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0273-2289/97/63-65—0159$11.50

Reaction Engineering Aspects of α-1,4-D-Glucan Phosphorylase Catalysis

Comparison of Plant and Bacterial Enzymes for the Continuous Synthesis of D-Glucose-1-Phosphate

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ABSTRACT

Some important process properties of α-1,4-D-glucan phosphorylases isolated from the bacterium *Corynebacterium callunae* and potato tubers (*Solanum tuberosum*) were compared. Apart from minor differences in their stability and specificity (represented by the maximum degree of maltodextrin conversion) and a 10-fold higher affinity of the plant phosphorylase for maltodextrin (K_M of 1.3 g/L at 300 m*M* of orthophosphate), the performances of both enzymes in a continuous ultrafiltration membrane reactor were almost identical. Product synthesis was carried out over a time course of 300–400 h in the presence or absence of auxiliary pullulanase (increasing the accessibility of the glucan substrate for phosphorolytic attack up to 15–20%). The effect of varied dilution rate and reaction temperature on the resulting productivities was quantitated, and a maximum operational temperature of 40°C was identified.

Index Entries: α-1,4-D-Glucan phosphorylase; *Corynebacterium callunae; Solanum tuberosum*; α-D-glucose-1-phosphate; continuous production.

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INTRODUCTION

α-1,4-D-Glucan phosphorylases are widespread in nature and catalyze the phosphorolytic degradation of α-1,4-linked oligo- or poly-D-glucose into α-D-glucose-1-phosphate (D-Glc-1-*P*). According to their natural substrates, the enzymes can be classified as glycogen, starch, or maltodextrin phosphorylases, and indeed phosphorylases isolated from various sources show striking differences in their specificity towards these substrates *(1,2)*. The phosphorolysis reaction is readily reversible in vitro as shown in equation 1 where N (≈ 10–25) denotes the degree of polymerization of the available linear chains in the respective glucan *(3)*.

$$(\alpha\text{-1,4-D-glucan})_N + P_i \Leftrightarrow (\alpha\text{-1,4-D-glucan})_{N-1} + \alpha\text{-D-Glc-1-}P \qquad (1)$$

The equilibrium constant is pH-dependent, but even in alkaline regions between pH 8.0–9.0 polymer synthesis is favored. Glucan phosphorylases have attracted much attention with regard to their catalytic mechanism, regulation, and evolution *(4–6)*. Their use as biocatalysts in an applied field of research is, however, rarely documented, and with the exception of starch phosphorylase from *Solanum tuberosum* (potato), the process properties of these enzymes have not been studied in much detail *(7,8)*. We have recently become interested in possible applications of microbial phosphorylases lacking allosteric and covalent regulation of enzyme activity like the potato enzyme and have been studying the synthesis of D-Glc-1-*P* by phosphorylases from *Corynebacterium callunae* and *Escherichia coli (9–11)*.

D-Glc-1-*P* has limited applications as such. Likely fields for the direct use of this compound are mostly medically oriented *(12)*. However, as an activated or naturally C1-protected sugar it may serve as an important intermediate or starting material in the synthesis of simple and complex carbohydrates. The use of D-Glc-1-*P* for the production of glucuronic acid and α,α-trehalose, that both are fine chemical with large scale applications in food industries, has been recently demonstrated *(13,14)*. Oligosaccharide syntheses by chemoenzymatic approaches are often dependent of glycosyl transferases requiring UDP- or ADP-D-glucose as substrates. These compounds in turn are derived from D-Glc-1-*P* and the corresponding nucleotide triphosphates *(15)*.

Pertaining to process engineering aspect in continuous enzyme catalysis, the use of soluble biocatalysts is promising especially when the optimization of productivities and space time yields at concomitant high specificity and selectivity is considered. Immobilization of the biocatalysts in their native state by employing ultrafiltration membranes represents the tool of process technology to accomplish this objective *(16,17)*. We have recently demonstrated that microbial glucan phosphorylases are stable and well-suited for conversions of maltodextrins and orthophosphate in continuous ultrafiltration membrane reactors *(9–11)*. In this study the

authors have compared the phosphorylase from *C. callunae* with the higher plant enzyme from *S. tuberosum*: The latter enzyme has already been used for the conversion of starchy material in soluble or solid-immobilized form *(7,8)*. Its application in membrane reactors, however, has so far not been studied.

