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KINETIC PROPERTIES OF TWO STARCH PHOSPHORYLASES FROM PEA SEEDS

N. K. MATHESON and R. H. RICHARDSON

Department of Agricultural Chemistry, University of Sydney, N.S.W. 2006, Australia

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Key Word Index—*Pisum sativum*; Leguminosae; phosphorylase; pea; pyridoxal 5′-phosphate; adenosine diphosphoglucose; enzyme kinetics.

Abstract—Both of the starch phosphorylase fractions from Victory Freezer pea seeds, that can be separated by DEAE–cellulose chromatography and purified by Sepharose 4B-starch affinity chromatography, contain pyridoxal 5′-phosphate. The addition of further quantities of pyridoxal 5′-phosphate causes inactivation. Both enzymes showed similar bi-substrate kinetics with D-Glc-1-P and varying amounts of amylopectin and also with P_i and varying amounts of amylopectin. In the direction of glucan sythesis the K_m for amylopectin with phosphorylase II was much higher than with phosphorylase I. However, the two enzymes differed in their behaviour on glucan degradation at varying concentrations of P_i. With phosphorylase II the K_m for amylopectin was dependent on the concentration of P_i but that for phosphorylase I was constant. Phosphorylase II was strongly inhibited by ADPG in the direction of glucan degradation but only slightly in the direction of glucan synthesis by both ADPG and UDPG. Phosphorylase I was only slightly inhibited by ADPG in both directions and by UDPG in synthesis. UDPG inhibited both enzymes moderately in glucan degradation,

INTRODUCTION

There have been a number of studies of the kinetic properties of starch phosphorylases (EC 2.4.1.1), e.g. potato [1–4], hybrid maize [5, 6], sweet corn [7, 8] and banana [9, 10]. K_m values for D-glucose 1-phosphate (Glc-1-P) have generally been in the range 1–5 mM and those for inorganic phosphate (P_i) 3–10 mM. The K_m value for α-glucans, acting as acceptors for glucose units in the direction of glucan synthesis and as glucose donors in the direction of degradation, depends on the type of glucan and large differences have been recorded [1, 4, 7, 8, 11, 12]. For example, for amylopectin acting as an acceptor, values as low as 2.2 μM glucose non-reducing end group [8] and as high as 120 μM [1] have been found. Activity of potato phosphorylase as a function of Glc-1-P concentration in α-glucan synthesis and of P_i concentration in α-glucan degradation, at varying levels of amylopectin concentration has been measured and the Lineweaver–Burk plots intersected to the left of the vertical axis [2]. From this and other data a mechanism of catalysis similar to that of mammalian phosphorylases was proposed.

Pyridoxal 5′-phosphate (P5′p) is a cofactor for potato phosphorylase [13, 14] as it is for glycogen phosphorylase and for all other α-glucan phosphorylases that have been examined and there are two molecules per active enzyme molecule (MW 207000). P5′p inactivates a number of enzymes for which it is not a co-factor, e.g. glutamic dehydrogenase, 6-phosphogluconate dehydrogenase, aldolase and 3-phosphoglycerate dehydrogenase as well as some in which it is, e.g. glutamate decarboxylase [15–17]. It also modifies the regulatory properties of muscle glycogen phosphorylase [18].

Mammalian glycogen phosphorylases have an elaborate allosteric control mechanism [19]. This does not operate in plant starch phosphorylases and reports have varied considerably on the effects of cell components that might modify the kinetic behaviour of this enzyme. For example, ATP has been found to act as an activator [11] an inhibitor [6], to have no effect [7] and to induce allosteric kinetics [9]. A number of other nucleotides have also been reported to modify the reaction rate and among these are the nucleoside diphosphoglucoses [6–8, 10, 20, 21] that are substrates for starch synthase. Phosphorylases from sweet and normal corns, spinach leaves, broad bean cotyledons, potato and banana fruits have all been shown to be inhibited.

In this paper the kinetics of two phosphorylases from Victory Freezer pea seeds, that can be separated by DEAE-cellulose chromatography [22] have been compared. The P5′p contents and the effect of excess amounts of this compound on their activity as well as the effects of nucleoside diphosphoglucoses on these enzymes acting in the direction of synthesis and degradation of amylopectin have been studied.

RESULTS AND DISCUSSION

Victory Freezer pea phosphorylase I was purified [22] by affinity chromatography on a Sepharose 4B-starch column, followed by chromatography on DEAE-cellulose and then chromatography on 8% agarose gel. Phosphorylase II was purified by DEAE-cellulose chromatography, affinity chromatography and DEAE-cellulose chromatography, affinity chromatography and DEAE-cellulose chromatography again. A final gel filtration step could also be included. The final sp. act. were in the region of 200 n*k* per mg of protein measured as inorganic phosphate released.

