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Isolation and Kinetic Properties of Phosphorylase from Yellow Yam Tuber *(Dioscorea cayenensis)*

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Abstract. Two forms of α–glucan phosphorylase were isolated from *Dioscorea cayenensis* by ammonium sulphate gradient solubilization and further purified using starch adsorption and ion exchange chromatography on DEAE-Sephadex A-25 colunm. Fraction DC_1 was purified 80 fold with specific activity of 400 μmol min^{-1} mg^{-1} protein, while fraction DC_2 showed 60 fold purification with specific activity of 300 μmol min^{-1} mg^{-1} protein. Both enzyme forms were activated by AMP, magnesium, calcium and inhibited by ATP, ADP, ADP-glucose and sodium sulphate. They showed absolute primer requirement and obeyed Michaelis-Menten kinetics. The two forms have different Km values and different pH optima. The presence of amino acids and intermediates of glycolysis had no effect on the activities of the enzymes. There are no unusual properties of the enzymes which suggest that they function primarily in starch biosynthesis in *D. cayenensis* tuber.

α–Glucan phosphorylase (EC 2.4.1.1, α-1,4-glucan orthophosphoglucosyltransferase) catalyses the stepwise phosphorolytic cleavage of starch to glucose-1-phosphate. Although the main function of phosphorylase in animals is degradative, data from some investigators suggest that the enzyme might have some synthetic function in plants (Tsai and Nelson 1968, Matheson and Richardson 1976, Stitt and Steup 1985).

Multiple forms of starch phosphorylase have been detected in a range of plant tissues (Tsai and Nelson 1968, Khana *et al.* 1971, Gerbrandy and Doorgeest 1972, Baxter and Duffus 1973, Singh and Sanwal 1976, Matheson and Richardson 1976). Properties of these isoenzymes have been reviewed (Matheson and Richardson 1976, Turner and Turner 1980).

Phosphorylase has been detected in yam tubers (Ugochukwu *et al.* 1977) and the distribution pattern in various defined physiological segments studied (Oluoha 1988). Recently, phosphorylase from *D. rotundata* was purified and its properties studied (Oluoha 1989). Although the enzyme can synthesize polyglucan from glucose-1-phosphate without a primer, it was demonstrated that the primary function of the enzyme is degradative (Oluoha 1989).

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Different forms of starch phosphorylase from various plant tissues have been shown to exhibit different kinetic properties (Gerbrandy and Doorgeest 1972, Khanna *et al.* 1971, Baxter and Duffus 1973, Singh and Sanwal 1976, Matheson and Richardson 1976). Furthermore our knowledge of plant phosphorylase lags behind that of the corresponding animal enzymes. It has become necessary therefore to study in some detail as many variants of plant phosphorylase as possible, hence this study of *D. cayenensis* tuber phosphorylase.

The aim of this study is to isolate, purity and examine the kinetic properties of different forms of phosphorylase from *D. cayenensis* tuber. The role of the enzymes in polysaccharide metabolism will also be examined.

MATERIAL AND METHODS

Materials

Yam tubers used in this study were obtained from one of the author's Experimental form X_2O, University of Benin, Benin City, Nigeria. They were planted in April and harvested in December, stored in a well aerated place at 28 °C and relative humidity of between 49 % and 50 % for 5 months. All reagents were of analytical grade and were obtained from Sigma except Sephadex G series which were the products of Pharmacia Fine Chemicals. Spectrophotometric measurements were made using Pye Unican SP 1800 double beam spectrophotometer.

Enzyme Extraction

Yam tuber (100 g) was homogenised in 400 cm^3 of chilled 0.5 M citrate buffer pH 6.5 containing insoluble PVP and 1.0 mM EDTA at 4 °C. The homogenate was passed through several layers of muslin and the extract centrifuged for 15 min at 16 000 *g*. All subsequent procedures including centrifugation were carried out at 4 °C. The supernatant was brought to 30 % saturation with solid $(NH_4)_2SO_4$ with constant stirring, left for 30 min and centrifuged. The precipitate was discarded and the supernatant was raised to 70 % saturation with solid ammonium sulphate, left for 30 min and centrifuged as before. The resulting precipitate was dissolved in 30 cm^3 distilled water and desalted using Sephadex G-15. The fractions showing phosporylase activity were pooled and concentrated.