MATERIALS AND METHODS

Chemicals

All chemicals were of reagent grade and obtained from Sigma (Deisenhofen, Germany) unless otherwise stated. The material for protein chromatography and electrophoresis was from Pharmacia (Uppsala, Sweden). Pullulanase (Promozyme) was from Novo (Bagsvaerd, Denmark) with a declared activity of 200 U per g liquid.

Enzyme Production and Isolation

Corynebacterium callunae DSM 20147 was used throughout this study and cultivated in shaken flasks or in a 10-L bioreactor system (MBR, Wetzikon, Switzerland) as previously described *(9)*. Glucose was used as a carbon source in the concentrations indicated. Maltodextrin or maltose were employed as the components inducing the synthesis of α-glucan phosphorylase. For monitoring the time course of enzyme production in bioprocess experiments, samples were taken at the times stated, and biomass formation and phosphorylase activity in cell extracts determined. The preparation of these extracts was as reported previously *(9)*.

The partial purification of *C. callunae* phosphorylase was accomplished by ammonium sulfate precipitation (25% saturation) followed by hydrophobic interaction chromatography on Phenylsepharose fast flow (decreasing linear gradient of 0 to 20% $(NH_4)_2SO_4$) to yield a stable enzyme preparation with a specific activity of 3.5 U/mg *(9,11)*.

The isolation of *S. tuberosum* phosphorylase was carried out as follows. Peeled and cut potato tubers were transferred into 10 m*M* P_i buffer pH 6.9 (supplemented with 0.1% sodium dithionite) and grounded for 1–2 min in a conventional kitchen mixer. The potato juice was centrifuged (7500 rpm, 15 min), and the slightly acidified (pH 5.8) supernatant treated with 5000 ppm biocryl processing aids (BPA 1050; Toso Haas, Stuttgart, Germany). The pelleted phosphorylase (6000 rpm; 15 min) was redissolved in 50 m*M* P_i pH 6.9 and brought to 25% saturation in $(NH_4)_2SO_4$. The supernatant recovered after ultracentrifugation (30,000 rpm, 40 min; 45.1 Ti rotor) was applied to a 25-mL XK 26 column of Phenylsepharose fast flow low sub. Bound protein was eluted at 2 mL/min using a decreasing step-gradient of 0, 7.5, 16, and 25% $(NH_4)_2SO_4$ in 50 m*M* P_i pH 6.9. Protein was detected at 280 nm, and phosphorylase was eluted at 7.5% $(NH_4)_2SO_4$. Concentration was carried out by crossflow (30 kDa Mini-Ultrasette;

Filtron, Northborough, MA) or dead-end ultrafiltration (Amicon stirred 50-mL cell equipped with 30 kDa membrane) as well as by using 30 kDa Centricon tubes (Millipore, Eschborn, Germany). Anion exchange chromatography of starch phosphorylase was carried out on DEAE membrane cartridges (Mem Sep 1000; Biorad, Hercules, CA). Phosphorylase was eluted by a linear gradient of 50–600 m*M* P_i pH 6.9. SDS electrophoresis was carried out on a Pharmacia Phast system using precast 8–25 gels and silver staining of protein bands.

Enzyme Characterization

Temperature optima, pH optima and the apparent kinetic constants (30°C) in the direction of α-glucan degradation (phosphorolysis, P-mode) were determined by reported methods using discontinuous assays and heat termination of reactions *(9,11)*. Incubations were carried out on a Thermomixer 5436 (Eppendorf, Munich, Germany) with gentle agitation at 5×100 min^{-1}. The mathematical analysis of the concentration dependence of the reaction rate was performed employing classic Michaelis-Menten models including terms for substrate inhibition when necessary and using non-linear regression for parameter estimation.

