the fructose-1-phosphate formed using 1-phosphofructokinase: 3 ml of the reaction mixture contained: 50 mM Tris–HCl buffer, pH 8.0; 3.3 mM $MgCl_2$; 1.0 mM ATP; 0.50 mM $NADH_2$; 4 μg aldolase; 0.3 ml of the clear supernatant. The reaction was started by the addition of 5 μg of 1-phosphofructokinase (300 U/mg of protein; purified from *Cl. pasteurianum*). ΔE was determined at 365 nm.

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## References

[1] Kundig, W., Ghosh, S. and Roseman, S. (1964) Proc. Natl. Acad. Sci. U.S. 52, 1067.

[2] Hanson, T. E. and Anderson, R. L. (1966) J. Biol. Chem. 241, 1644.
[3] Fraenkel, D. G. (1968) J. Biol. Chem. 243, 6458.
[4] Kotzé, J. P. (1968) S. Afr. Agric. Sci. 11, 349.
[5] Patni, N. J. and Alexander, J. K. (1971) J. Bacteriol. 105, 226.
[6] v. Hugo, H., Schoberth, S., Madan, V. K. and Gottschalk, G. (1972) Arch. Mikrobiol. 87, 189.
[7] Bornstein, B. T. and Barker, H. A. (1948) J. Bacteriol. 55, 223.
[8] Andreesen, J. R., Gottschalk, G. and Schlegel, H. G. (1970) Arch. Mikrobiol. 72, 154.
[9] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265.
[10] Schmidt, K., Liaaen-Jensen, S. and Schlegel, H. G. (1963) Arch. Mikrobiol. 46, 117.
[11] Anthony, C. and Guest, J. R. (1968) J. Gen. Microbiol. 54, 277.
[12] Kelker, N. E., Hanson, T. E. and Anderson, R. L. (1970) J. Biol. Chem. 245, 2060.
[13] Hsu, D. S. and Reeves, R. E. (1970) Arch. Biochem. Biophys. 137, 59.