Enzyme Fractionation and Purification

Ammonium sulphate gradient solubilization was adopted from the method used by King (1972) with modifications. The protein solution (20 cm^3) was added with constant stirring to 60 cm^3 of Sephadex G-15 already equilibrated with 80 % saturated ammonium suplhate solution in 0.1 M Tris/HCl buffer (pH 7.5). Stirring was continued for 40 min. The Sephadex mixture was filtered, dried, resuspended in saturated ammonium sulphate solution (80 % and packed

in a column of 2×20 cm. The column was eluted with ammonium sulphate gradient decreasing from 80 % to 10 % saturation. A flow rate of 40 cm^3h^{-1} was maintained and fractions (4.0 cm^3) were collected. Fractions constituting different peaks showing phosphorylase activities were separately pooled and dialysed. The dialysed enzymes were centrifuged and any precipitates discarded. The supernatants were concentrated. Adsorption onto starch was adopted from the method of Burr and Nelson (1975) except for the use of yam starch and temperature of -2 and -10 °C. Each precipitate was dissolved in 30 cm^3 of 0.01 M Tris/HCl buffer, pH 7.5 and applied separately to 1.5×30 cm DEAE-Sephadex A-25 ion exchange column already equilibrated with the same buffer. The column was washed with 80 cm^3 20 mM Tris/HCl buffer, pH 7.5 containing EDTA (1.0 mM) and eluted with a linear gradient of 0 to 0.5 M NaCl in the same buffer. A flow rate of 30 cm^3h^{-1} was maintained and 5.0 cm^3 fractions were collected. The fractions making up the peak activity were pooled, centrifuged and the enzymes precipitated. The precipitated proteins were dissolved in 30 cm^3 of citrate buffer pH 6.3 for DC_1 and 6.5 for DC_2 and used as purified enzymes.

Analytical Methods

Protein was determined using the protein-dye binding method decsribed by Bradford (1976). Bovine serum albumin was used as standard protein. Sodium was estimated using a flame photometer, while NH_4^+ was estimated employing nesslerization (Varley 1969).

Phosphorylase Activity in the Direction of Starch Synthesis

Phosphorylase activity in the direction of starch synthesis was assayed as previously described (Oluoha 1989).

Detection of Phosphorylase Activity by Production of Iodine Staining Glucan

Purified enzyme (0.1 cm^3) was incubated at 28 °C with 0.005 % soluble starch and 10 mM glucose-phosphate in 0.1 M citrate buffer pH 6.3 for DC_1 or pH 6.5 for DC_2 (0.1 cm^3) for 10 min. Dilute I_2 (0.01 % I_2 in 0.1 % KI) in 0.1 M citrate buffer pH 4.0 (3.8 cm^3), was added and absorbance at 600 nm was measured.

Detection of Phosphatase Activity

0.1 cm^3 of appropriate enzyme fraction was incubated with 0.1 cm^3 of 10 mM glucose-1-phosphate and 0.8 cm^3 buffer pH 6.3 or 6.5 for 10 min. 1.0 cm^3 of 10 % w/v TCA was added and the mixture centrifuged. Glucose liberated in the supernatant was assayed using glucose oxidase-peroxidase system with 4-aminophenazone as oxygen acceptor (Wootton 1974).

pH Optima

pH optima of the enzymes were determined in citrate buffer (0.1 M) in pH range varying from 5 to 7.5 using the assay method described. Other buffers used were maleate (0.1 M), Tris/maleate (0.05 M), imidazole (0.2 M) and glycerophosphate (0.1 M).

Effect of Temperature

Phosphorylase activity was determined at various temperatures ranging from 25 °C to 50 °C using the assay method.

Phosphorylase Activity in Direction of Starch Degradation

Phosphorylase activity in the direction of starch degradation was followed by disappearance of starch-iodine coloured complex (Singh and Sanwal 1976). The incubation mixture contained 0.5 cm^3 of 1 % soluble starch, 0.2 M phosphate buffer, pH 6.3 for DC_1 and 6.5 for DC_2 and 0.1 cm^3 enzyme preparation in a total assay volume of 6.0 cm^3. While the reaction mixtures were incubating at 28 °C, 1.0 cm^3 aliquots were removed at time intervals and mixed with 0.1 cm^3 I_2/KI reagent. Resulting solution was diluted to 10 cm^3 with distilled water and absorbance at 600 nm was read against the blank.

Table 1
Summary of purification procedures

Fraction	Total protein [mg]	Total activity [μmol min^{-1}]	Specific activity [units min^{-1} mg^{-1} (protein)]	Purification (fold)	Yield [%]
Crude extract	600	3000	5.0	1	100
0–30% $(NH_4)_2SO_4$ precipitation (Supern)	200	2500	12.5	2.5	83.3
30–70% $(NH_4)_2SO_4$ precipitation (precipt)	100	1800	18.0	3.6	60.0
$(NH_4)_2SO_4$ gradient solubilization Fraction DC_1	5.5	1700	366.6	73.3	56.6
Starch absorption and DEAE-Sephadex chromotography (Fraction DC_1)	4.0	1600	400.0	80.0	53.3
$(NH_4)_2SO_4$ solubilization. Fraction DC_2	8.0	1600	200	40.0	53.3
Starch absorption and DEAE-Sephadex Chromato. (DC_2)	6.0	1500	300	60	50.0