Assays

Phosphorylase activity was measured in P-mode at 30°C by a continuous coupled assay described recently *(9)* using 30 mg/mL maltodextrin DE 19.4 (Agrana, Vienna, Austria) and 50 m*M* orthophosphate as substrates. Accordingly, the concentration of D-Glc-1-*P* was quantitated in a discontinuous assay. One unit of phosphorylase activity refers to 1 μmol NADH formed per minute, and the concentration of D-Glc-1-*P* is determined from the amount of NADH produced in the discontinuous assays. Orthophosphate was quantitated spectrophotometrically using a commerical kit (Spectroquant; Merck, Darmstadt, Germany). Protein was measured according to Bradford *(18)*.

Discontinuous and Continuous Synthesis of D-Glc-1-P

Discontinuous syntheses of D-Glc-1-*P* were carried out at 30°C essentially as previously described *(9)*. The time-course of product formation was monitored until apparent equilibration was attained. Continuous experiments were carried out at temperatures from 25 to 45°C in a convective, well-mixed, stirred tank enzyme reactor with a total volume of 40 mL. A schematic representation of the reaction system employed in this study is shown in Fig. 1. The vessel (equipped with a heating mantle for temperature control using an external water bath) had a flat membrane configuration thus requiring dead-end filtration, and a 10 kDa ultrafiltration membrane (NMWL 10,000) was used to retain phosphorylase during continuous operation. In addition, the reactor was

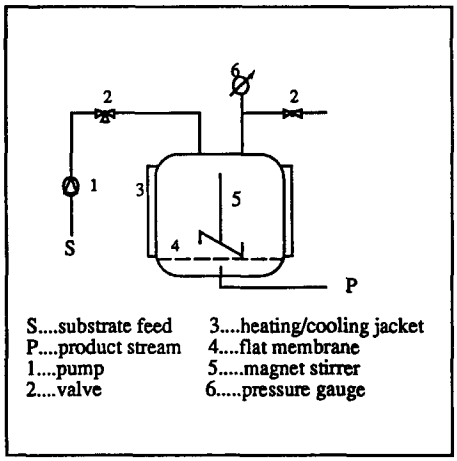

Fig. 1. Schematic representation of the enzyme reactor equipped with a flat 10 kD ultrafiltration membrane used in this study.

fitted with a conductivity electrode and a pressure sensor. Substrate was fed through a sterile filter at flow rates of 3 to 20 mL/h equivalent to average residence times of 2–13 h, and mixing was accomplished by magnetic stirring at 3–5 s^{-1} Equilibration with substrate in buffer was always allowed to proceed overnight, and the reaction was then started by the injection of a concentrated enzyme preparation (30–50 U/mL) through a septic seal. Samples were then periodically taken at the reactor outlet or, for determining the enzyme activity in the reactor, directly from the reactor through the seal. Samples were gelfiltered on NAP-5 desalting columns prior to measuring residual phosphorylase activity to remove D-Glc-1-*P* produced. Pretreatment of maltodextrin by pullulanase was accomplished by an enzyme dosage of 120 U/g dextrin *(9,10)*.

RESULTS AND DISCUSSION

Enzyme Production

Phosphorylase synthesis in *C. callunae* is inducible by maltodextrins or maltose and starts late in the exponential growth phase when the easily metabolizable carbon source such as glucose is already being depleted (Fig. 2). Table 1 summarizes results we have obtained in several bioprocess experiments aimed at the improvement and partial optimization of phosphorylase production by *C. callunae*. The data are results of bioreactor cultivations of the organism at 30°C, a constant pH of 7.4 and dissolved oxygen at 40% saturation *(9,11)*. Supplementation of the basal growth medium with glucose allows to increase the formation of wet biomass substantially with values in the range of 100 g/L of medium being achievable. The maximum phosphorylase activity produced by *C. callunae* is in the

