# THE BACTERIAL PHOSPHOENOLPYRUVATE DEPENDENT PHOSPHOTRANSFERASE SYSTEM (PTS)

## Solubilisation and kinetic parameters of the glucose-specific membrane bound enzyme II component of *Streptococcus faecalis*

Hendrik HÜDIG and Wolfgang HENGSTENBERG

*Abteilung Biologie, Arbeitsgruppe Physioloie der Mikroorganismen, Ruhr-Universität Bochum, Postfach 10 2148, D-4630 Bochum, FRG*

Received 21 March 1980

### 1. Introduction

The first step in glucose metabolism in many Gram-positive and Gram-negative bacteria is its uptake by vectorial phosphorylation via the phosphotransferase system (PTS), which consists of 2–3 soluble phosphocarrier proteins and membrane bound sugar specific components (Enzyme II) [1–4]. Recently, it has been shown that one of the Enzyme II complexes catalyses a sugar phosphate: sugar transphosphorylation reaction which is independent of the soluble components of the PTS [5].

In this letter we report the solubilisation of the glucose-specific Enzyme II component of *Streptococcus faecalis* with the non-ionic detergent Aminoxid WS 35 and kinetic parameters of PTS phosphorylation and transphosphorylation from glucose-6-phosphate and from galactose-6-phosphate to glucose.

### 2. Experimental procedures

#### 2.1. *Preparation of Enzyme II-membrane fragments*

*Streptococcus faecalis* 26487 (kindly provided from the Streptococcen-Zentrale, Kiel) was grown in a 100 l-fermentor on a medium containing 1 kg yeast extract, 200 g Tryptone, 10 g $MgSO_4$, 1 kg glucose, 200 g $Na_2HPO_4 \times 2\ H_2O$ at pH 7.0. At $A_{580}$ 10 the yield of wet cell paste was about 1.2 kg.

200 g of cells (wet weight) were suspended in 1 l of standard buffer (50 mM Tris–HCl, pH 7.5, $10^{-4}$ M EDTA, $10^{-3}$ M dithiothreitol, $10^{-4}$ M phenylmethylsulfonylfluoride) containing 5 mg DNase (Boehringer) and pumped through a continuous-flow vessel of a Dyno-Mill type KDL (W. A. Bachofen, Basel, Switzerland) at a flow rate of 2 l/h (glass beads: 0.3 mm). Cell debris was removed by centrifugation at 12 000 × *g* for 2 h. The crude extract was applied to a DEAE-cellulose column (9 × 19 cm, Whatman DE 23) equilibrated with standard buffer. The column was eluted with 2 l of standard buffer. Membrane fragments eluted without retention from the column. The soluble PTS-components finally elute under a 6 l gradient of 0–0.6 M NaCl [6]. The membrane fragments were precipitated with ammoniumsulfate (70%) and centrifuged at 12 000 × *g*. The pellet was resuspended in standard buffer and 3 × centrifuged at 350 000 × *g* for 1.5 h, 5°C. Purified membrane fragments, stored at –20°C, contain Enzyme $II^{gluc}$-activity, stable for several months.

#### 2.2. *Solubilisation of Enzyme $II^{gluc}$*

500 mg membranes (wet weight) with about 80 mg protein (Lowry), were resuspended in 100 ml standard buffer, pH 7.5 or 0.08 M Tris-glycine buffer, pH 9.3. Aminoxid WS 35 (1-alkoylamino-3-dimethylaminopropane-3-*N*-oxide (Th. Goldschmidt, AG, Essen, FRG) was added. The final concentration was 0.1% Aminoxid. After incubation for 0.5 h at 25°C nonsolubilized Enzyme II was sedimented by ultracentrifugation (150 000 × *g*, 1 h, 10°C). The supernatant contained about 50% solubilized Enzyme II.

2.3. *Enzyme II-assay*

The glucose-phosphorylating activity was assayed in vitro by the NADP-dependent glucose-6-phosphate dehydrogenase (G-6-P-DH). The total volume of the assay was 705 μl containing 5 μmol $MgCl_2$, 5 μmol PEP, 600 μg enzyme I, 12.5 nmol HPr, 5 mg membranes (wet weight), 1 μmol glucose, 2 μmol $NADP^+$ (Boehringer) and 2.5 μg G-6-P-DH (Boehringer). Enzyme I from *Staphylococcus aureus* was used in a partially purified preparation as described in [7]. $NADPH_2$ was measured in an Eppendorf-Photometer at 366 nm at 25°C. For measuring the transphorylating activity of Enzyme II the assay contained 1 μmol galactose-6-P (Boehringer). 1 μmol glucose, 5 mg membranes (wet weight), 5 μmol $MgCl_2$, 2 μmol $NADP^+$ and 2.5 μg G-6-P-DH (Boehringer) in a final vol. of 705 μl.