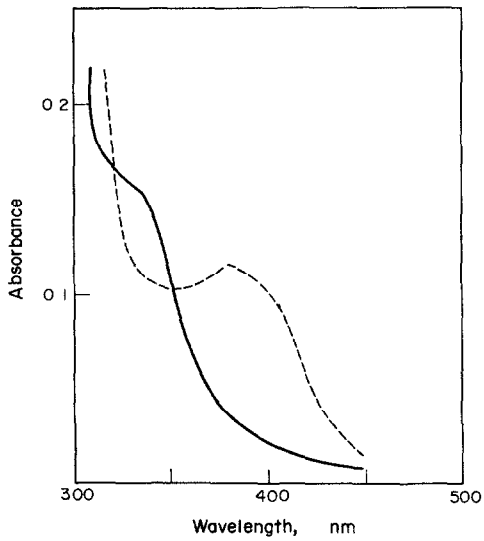

Fig. 1. Absorption spectra of phosphorylase I from Victory Freezer pea seeds at neutral and alkaline pH. —— pH 7, - - - pH 13.

P5'p in phosphorylase can be detected by spectral analyses at high protein concentrations. Neutral solutions contain an absorption band at 330 nm, which is replaced below pH 4.5 or above 9.5 by another maximum near 400 nm [6, 7, 13, 14]. The methods of preparation and properties of starch phosphorylases that have been previously investigated indicate that they may have been similar to the type II enzyme [23]. In view of this the spectral behaviour of Victory Freezer pea phosphorylase I was studied. As shown in Fig. 1, at a concentration of 5 mg protein per ml, the spectrum at pH 7 has a shoulder at 330 nm; that disappeared at pH 13 when a new peak at 385 nm became evident. A difference spectrum performed with solutions at pH 7 and 13 showed a maximum at 390 nm and a minimum at 335 nm. Phosphorylase II solutions at pH 7 also had a shoulder in their spectra at 330 nm indicating the presence of P5'p.

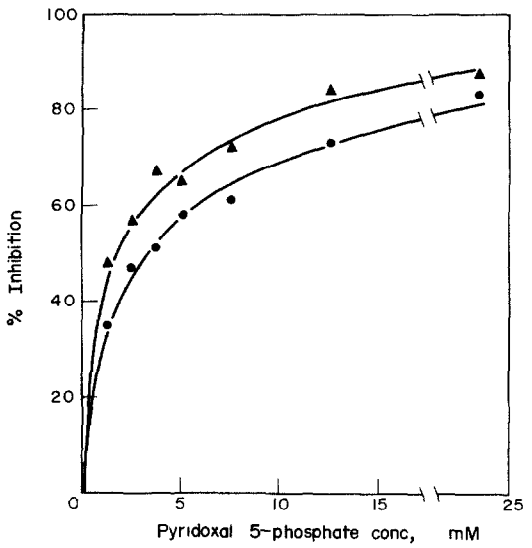

Fig. 2. The effect of pyridoxal 5'-phosphate on the activity of phosphorylases I and II from peas (Victory Freezer).

The effect of the addition of extra P5'p to both enzymes was then studied and inactivation was observed. The concentration of enzyme was about 1 μM and P5'p concentrations were in the range of 1 to 25 mM. Enzyme and P5'p were incubated in the dark at 30° in Tris buffer, pH 6.5. In preliminary studies the change in activity with time of incubation was followed for phosphorylase II and, at 20 mM P5'p, more than half of the final inactivation occurred in 1 min. In subsequent studies, enzyme and P5'p were incubated for 60 min and the concentration of active enzyme was sufficient to allow determination of activity after less than 10 min incubation with substrate. The pattern of inactivation in the dark of phosphorylases I and II at various P5'p concentrations is shown in Fig. 2. Both enzymes were similarly inactivated and 5 mM P5'p gave 50 % inactivation. Two types of inactivation by P5'p have been found. In the light there is photo-oxidation of the imidazole side chain of histidine but in the dark the ε-amino of lysine reacts [16].

The inhibitions by a number of compounds containing some of the structural features of P5'p were compared by incubating enzyme and compound for 70 min and assaying for 7 min. Enzyme II was strongly inactivated by P5'p (74 %). Pyridine 3- and 4-aldehydes, pyridoxine, deoxy pyridoxine, pyridoxamine, pyridoxamine 5'-phosphate and pyridoxal all showed less than 5 % inactivation. Enzyme I was also strongly P5'p (76 %) and the other compounds gave inactivations between 7 and 12 %, apart from pyridine 4-aldehyde and pyridoxal, when the values were 26 and 27 %.