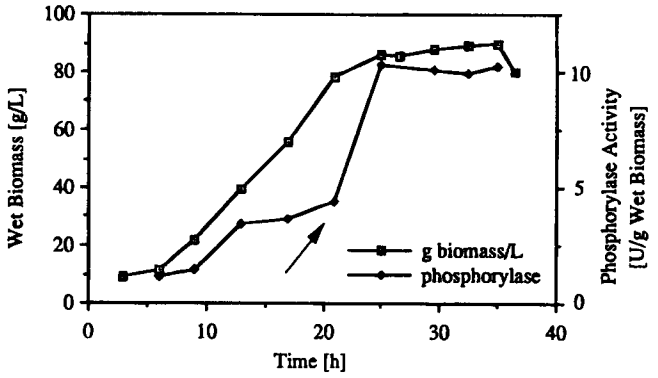

Fig. 2. Fermentation time course of *Corynebacterium callunae* on basal medium using 60 g/L glucose and 20 g/L maltose as the component inducing phosphorylase synthesis. The arrow indicates depletion of glucose. Other conditions: 30°C, 650 rpm, 40% oxygen saturation, pH 7.4.

Table 1
Production of α-1,4-D-Glucan Phosphorylase from *Corynebacterium callunae*

Glucose %	Inducing component %	Cultivation time h	Wet biomass g/L	Phosphorylase U/g biomass	Specific activity U/g protein
none	G_x, 1.6	10	15	10.5	0.25
none	G_x, 16.0	24	50	4.9	0.18
4	G_x, 16.0	36	98	6.7	0.16
12	G_x*, 3	45	110	1.8	n.d.
6	G_2, 2	36	92	10.2	0.24

Abbreviatons: G_x, maltodextrin; G_2, maltose; n.d., not determined; * induction at the end of the exponential growth phase.

range of 10 U/g biomass. In previous experiments it was found that the addition of a four fold excess of maltodextrin over glucose is necessary to obtain specific phosphorylase production of 6–8 U/g (wet) biomass (cf Table 1). The most likely explanation for this fact is that only certain limited components in a heterogeneous substrate such as a commercial maltodextrin are capable of inducing phosphorylase synthesis. Analysis of the fermentation medium by thin layer chromatography corroborates at least qualitatively, that the major fraction of the maltodextrin is not metabolized by the organism. Especially when high cell density cultivations of the organims are considered which require glucose concentrations of at least 40–60 g/L, the use of maltodextrin (in concentrations of 160–250 g/L) does

not seem feasible and practical (because of the high viscosity of the fermentation medium). Induction of phosphorylase synthesis by maltose is thus more favorable, and using a cocentration of 20 g/L of maltose, the phosphorylase activities obtained per gram wet biomass are indeed in a range so far found to be maximal in *C. callunae* (Table 1). According to the data in Fig. 2 and Table 1, 1 L of fermentation medium yields approx 900 U of phosphorylase. When a comparison with potato tubers is made, these results show that 1–1.5 kg of the plant material need to be processed to obtain equal enzyme activities (0.60–0.75 U/g of tuber).

Enzyme Isolation

A minimal purification of *C. callunae* phosphorylase has recently been established that eliminates contaminating activities, especially that of phosphatase that hydrolyzes D-Glc-1-*P* to D-Glc and P_i. A strategy found to be efficient in processing starch phosphorylase is summarized in Table 2 that represents a considerable improvement of other literature protocols pertaining to the isolation of partially pure plant phosphorylase from potato tubers *(7)*. Hydrophobic followed by anion exchange chromatography is usually sufficient to remove all amylase, phosphoglucomutase, and phosphatase activities. As judged from SDS PAGE (not shown) the majority of contaminating proteins is removed after the ion exchange step resulting in an overall purification factor of approx 28–30 (Table 2). The yields in the purification sequences of both phosphorylases are comparable with approx 60–70%. If not indicated otherwise, a preparation of potato phosphorylase with a specific activity of 2.8 U/mg was used in the following conversion studies (Table 2).