## 3. Results and discussion

3.1. *Dependence of G-6-P formation on PTS components*

To assay the G-6-P formation we have chosen the NADP-dependent G-6-P-DH. Phosphorylating of glucose only occurred in the presence of membrane fragments of *S. faecalis*, HPr from *Strep. faecalis* or *S. aureus*, Enzyme I from *S. aureus* and phosphoenolpyruvate. Preliminary experiments showed that Enzyme I from *S. faecalis* can be used in the *Staphylococcus aureus* mutant complementation assay [8] as well as homologeous Enzyme I.

3.2. *Solubilisation and partial purification of the membrane bound Enzyme II specific for glucose with the non-ionic detergent Aminoxid WS 35*

Solubilisation. Successful solubilisation of EII activity was achieved in 0.08 M Tris-glycine buffer, pH 9.3 at a protein detergent ratio of about 10:1 in 0.1% solution, yielding 50–60% of soluble enzyme activity. Similar results were obtained with Genapol X-100 (Farbwerke Hoechst). The detergent Triton X-100, which is widely applicated in solubilisation experiments destroyed Enzyme II activity at concentrations of 0.1–1%. Triton has been successfully applied in the solubilisation and purification of Enzyme II specific for galactosides of *S. aureus* [9]. As already observed during our studies with the staphylococcal Enzyme II specific for galactosides the addition of non-ionic detergent to the membrane suspensions stimulated the biological activity of Enzyme II specific for glucose 1.5-fold. One trivial explanation may be that binding sites for HPr or sugar, which are hidden in the vesicular structures of the membrane fragments [10] became accessible after detergent treatment. The solubilized Enzyme II activity had a half life of 2 days at 4°C. Glucose at $5 \times 10^{-3}$ M did not protect from inactivation.

Partial purification of the solubilized Enzyme II by ion exchange chromatography. Soluble Enzyme II bound to DEAE cellulose at pH 9.3 in 0.1% Aminoxid. Enzyme II activity was eluted in several peaks as shown in fig.1. Peak III contained the highest specific activity of Enzyme II. An electrophoretic analysis in SDS of the peaks with Enzyme II activity revealed that the protein pattern consisted of only two major bands with minor impurities. Since strains of *S. faecalis* with deletions of the constitutive Enzyme $II^{gluc}$ are not available we cannot identify the band in the SDS gel pattern which is responsible for the Enzyme II activity.

3.3. *Evidence of direct phosphotransfer from phospho-HPr to the sugar by the membrane component*

In *E. coli* two glucose specific PT systems have been characterized: The high-affinity system operates with a second phosphocarrier component (Enzyme III) which is cytoplasmic and soluble (MW 25 000) [4].

The low-affinity system is composed of two membrane-bound components one of which possesses the sugar specificity. According to Saier et al. [5] a single membrane-bound sugar specific component is responsible for the mannitol phosphorylation in *E. coli*. We suggest that the glucose specific PTS of *S. faecalis* is similarly arranged as the *E. coli* mannitol system: The phosphogroup is directly transferred to the sugar via the membrane component.

The following data support this suggestion. Enzyme I used in our assays was free of low MW proteins (Sephadex cut from 60–100 000 daltons). HPr of *S. faecalis* was a homogeneous preparation [11]. Even after solubilisation and ion exchange chromatography PEP-dependent glucose phosphorylation could be demonstrated in the complete system.