A difference between the inactivation by pyridoxal and its 5'-phosphate has been found for 6-phosphogluconate dehydrogenase [16] and rabbit muscle aldolase [15, 16]. These observations and also that most of the substrates for enzymes sensitive to P5'p contain phosphate ester groups has led to the suggestion that the phosphate group in P5'p functions by orienting the aldehyde group in pyridoxal towards the active site. Excess P5'p has been shown to change the allosteric properties of glycogen phosphorylase [18].

There is no evidence in starch phosphorylases for a control system similar to the elaborate mechanism with glycogen phosphorylase and, although the latter enzyme is considered to function only in degradation *in vivo*, it has been proposed that starch phosphorylase also synthesizes starch [20, 21, 24, 25].

The bisubstrate kinetics of the two Victory Freezer pea phosphorylases were measured and some possible inhibitors tested. Initial reaction rates of glucan degradation were determined by enzymic estimation of Glc-1-P formation and of glucan synthesis by colorimetric estimation of P_i released. The Lineweaver–Burk plots for the initial rates of P_i release by phosphorylases I and II, at varying concentrations of Glc-1-P and potato amylopectin, i.e. in the direction of glucan synthesis, are shown in Fig. 3. The apparent K_m for Glc-1-P with each enzyme was unaffected by the concentration of amylopectin. Similar behaviour has been found for potato phosphorylase [2]. The secondary plots of the increase of V_{max} vs the increase of amylopectin concentrations was linear ($1/V = 8.81 \times 10^{-5}\ 1/S + 0.124$; V in mkat/ml and S in % amylopectin, correlation coefficient 0.999 for phosphorylase I: $1/V = 8.08 \times 10^{-4}\ 1/S + 0.0824$, correlation coefficient 0.997 for phosphorylase II) and the K_m value for amylopectin with phosphorylase I was 1.8 μM glucose non-reducing end groups (0.0007 %) and

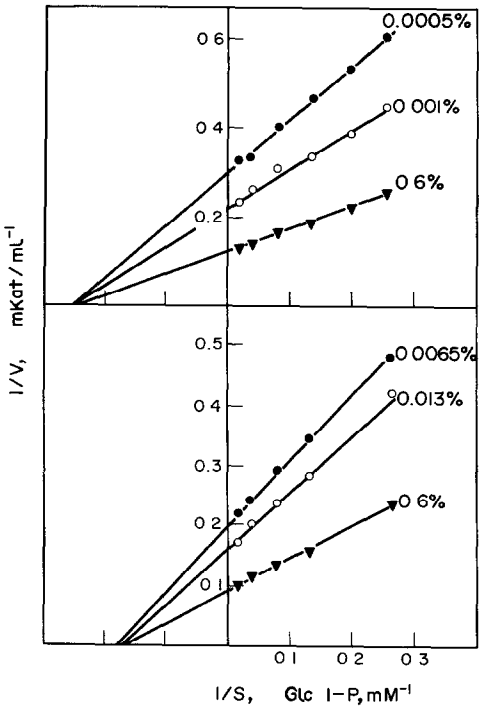

Fig. 3. Effect of amylopectin concentration on double reciprocal plots of Victory Freezer pea phosphorylases I and II in the direction of glucan synthesis.

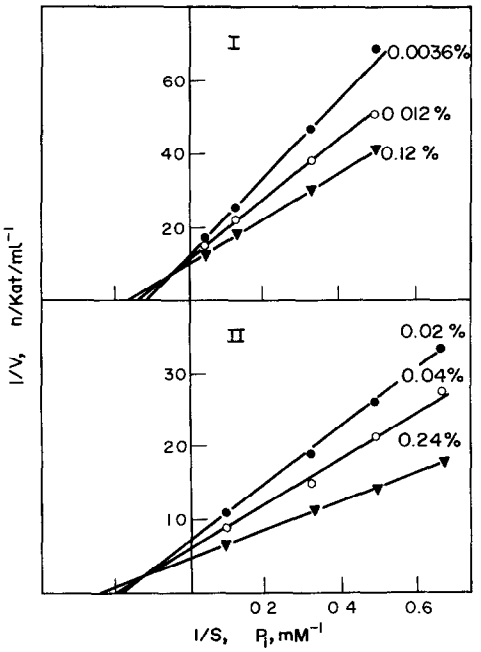

Fig. 4. Effect of amylopectin concentration on double reciprocal plots of Victory Freezer pea phosphorylases I and II, in the direction of glucan degradation.

with phosphorylase II 24 μM (0.0098%), similar to previously described values for glycogen [22]. The K_m values for Glc-1-P, estimated from the primary plots were 4 and 5.5 mM, slightly different to the values with soluble starch [22]. In the cell, to maintain a concentration of Glc-1-P of 4 mM the concentrations of Glc-6-P and Fru-6-P, calculated from the known K_{eq} values of phosphoglucomutase and phosphoglucoisomerase, would be 70 and 30 mM respectively, giving a total hexose-6-P concentration of 100 mM. The concentrations of these compounds in illuminated leaves have been estimated at much lower levels than this [26]. Also, K_m values reported for Glc-1-P of two competing enzymes, phosphoglucomutase [27] and ADPG pyrophosphorylase [28] are much lower, 5 μM and 100 μM, so these two enzymes would compete successfully for this substrate.