Enzyme Characterization

Pertaining to their temperature optima the phosphorylases from *S. tuberosum* and *C. callunae* show very similar dependences of initial rate and stability on reaction temperature. The classic temperature optimum is found at 50–55°C (50 m*M* P_i, pH 7.5) certainly not coinciding with the operational optimum. The operational stability of both phosphorylases was studied in 300 and 600 m*M* P_i at 30 and 45°C. The corresponding half-lives are 13 and 20 d in case of starch phosphorylase from potato whereas values of 9 and 18 d were determined for the bacterial enzyme. Both phosphorylases are unstable in the absence of P_i, e.g., in TrisAc buffer the half-lives are <12 h at 4°C. Potato phosphorylase is destabilized by 10 m*M* chloride ions that in turn seem not to affect the stability of the bacterial enzyme at 4°C. The pH optimum of *S. tuberosum* phosphorylases is found at pH 7.8–8.0 and is shifted towards the alkaline region by nearly 1 U of pH when comparison is made with the *C. callunae* enzyme. The apparent kinetic constants for maltodextrin were determined at otherwise realistic (operational) conditions, i.e., 300 m*M* P_i and

Table 2
Partial Purification of Starch Phosphorylase from *Solanum tuberosum*

	Total activity (U)	Specific activity (U/mg)	Yield %	Purification factor (-fold)
Crude cell extract	860	0.24	100	1.0
BPA 1050 precipitation	743	0.77	86	3.3
HIC Phenyl-sepharose	610	2.76	71	11.7
DEAE ionexchange	525	6.50	61	27.6

Abbreviations: HIC, hydrophobic interaction chromatography; BPA, biocryl processing aids.

Table 3
Apparent Kinetic Constants for Maltodextrin Determined at Operationally Relevant Conditions: 300 m*M* P_i (saturation), 30°C, pH 7.5

Kinetic constant (range 0 - 100 g/L)	Starch phosphorylase (*S. tuberosum*)	Maltodextrin phosphorylase (*C. callunae*)
Maximum rate (V_{max}) (U/mg)	3.0	5.3
Apparent K_M (g maltodextrin/L)	1.29	12.0
Substrate inhibition K_{IS} (g/L)	131	none

pH 7.5 (Table 3) (*9*). The K_M-values for maltodextrin differ by a factor of almost 10, and it seems thus likely that the potato phosphorylase will outperform the bacterial enzyme in terms of catalytic efficiency at low concentration of the glucan substrate. Substrate inhibition by maltodextrin was significant only in case of *S. tuberosum* phosphorylase. The affinities of both enzymes for untreated maltodextrin and maltodextrin that had been obtained after exhaustive treatment with pullulanase are identical as are the K_M values for orthophosphate (20–25 m*M* determined in P_i buffer using 80 g/L maltodextrin). Pretreatment was monitored by measurements of the formation of reducing sugars and by the iodine-starch reaction (*10*).

Discontinuous Synthesis of D-Glc-1-*P*

The results of the kinetic characterization point to substrate inhibition of starch phosphorylase from *S. tuberosum* by maltodextrin. To determine whether this inhibition affects the synthesis of D-Glc-1-*P*, conversion experiments were carried out at varying concentrations of maltodextrin with orthophosphate kept constant at 600 m*M*. The amount of product formed was monitored after 90 and 240 min reaction time. It becomes obvious from the data in Figs. 3A and B that in case of potato phosphorylase the time-dependent product yields are decreased when the maltodextrin concentrations exceed 120 g/L. However, this effect does not reduce maximum attainable product concentrations in comparison to the results obtained with phosphorylase from *C. callunae* (Fig. 3A) and is of minor practical significance because the applied glucan concentrations will seldom be higher than 100 g/L.

In phosphorolysis direction of catalysis either P_i or the glucan substrate may be the limiting component determining the maximum concentration of the product D-Glc-1-*P*. In the presence of a molar excess of glucan (i.e., based on the concentration of anhydroglucose units), the attainable product yield is governed by the equilibrium constant $[P_{i,eq}/Glc\text{-}1\text{-}P_{eq}]$ *(9,10)*. In contrast, to study the maximum degradation of various α-glucans by the actions of plant and bacterial phosphorylase, an excess of orthophosphate had to be employed, usually 300 m*M* P_i at 10 g/L glucan, i.e., theoretically a complete degradation of the glucan should be possible even when the maximum conversion of the initial P_i in Glc-1-*P* cannot exceeed 18 to 20% (pH 7.5, 30°C). Among the substrates tested including various maltodextrins differing in their dextrose equivalent-values, soluble starch as well as pullulanase-pretreated material, all were more substantially (10 to 20%) degraded by starch phosphorylase from *S. tuberosum* as compared to the *Corynebacterium* enzyme. Typically the maximum degrees of glucan conversion are 30 to 45% for untreated- and 50 to 65% for pullulanase-treated substrates when starch phosphorylase is employed. The different maximal degrees of glucan conversion seen with bacterial and plant phosphorylase do not result from a more complete degradation of a linear α-glucan chain by the *S. tuberosum* enzyme. The limit dextrin of both phosphorylases, i.e., the smallest oligomeric substrate converted at significant rates in P-mode direction, is maltopentose (Grießler and Nidetzky, unpublished results).