RESULTS

α-Phosphorylase extracted from *D. cayenensis* tuber was fractionated using ammonium sulphate gradient solubilization and further purified by starch absorption and DEAE-Sephadex ion exchange chromatography. A summary of purification procedure is shown in Table 1. Fraction DC_1 was purified 80 fold with specific activity of 400 μmol min^{-1} mg^{-1} protein and a recorvery of 53.3 %. For fraction DC_2 60 fold purification was obtained, with specific activity of 300 μmol min^{-1} mg^{-1} protein and a recorvery of 50 % relative to the initial starting activity.

Fig. 1 shows ammonium gradient solubilization profile of the enzymes. Three protein peaks were detected. The protein peaks designated DC_1 and DC_2 showed phosphorylase activity. DC_1 and DC_2 denote *Dioscorea cayenensis* tuber phosphorylase fractions, while 1 and 2, the order in which the enzyme forms eluted from the column. The protein peak P showed some phosphatase activity and eluted between two phosphorylase peaks. DC_1 was solubilized between 51 % and 47.9 % salt saturation with peak position at 49.5 %. The fractional

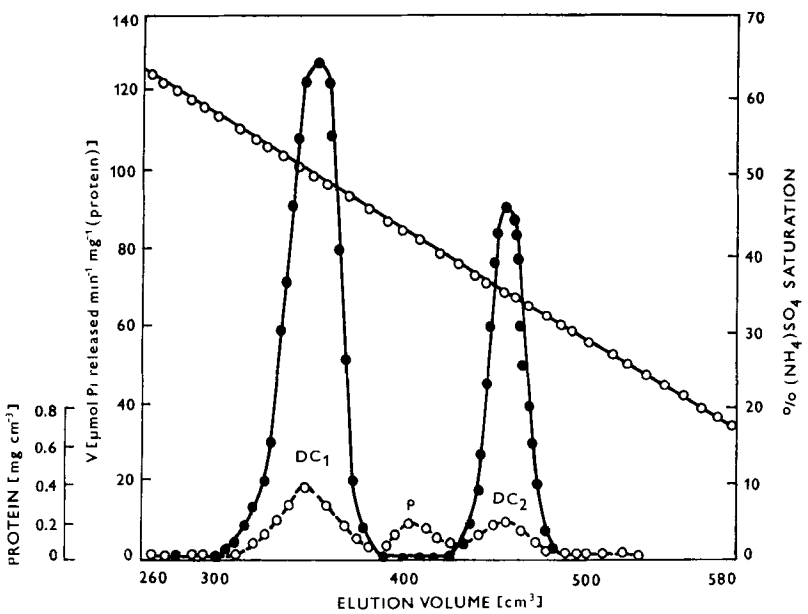

Fig. 1. Separation of phosphorylase forms by ammonium sulphate gradient solubilization.
The column was eluted at 28 °C with gradient decrease from 80 % to 10 % ammonium sulphate saturation buffered with 50 mM Tris/HCl pH 7.5. A flow rate of 40 cm^3 per h was maintained and fractions (4.0 cm^3) were collected. Fractional volumes between 332 cm^3 and 368 cm^3 were pooled for DC_1 and fractions between 444 cm^3 and 468 cm^3 were pooled for DC_2. Enzyme activity was assayed in direction of starch synthesis by determining Pi released as described under materials and methods. Enzyme activity ●—●—●, Protein ○– –○– –○, $(NH_4)_2SO_4$ ○—○—○

volumes between 332 cm^3 and 368 cm^3 were collected and pooled. DC_2 was eluted between 36 % and 33.5 % salt saturation with peak position at ammonium sulphate saturation of 34.9 %. The fractions between 444 cm^3 and 468 cm^3 were collected and pooled.

The elution behaviours of DC_1 and DC_2, separately chromatographed on DEAE-Sephadex ion exchange column are shown in Figs 2A and 2B respectively. DC_1 was eluted between 0.12 and 0.188 M NaCl and fractions between 140 cm^3 and 190 cm^3 were pooled and precipitated with 60 % saturation in ammonium sulphate, while DC_2 eluted between 0.115 and 0.168 M NaCl and