Initial rates of phosphorolysis, as catalysed by the two enzymes at various levels of P_i and amylopectin, are shown in Fig. 4. For both enzymes the plots for different amylopectin concentrations intersected to the left of the 1/V axis and slightly above the 1/S axis. Thus, the apparent K_m values for P_i and the V_{max} values were only slightly affected by changes in amylopectin concentration and the ranges of apparent K_m values differed little for the two enzymes, viz. 6 to 9 mM for I and 4 to 5 mM for II. Reported levels of P_i in plants are generally of this order and higher [29–32] than these K_m values, consistent with phosphorolysis occurring.

Similar plots of the effect of Glc-1-P concentration on the rate of P_i release, in the direction of glucan synthesis, at various amylopectin concentrations are shown in Fig. 5. Phosphorylase I has a very low K_m towards

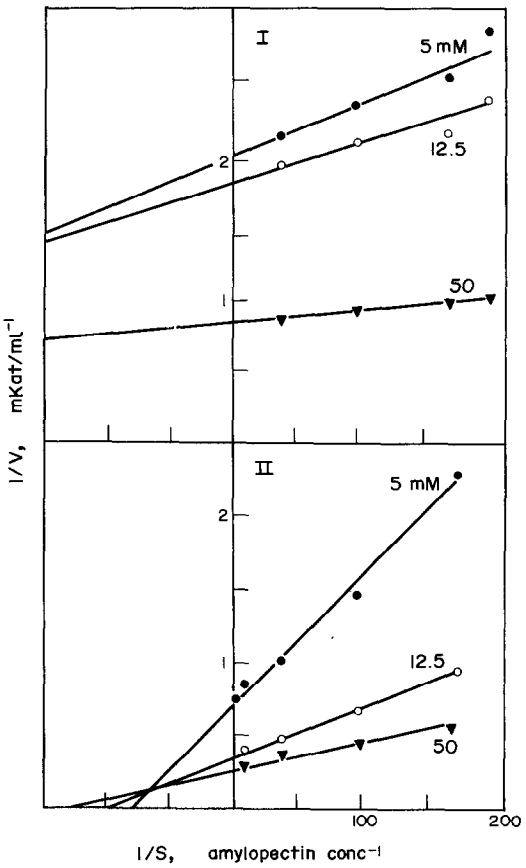

Fig. 5. Effect of Glc-1-P concentration on double reciprocal plots of Victory Freezer pea phosphorylases I and II in the direction of glucan synthesis.

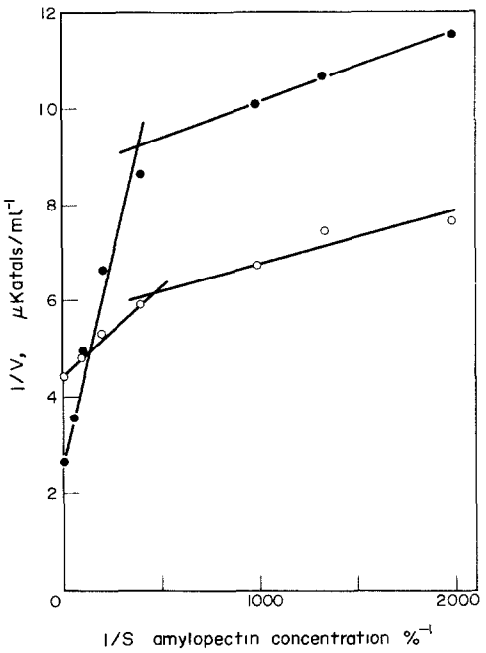

Fig. 6. Lineweaver–Burk plots of the rate of elongation of amylopectin, catalysed by Victory Freezer pea phosphorylase, using an extended range of amylopectin concentrations. [Glc-1-P] = 50 mM (○ phosphorylase I; ● phosphorylase II).