Continuous Conversion in Membrane Reactors

The continuous synthesis of D-Glc-1-*P* in the reaction system represented in Fig. 1 was studied using partially purified *S. tuberosum* or *C. callunae* phosphorylase. In each experiment the attainable product concentration was limited by the amount of either orthophosphate or glucan in the substrate feed. Flow rates (dilution rates) were varied, and the

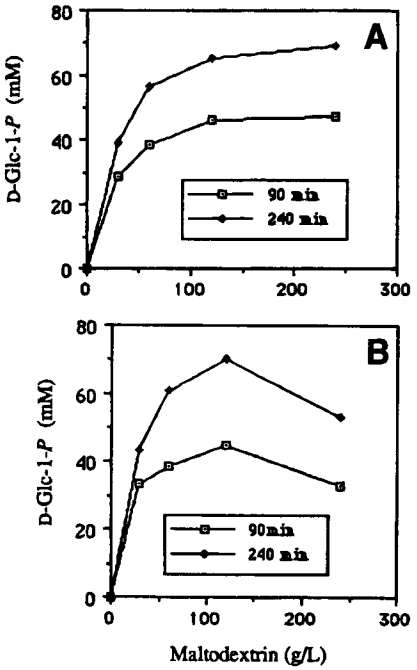

Fig. 3. Maltodextrin-dependent synthesis of D-Glc-1-*P* at 30°C and pH 7.5 using 600 mM P_i as a constant cosubstrate. **(A)** 0.8 U/mL *C. callunae* phosphorylase. **(B)** 0.8 U/mL *S. tuberosum* phosphorylase.

concomitant effect on the reactor productivity evaluated. The time courses of substrate conversion shown in Figs. 4 and 5 allow to compare the action of plant and bacterial phosphorylase with regard to the extent of maltodextrin degradation at several different operation conditions in the membrane reactor (glucan limit). The data indicate that the performances of both phosphorylases are very similar under the reaction conditions employed even though their kinetic constants, e.g., K_M-values, differ significantly. A more complete degradation of the glucan substrate by *S. tuberosum* phosphorylase noticed in the discontinuous experiments was not detected in the continuous conversions (cf Figs. 4 and 5), most probably because a maximum phosphorolytic degradation of the respective glucan can be achieved only at very low dilution rates. The pretreatment of maltodextrin by pullulanase has some effect on the attainable degrees of conversion (15 to 20% increase), and simultaneous or sequential enzyme action are equally effective (cf Figs. 4 and 5). When pretreatment and phosphorolysis are accomplished in a simultaneous manner, supplementation with fresh pullulanase approximately each 50 h is required (Fig. 5) pointing to some inactivation of the auxiliary biocatalyst. The dependence of the productivity on the applied dilution rate is roughly linear as shown in Fig. 6, and an increase of these values beyond 0.6 h^{-1} (corresponding to 25 mL/h) is expected to result in a further increase of productivity (at the cost