3.4. *Substrate specificity at the PEP-dependent phosphorylation*

Transphosphorylation from galactose-6-P to glu-

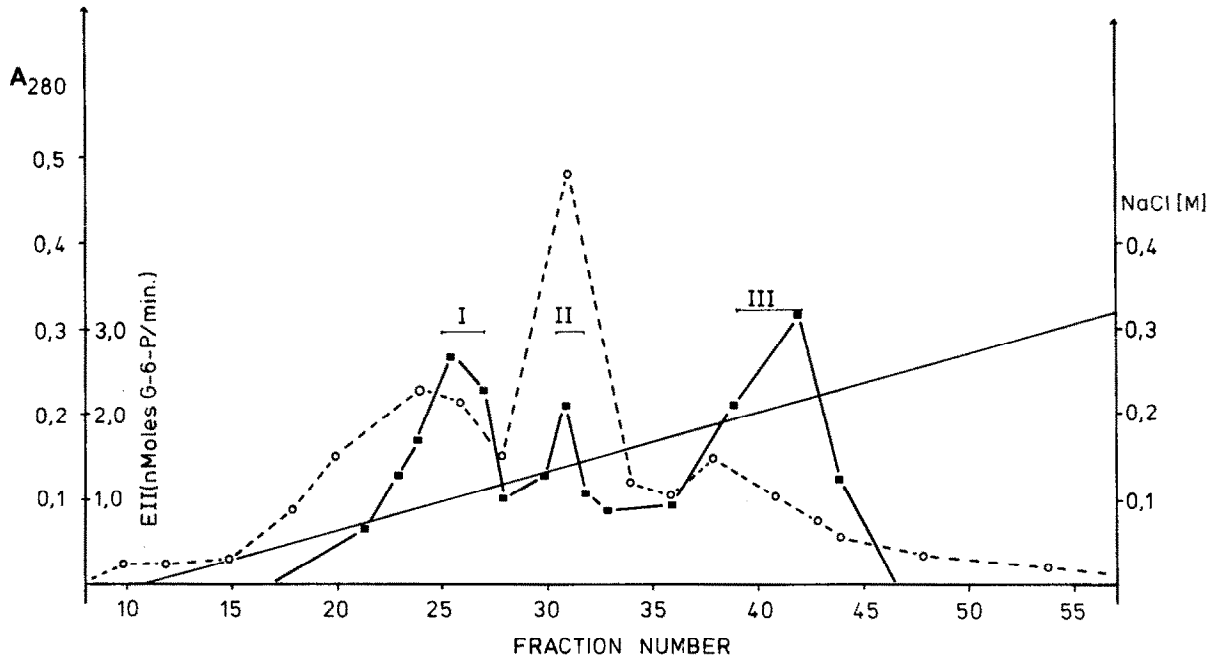


Fig.1. Ion exchange chromatography on DEAE-cellulose column (2.5 × 18.5 cm; Whatman, DE 23) in 0.1% Aminoxid and 0.08 M Tris–glycine buffer, pH 9.3. –■–■–, Enzyme II-activity; ––o––o––, $A_{280}$; —— NaCl-gradient (0–0.5 M; 2 × 300 ml). Fractions of 8 ml.

cose. As listed in table 1 only free hexoses are accepted as substrates for the glucose-specific PTS. Epimers at C(2) like mannose or 2-deoxyglucose are substrates whereas the C(4) epimer galactose does not inhibit glucose phosphorylation. The analogue 5-thioglucose is a potent inhibitor, which means that the ring oxygen of the pyranose is not necessary for substrate recognition. Glycosides of the α- and β-configuration did not reduce glucose phosphorylation. α-Methylglucoside which is commonly used as a glucose analog for transport assays does not inhibit even at a 20-fold molar excess over a glucose concentration of $10^{-4}$ M.

Transphosphorylation: As already observed for the mannitol system of *E. coli* where mannitol-phosphate can serve as phosphodonor for free mannitol G-6-P or even galactose-6-P phosphorylate free glucose in presence of *S. faecalis* membrane fragments. Galactose-6-phosphate is an exceptionally suitable phosphoryl donor for glucose because G-6-P formation can easily be estimated in the presence of galactose-

Table 1

| | *Strep. faecalis*[a] | | *E. coli* [12] |
|---|---|---|---|
| | $K_m$ (M) | $K_i$ (M)[b] | $K_m$ (M) |
| Glucose | $4 \times 10^{-5}$ | | $4 \times 10^{-4}$ |
| 2-Deoxyglucose | $4 \times 10^{-5}$ | | – |
| Galactose | | | – |
| Mannose | | $1 \times 10^{-4}$ | $6 \times 10^{-4}$ |
| 5-Thioglucose | | $6.2 \times 10^{-4}$ | – |
| *N*-Acetylglucosamine | | | $9 \times 10^{-4}$ |
| Methyl-α-glucopyranoside | | | $7 \times 10^{-4}$ |

[a] Values calculated according to the method of Lineweaver and Burk or Eadie and Hofstee

[b] $K_i$ values represent competitive inhibition of glucose phosphorylation

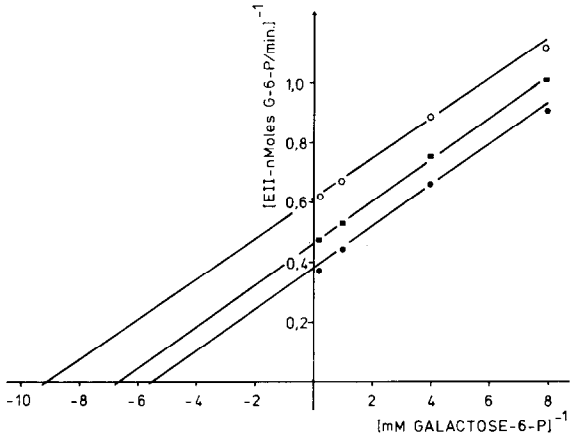


Fig.2. Shows the determination of the $K_m$ for galactose-6-phosphate at various glucose concentrations. According to Cleland the above kinetics can be best interpreted as a double displacement (ping pong) reaction [13], which suggests formation of a covalently bound phosphogroup at Enzyme $II^{gluc}$. –o–o–, 0.5 mM glucose; –▪–▪–, 2 mM glucose; –•–•–, 4 mM glucose.