amylopectin (0.0007 %) so that over the range of amylopectin concentrations studied, the change in velocity is small and the estimation of apparent K_m difficult. However, some observations could be made. The apparent K_m was only slightly affected by increasing Glc-1-P concentrations from 5 to 50 mM but V_{max} increased *ca* 2 to 3 fold. Similar results were found for phosphorylase II but the K_m values were much higher. When the reaction velocities of both enzymes were determined at a saturating Glc-1-P concentration (50 mM) over a wider range of amylopectin concentrations (0.6 to 0.0005 %), an inflexion point in the slope of the double inverse plots appeared (Fig. 6). At lower concentrations than those used in the bisubstrate kinetic experiments, the velocity was greater than would have been predicted from extrapolation of the linear inverse plot at high concentrations. The deviation occurred at a concentration of 0.0025 % amylopectin for both enzymes and the apparent K_m values calculated from data below this concentration are extremely low, 0.46 μM (0.00021 %) for I and 0.38 μM (0.00017 %) for II. Whereas the apparent K_m values for amylopectin determined for the two enzymes at high concentrations of amylopectin differed tenfold, those determined at low concentrations were similar. A similar effect has been found previously with potato phosphorylase [33]. Two possible reasons for the change in apparent K_m are the presence of two enzymes in both preparations that have distinctly different K_m values toward the substrate or small amounts of glucan substrate associated with the enzyme or substrate. Both enzymes had been purified to give a single band of phosphorylase activity on polyacrylamide gel electrophoresis. The Glc-1-P was a commercial sample but without glucan the enzyme plus Glc-1-P always showed a lag period [22]. Some purified phosphorylase preparations have been shown to apparently contain bound primer [34].

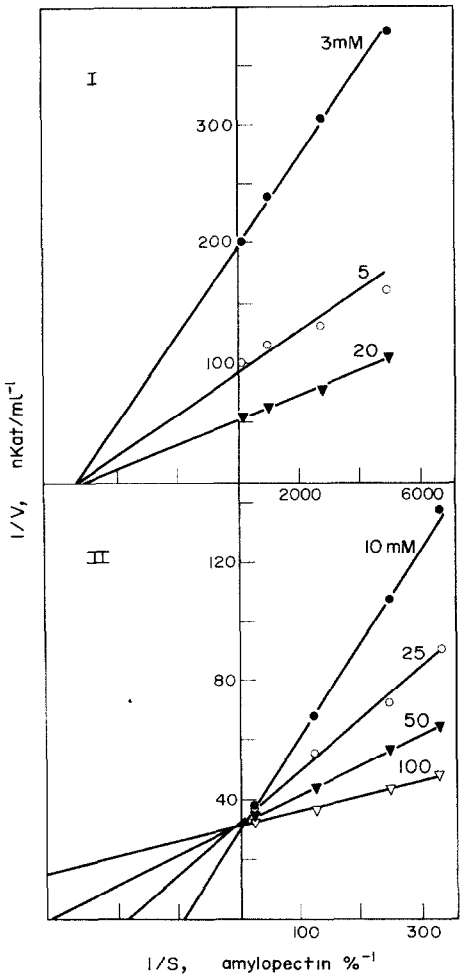

Fig. 7. Effect of P_i concentration on double reciprocal plots of Victory Freezer pea phosphorylases I and II in the direction of glucan degradation.

When the effect of varying P_i concentration on glucan degradation with changing amylopectin concentrations was studied, the two enzymes responded differently (Fig. 7). With phosphorylase I the apparent K_m for amylopectin was unchanged at different P_i concentrations but that with phosphorylase II showed a 7 fold change (cf. [35]). The K_m for amylopectin with I was *ca* 0.5 μM (0.0002 %). The V_{max} increased 4 fold over the range of P_i concentrations from 3 to 20 mM. The apparent K_m for amylopectin of phosphorylase II was 3.5 μM (0.0014 %) at 100 mM but 26 μM (0.011 %) at 10 mM P_i, whereas the V_{max} was unchanged. The secondary plot of apparent K_m against the reciprocal of P_i concentration for phosphorylase II was linear in the concentration range studied (K_m amylopectin % = 0.0932 × 1/S + 0.00092, where S is the concentration of P_i in mmol, correlation coefficient 0.996). Thus, if the concentration of P_i in the cell increased, phosphorylase II would maintain a similar velocity at decreasing amylopectin concentrations. Phosphorylase II is the main enzyme present at different stages in a number of plant tissues, except for germinating peas [23] and high P_i concentrations would be likely to be associated with a need for an increased supply of glycolytic substrates produced from starch reserve.

Conversely, decreasing P_i concentrations increase the K_m for amylopectin. If the linear relationship of the inverse of P_i concentration and K_m for amylopectin holds below 10 mM P_i, then the K_m at 5 mM P_i would increase to 0.02% and at 1 mM P_i to 0.09%.