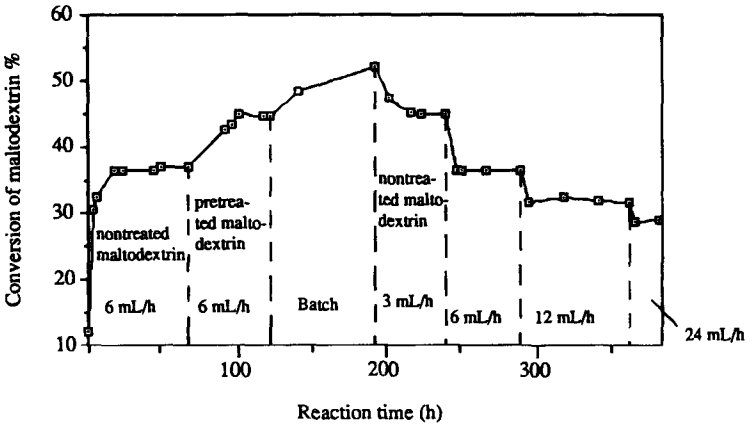

Fig. 4. Continuous conversion of 9.5 g/L maltodextrin at 30°C using 260 m*M* P_i (glucan limit) at varying operating conditions (pH 7.5). An initial *C. callunae* phosphorylase activity of 0.9 U/mL was employed.

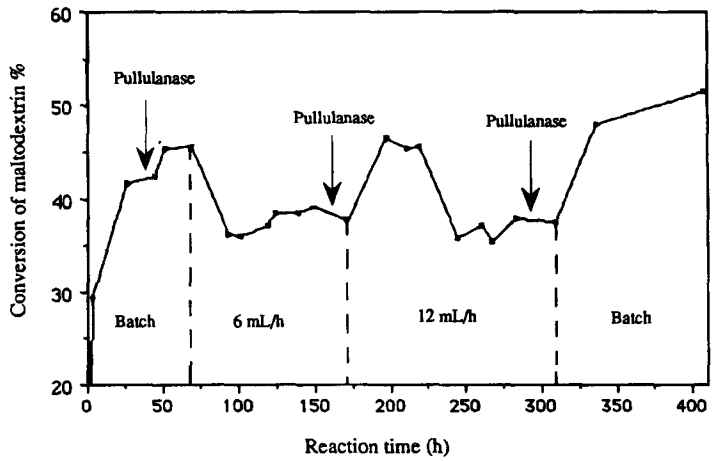

Fig. 5. Continuous conversion of 9.5 g/L maltodextrin at 30°C using 260 m*M* P_i (glucan limit) at varying operating conditions (pH 7.5). An initial *S. tuberosum* phosphorylase activity of 0.8 U/mL was employed.

of the extent of substrate conversion). Synthesis of D-Glc-1-*P* when employing P_i as the limiting substrate yields two to three-fold improved productivities with the maximum conversion of orthophosphate being in a range of 12 to 18% (Fig. 7). Again the results achieved, even on a quantitative basis, were nearly identical for plant and bacterial phosphorylase (not shown). The range of applicable flow rates in the reaction system used throughout this study was limited because of the retention of the polymeric substrate in dead-end ultrafiltration (cf Fig. 1). As possible alternatives other (external) membrane configurations are considered and immobilization of phosphorylase from *C. callunae* is currently being studied. Binding

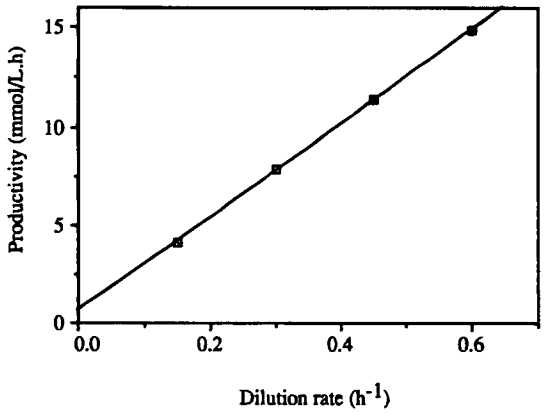

Fig. 6. Evaluation of reactor productivities at various dilution rates. Other conditions: 30°C, 260 m*M* P_i, 10 g/L maltodextrin, initial *C. callunae* phosphorylase activity of 0.9 U/mL.