6-P which is not a substrate for G-6-P-DH. HPr ($K_m$ for Enzyme II: $3.2 \times 10^{-6}$ M) competitively inhibits G-6-P formation from galactose-6-P ($K_m$ $1.5 \times 10^{-4}$ M at 2 mM glucose).

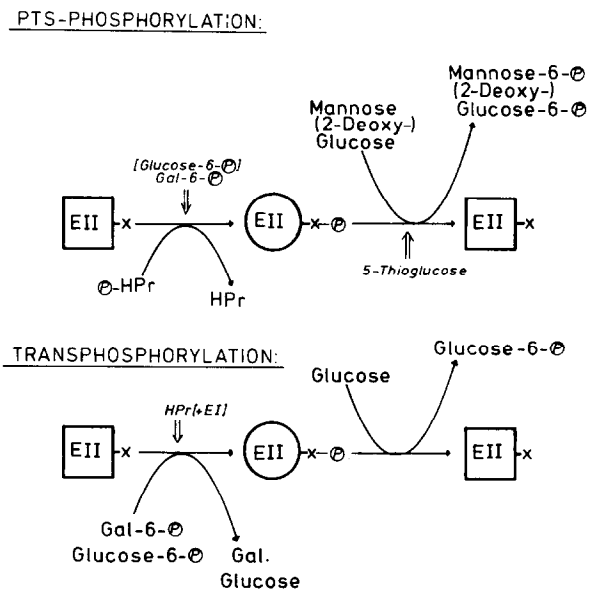


Fig.3. Summary of Enzyme II catalysed reactions. The upper part of the scheme describes the PTS-dependent phosphorylation. Galactose-6-P and G-6-P inhibit glucose phosphorylation, an influence which has already been noted by Kaback et al. in *E. coli* [14] and which is plausible from the physiological point of view. 5-thioglucose is not listed as a 6-phosphate, since 5-thioglucose-6-phosphate has not yet been identified in PTS reactions. The transphosphorylation scheme illustrates the competition between HPr and galactose-6-P at the Enzyme II level which also can be interpreted as further evidence for the direct phosphotransfer from phospho-HPr to the sugar by the membrane component.

## References

[1] Saier, M. H. jr (1974) Bacteriol. Rev. 41, 856–871.
[2] Hengstenberg, W. (1977) Curr. Top Microbiol. Immunol. 77, 98–121.
[3] Robillard, G. T., Dooijewaard, G. and Lolkema, J. (1979) Biochemistry 18, 2984–2990.
[4] Kundig, W. (1974) J. Supramol. Struct. 2, 695–714.
[5] Jacobson, G. R., Lee, C. A. and Saier, M. H. jr (1979) J. Biol. Chem. 254, 249–252.
[6] Beyreuther, K., Raufuss, H., Schrecker, O. and Hengstenberg, W. (1977) Eur. J. Biochem. 75, 275–286.
[7] Stein, R., Schrecker, O., Lauppe, H. F. and Hengstenberg, W. (1974) FEBS Lett. 42, 98–100.
[8] Hengstenberg, W., Penberthy, W. K., Hill, K. L. and Morse, M. L. (1969) J. Bacteriol. 99, 383–388.
[9] Foohs, A., Hengstenberg, W. and Schrecker, O. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 1082–1083.
[10] Lengsfeld, A. H., Alexander, E. I., Hengstenberg, W. and Korte, T. (1973) Exp. Cell Res. 76, 159–169.
[11] Bernsmann, P., Muss, P., Hengstenberg, W., Beyreuther, K. and Kalbitzer, H. R. (1980) Hoppe-Seyler's Z. Physiol. Chem., 361, 220–221.
[12] Kundig, W. and Roseman, S. (1971) J. Biol. Chem. 246, 1353–1406.
[13] Cleland, W. W. (1970) in: The Enzymes (P. D. Boyer, ed) 3rd/edn., vol. 2, pp. 1–65, Academic Press, New York.
[14] Kaback, H. R. (1970) Curr. Top. Membr. Transp. 1, 35–99.