There have been a number of studies of possible compounds that regulate plant starch phosphorylases [5–9, 11, 20, 36]. Some inhibition in the direction of glucan synthesis has been found with nucleotides and glycolytic intermediates. Fewer studies have been made in the direction of glucan degradation [6]. The inhibition of Victory Freezer phosphorylase II was tested in the direction of glucan degradation. The substrates were supplied at concentrations near to their K_m values (10 mM P_i, 22 μM amylopectin) and the buffer was Tris–HCl. The inhibitions produced at 1 mM concentration for AMP, ADP, ATP, 2, 3, diPGA, Fru-6-P, Fru-1, 6diP and PEP were all less than 12% but ADPG and UDPG showed higher values (35 and 18%). The effects of these two compound were further studied with both enzymes acting in the direction of glucan degradation and also glucan synthesis. The substrate concentration for Glc-1-P was 10 mM. The per cent inhibitions at varying nucleoside diphosphoglucose levels are shown in Fig. 8. In the direction of glucan synthesis (Fig. 8a) each enzyme was only weakly inhibited by both compounds but ADPG was slightly more effective than UDPG. In contrast, in glucan degradation ADPG caused a much stronger inhibition of phosphorylase II (Fig. 8b). Approximately 1.3 mM ADPG gave 50% inhibition. Phosphorylase I was inhibited less than 10% at this concentration. The inhibition by UDPG in glucan degradation was similar for both enzymes and was less than the ADPG inhibition of phosphorylase II. A concentration of 2–3 mM gave 50% inhibition. However,

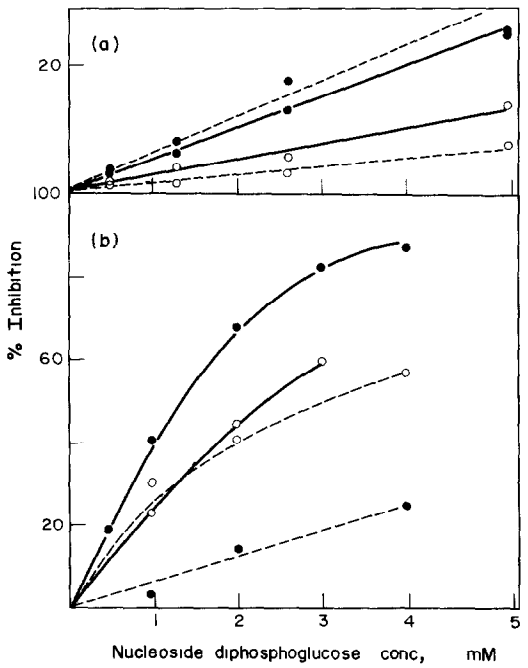

Fig. 8. The inhibition by ADPG and UDPG of Victory Freezer pea phosphorylase: (a) in the direction of glucan synthesis; (b) in the direction of glucan degradation with [P_i] = 10 mM. – – – enzyme I; —— enzyme II; ● ADPG; ○ UDPG.

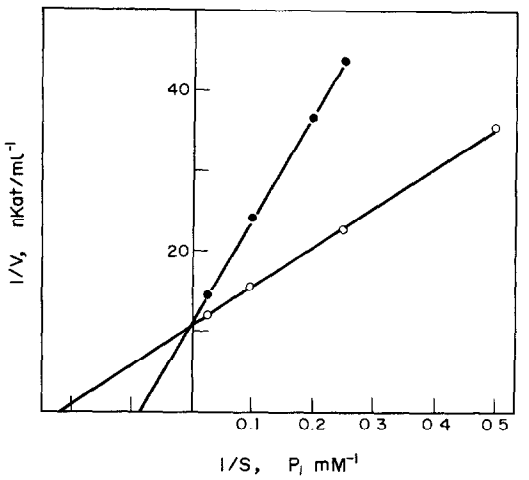

Fig. 9. Lineweaver–Burk plot of Victory Freezer pea phosphorylase II in the direction of glucan degradation (● with 1 mM ADPG; ○ no ADPG).

it was considerably higher than the inhibition of both enzymes acting in the direction of glucan synthesis. For phosphorylase II, in glucan degradation, inhibition by 1 mM ADPG was competitive towards P_i in the range of 4 to 40 mM P_i (Fig. 9).

There have been previous reports of inhibition by nucleoside diphosphoglucoses of phosphorylase acting in the direction of glucan synthesis. Phosphorylases from spinach leaves and broad beans were inhibited *ca* 40% when the concentration of ADPG was twice that of Glc-1-P and UDPG was half as effective. Both ADPG and UDPG inhibited an unprimed activity from potato but had no effect on the primed enzyme [21]. The activity from the embryo of sugary maize was inhibited 58% by ADPG and 40% by UDPG at 5 mM concentration [8] and ADPG but not UDPG inhibited a phosphorylase from sweet corn endosperm [7]. 5 mM ADPG caused a 30% inhibition in the presence of 20 mM Glc-1-P and it was suggested that regulation of phosphorylase activity by the substrate of starch synthase would seem to be in keeping with a degradative role for phosphorylase. A purified normal maize phosphorylase, that was probably type II, was inhibited by ADPG and to a lesser extent by UDPG [6]. The inhibition by ADPG did not follow Michaelis–Menten kinetics and the Hill number was 1.3. In phosphorolysis the same enzyme was competitively inhibited by both ADPG and UDPG and K_i values of 1.5 mM and 2.8 mM were found and it was suggested that, at least in developing maize endosperms, that phosphorylase can only function primarliy as a degradative enzyme.