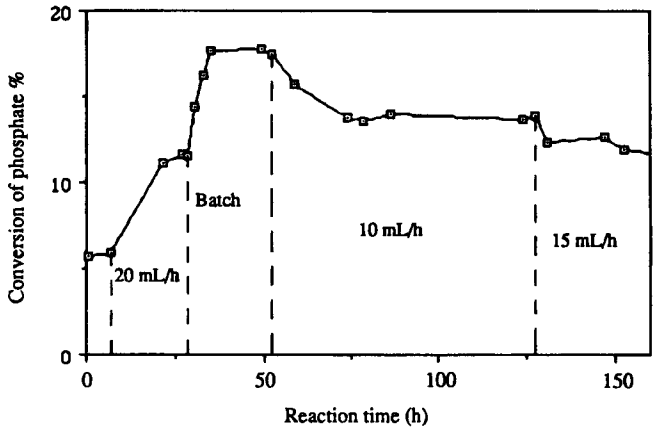

Fig. 7. Continuous conversion of 260 m*M* P_i at 30°C using 60 g/L maltodextrin (P_i limit) at varying operating conditions (pH 7.5). An initial *C. callunae* phosphorylase activity of 0.9 U/mL was employed.

of phosphorylase from *C. callunae* on weak or strong anion exchange resins has been accomplished, and production of D-Glc-1-*P* at substantially higher dilution rates than applied in the membrane reactor shown in Fig. 1 was indeed possible. However, preliminary results point to a rather low specific activity of the immobilized enzyme probably a result of intrinsic effects of matrix-binding of the phosphorylase (not shown).

Effect of Reaction Temperature

In the absence of rate limitations by external or internal mass transfer (which is assumed to be the case when soluble enzymes are employed), the reaction temperature is expected to be an important

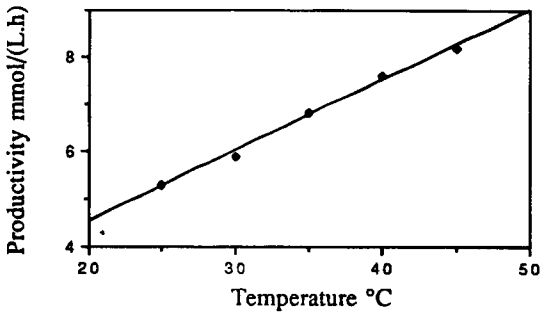

Fig. 8. Temperature effect on the continuous D-Glc-1-*P* synthesis by *C. callunae* phosphorylase. Other conditions: 600 m*M* P_i, 60 g/L maltodextrin (P_i limit), dilution rate 0.25 h^{-1}, initial *C. callunae* phosphorylase activity of 0.19 U/mL.

determinant of conversion rate and productivity. The continuous synthesis of D-Glc-1-*P* by *C. callunae* phosphorylase using orthophosphate-limited reaction conditions was followed in a temperature range of 25 to 45°C when applying a constant flow rate of 10 mL/h (0.25 h^{-1}). The enzymatic system was allowed to equilibrate at the corresponding temperature for at least 5 reactor cycles (i.e., 20 h), and during that period four to six samples were taken and product formation as well as enzyme activities quantitated. The product concentration in the filtrate remained fairly constant during each period of constant temperature as did the volumetric phosphorylase activity in the reactor. During the entire course of the experiment approx 20% of the initial activity were lost, and all productivities are corrected for the enzyme inactivation. The results shown in Fig. 8 clearly suggest that a well-defined linear relation holds for the dependence of productivity on the reaction temperature within the experimental range of 25 to 45°C. The operational (thermal) stability of both plant and bacterial phosphorylase allows their use at a reaction temperature up to 40 but not 45°C.

CONCLUSIONS

Starch phosphorylase from *S. tuberosum* and maltodextrin phosphorylase from *C. callunae* exhibit nearly identical process-relevant properties with regard to their application in a continuous synthesis of D-Glc-1-*P*. Despite some differences pertaining to thermal stability, kinetic properties (K_M-value for maltodextrin) and specificity (reflected by a different maximum extent of α-glucan substrate degradation), the performance of both enzymes during phosphorolytic conversion of maltodextrin and orthophosphate in an ultrafiltration membrane reactor are very well comparable. One additional discerning feature among both systems is the initially more than 10-fold higher phosphorylase activity in the bacterial biomass as compared to the plant material.

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