For the nucleotide diphospho-glucose compounds to regulate the catabolism of starch by phosphorylase *in vivo*, their concentrations would need to approach 50% inhibition values. UDPG contents of 120 μmol per kg in strawberry leaves [26] and 0.5 mmol per kg fr. wt in germinating pea cotyledons [37] have been found. In *Chlorella*, the amount of ADPG has been shown to increase on illumination with the concentration falling to an undetectable level in the dark [38].

The present results indicate that two possible factors in the control of phosphorylase II in the catabolism of starch in Victory Freezer pea seeds are the level of P_i,

which modifies the K_m for amylopectin and the level of ADPG which inhibits the enzyme. UDPG is also effective. The effect of change in the concentration of P_i and an opposite change in ADPG concentration would be complementary in the control of the rate of formation of Glc-1-P. A decrease in the concentration of P_i combined with an increase in ADPG would result in a decrease in Glc-1-P formation and three factors would contribute to this. Firstly, a lowered substrate (P_i) concentration, secondly, an increase in the K_m for the glucan substrate and thirdly, an increased percentage inhibition by ADPG. During photosynthesis, photophosphorylation would keep P_i levels low and the synthesis of glycolytic intermediates would stimulate the synthesis of ADPG [19, 38]. High levels of P_i and reduced ADPG, caused by darkness or an energy deficit would lead to an increased rate of formation of Glc-1-P.

EXPERIMENTAL

Plant material. Pisum sativum Victory Freezer was grown and harvested as described previously [22, 23].

Purification of phosphorylases I and II. Phosphorylase I was prepared from germinated peas, after homogenization and centrifugation, by chromatography on a Sepharose 4B-soluble starch affinity column, followed by chromatography on DEAE-cellulose and gel filtration. Phosphorylase II was prepared from mature peas and after homogenization, centrifugation and dialysis was purified by DEAE-cellulose chromatography, Sepharose 4B-soluble starch affinity chromatography, DEAE-cellulose chromatography and in some preparations gel filtration The details have been described [22, 23].

Enzyme assays. P_i release was measured colorimetrically and Glc-1-P formation enzymically as described previously [22, 23].

Preparation of amylopectin. Isolated potato starch granules (10 g) were dispersed in DMSO (50 ml) at 35° for 24 hr. The clear paste was dispersed in 0.1 M NaCl (500 ml) and heated at 100° under N_2 for 15 min. On cooling, *n*-BuOH (50 ml) was added and the mixture stored at room temp. for two days. The ppt. was removed by centrifuging (14 000 *g*, 25 , 30 min). The supernatant was dialysed for 24 hr and added to 4 vol. EtOH. The ppt. was collected by centrifugation and washed with EtOH, Me_2CO and Et_2O and dried. Solns were prepared by wetting with EtOH followed by H_2O and heating at 100°

Measurement of absorption spectra. Solns were scanned from 500 to 300 nm in 1 cm quartz cells and a full scale expansion of 0 to 0.1 optical density units.

Measurement of inhibition of phosphorylase by pyridoxal 5'-phosphate and analogues. Compounds tested were dissolved in H_2O and the pH adjusted to 6.5. Aliquots (0.1 ml) were incubated with enzyme (0.1 ml) at 30° for 60 or 70 min in the dark in 10 mM Tris buffer, pH 6.5. Substrate (0.2 ml) was added and the release of P_i measured colorimetrically. Blanks containing inhibitor or substrate and control containing H_2O in place of inhibitor were run concurrently

Determination of inhibition of phosphorylase by cellular intermediates. Preliminary studies were performed in 20 mM Na citrate buffer, pH 6.5. The inhibitions of the enzymes by ADPG and UDPG were measured in 10 mM Tris buffer pH 6.5. Substrate was added to the enzyme immediately after the addition of inhibitor. In the direction of starch synthesis the final vol. was 0.4 ml and activity was assayed by colorimetric estimation of P_i released In the direction of degradation the final vol. was 1 ml and Glc-1-P assayed enzymically. Controls omitting effector were run concurrently.

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REFERENCES

1. Hollo, J., Laszlo, E. and Hoschke, A. (1971) *Plant α(1-4) Glucan Phosphorylase.* Hungarian Academy of Sciences.
2. Gold, A. M., Johnson, R M. and Sanchez, G. R. (1971) *J. Biol. Chem.* **246**, 3444
3. Ariki, M. and Fukui, T. (1975) *J. Biochem. (Tokyo)* **78**, 1191.
4 Lee. Y -P. (1960) *Biochim Biophys. Acta* **43**, 18.
5. Tsai, C. Y. and Nelson, O. E. (1968) *Plant Physiol.* **43**, 103.
6. Burr, B. and Nelson, O. E. (1975) *European J. Biochem.* **56**, 539.
7. Lee, E. Y. C. and Braun. J J (1973) *Arch Biochem. Biophys.* **156**, 276.
8. Tandecarz, J., Lavintman, N. and Cardini, C. E. (1973) *Carbohyd. Res.* **26**, 385.
9. Singh, S. and Sanwal, G. G (1976) *Biochim. Biophys. Acta* **309**, 280.
10. Singh, S. and Sanwal, G. G. (1976) *Phytochemistry* **15**, 1447.
11 Khanna, S. K., Krishnan, P. S. and Sanwal, G. G. (1971) *Phytochemistry* **10**, 551.
12. Pfannemuller, Von B. and Burchard, W. (1969) *Makromol. Chem.* **121**, 1.
13. Kamogawa, A. (1969) *Biochim. Biophys. Acta* **178**, 459.
14. Lee, Y.-P. (1960) *Biochim. Biophys. Acta* **43**, 25
15. Rippa, M. and Pontremoli, S (1969) *Arch. Biochem. Biophys* **103**, 112.
16. Davis, L. C., Brox, L. W., Gracy, R. W., Ribereau-Gayon, G. and Horecker, B. L (1970) *Arch. Biochem. Biophys.* **140**, 215.
17. Cozzani, I., Santani, C., Jori, G., Gennari, G. and Tamburro. A. M. (1974) *Biochem J.* **141**, 463.
18. Avramovic-Zikic, O. and Madsen, N. B. (1972) *J. Biol. Chem.* **247**, 7005.
19. Krebs, E. G. and Preiss, J J (1976) *Biochemistry of Carbohydrates* (Whelan, W. J ed) Vol. 5, p. 337. Butterworths, London
20. De Fekete, M. A. R. (1968) *Planta* **79**, 208
21 Frydman, R. B. and Slabnik, E. (1973) *Ann. N.Y. Acad Sci.* **210**, 153
22. Matheson, N. K. and Richardson, R. H. (1976) *Phytochemistry* **15**, 887.
23. Richardson, R. H. and Matheson, N. K. (1977) *Phytochemistry* **16**, 1875.
24 Badenhuizen, N. P. (1973) *Ann. N.Y. Acad. Sci.* **210**, 11.
25. De Fekete, M. A. R. and Vieweg, G. H. (1974) in *Plant Carbohydrate Biochemistry* (Pridham, J. B. ed) p. 127. Academic Press, New York.
26. Isherwood, F. A. and Selvendran, R. R. (1971) *Phytochemistry* **10**, 579.
27. Ray, W. J., Roscelli, G. A. and Kirkpatrick, D. A. (1966) *J. Biol. Chem.* **241**, 2603.
28. Preiss, J., Ghosh, H. P. and Wittkop, J. (1967) in *Biochemistry of Chloroplasts* (Goodwin, T. W. ed.) Vol. 2, p. 131. Academic Press, New York
29 Santarius, K. A. and Heber, U. (1965) *Biochim. Biophys. Acta* **102**, 39.
30. Walsh, P. J. and Rowan, K S. (1973) *Australian J. Biol. Sci.* **26**, 685.
31. Ewart, M. H., Siminovitch, P. and Briggs, D. R. (1954) *Plant Physiol.* **29**, 407
32. Abbott, I. R. and Matheson, N. K. (1972) *Phytochemistry* **11**, 1261.
33. Pfannemuller, Von B. (1968) *Stärke* **20**, 351
34. Kamogawa, A., Fukui, T. and Nikuni, Z. (1968) *J. Biochem. (Tokyo)* **63**, 361.
35. Medicus, R. and Mendicino, J. (1973) *European J. Biochem.* **40**, 63
36 Baxter, E. D. and Duffus, C. M. (1973) *Phytochemistry* **12**, 2321
37. Sung, H-Y. and Su, J-C. (1973) *J. Chinese Agric Chem Soc.* Special Issue, 1.
38. Kanazawa, T., Kanazawa, K., Kirk, M. R. and Bassham, J. A (1972) *Biochim. Biophys. Acta* **256**, 656.