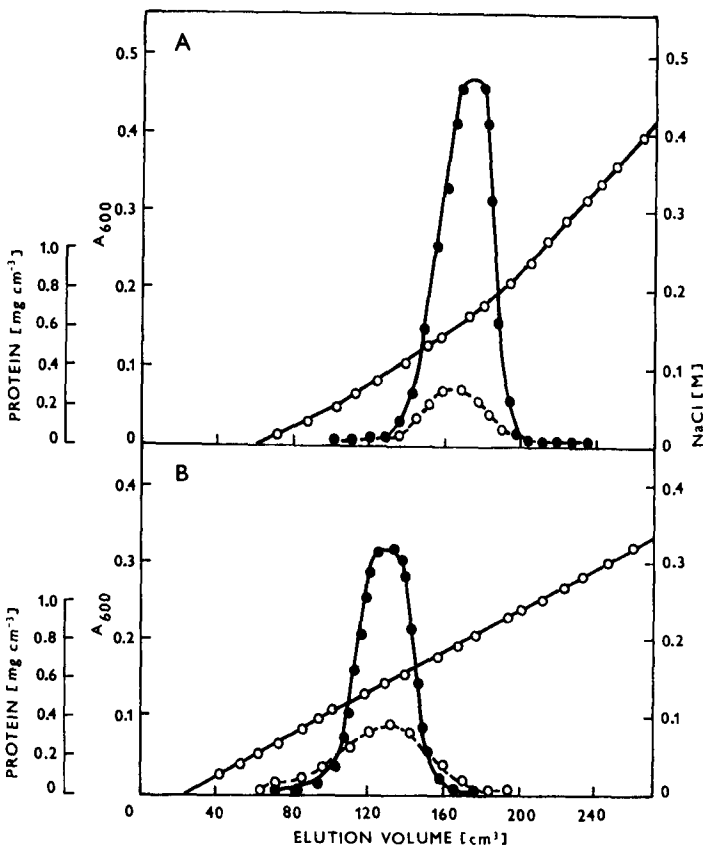

Fig. 2. Elution profile of DC_1 and DC_2, separately chromatographed on DEAE-Sephadex A-25. A flow rate of 30 cm^3 per h was maintained and fractions (5 cm^3) were collected. Enzyme activity was assayed in the direction of starch synthesis by measuring iodine staining glucan produced as described under Materials and Methods.

A: Elution profile of DC_1. Fractional volumes between 140 cm^3 and 190 cm^3 were pooled and proteine precipitated with 60 % $(NH_4)_2SO_4$ saturation.

B: Elution profile of DC_2. Fractions between 100 cm^3 and 150 cm^3 were pooled and proteins precipitated with 40 % $(NH_4)_2SO_4$ saturation. Enzyme activity (absorbance at 600 nm) ●- ●- ●, Protein ○- - ○- - ○, NaCl ○— ○— ○.

fractions between 100 cm^3 and 150 cm^3 were collected, pooled, dialysed and precipitated with solid ammonium sulphate (40 % saturation).

pH Optimum

The pH optima found for DC_1 and DC_2 were 6.3 and 6.5 respectively.

Effect of Temperature

The temperature optium for the two enzyme forms was 40 °C and above this temperature the enzymes became progressively inactivated. In the direction of degradation, the activities of the enzymes were linear with time up to at least 30 min using 3 μg of enzyme protein. Both enzyme forms were inactive in the absence of prime and showed Michaelis-Menten kinetics. No lag phase observed. Fig 8 shows the activity of phosphorylase during sprouting and yam tuber formation.

Kinetic Studies

The effects of ADP, ADP-glucose and metal salts are shown in Figs 3 to 7. Table 2 shows the kinetic constants of DC_1 in the presence of inhibitors and activators, extrapolated from the double reciprocal plots on Figs 3 and 4. K_m obtained in the presence of ADP and ADP-glucose increased while the V_{max} remained unchanged. The presence of ATP increased the K_m but decreased the V_{max}. This indicates that ADP and ADP-glucose are competitive inhibitors with respect to glucose-1-phosphate with K_i of 3.13 mM and 1.19 mM respectively (Figs 3 and 4), while ATP is a mixed-type competitive-non-competitive inhibitor with K_i of 4.88 mM. AMP activated the enzyme as is shown by decreased K_m and highly increased V_{max} (Fig 3). In the presence of magnesium and calcium, the K_m also decreased and V_{max} increased (Table 2 and Fig 4). This demonstrated that the two cations were activators of phosphorylase from *D. cayenensis* tuber.

In Table 3 the kinetic constants of DC_2 are reported. In the presence of ADP, ATP and ADP-glucose, the K_m increased while V_{max} remained constant with K_i values of 3.27, 4.5 and 2.19 mM respectively. This shows that these nucleotides

Table 2

Kinetic constants of fraction DC_1 of phosphorylase from *D. cayenensis*. Enzyme activity was assayed as described under Material and Methods with additions to the assay as indicated. The kinetic constants reported here were extrapolated from double reciprocal plots in Figs 3 and 4.

Addition to the assay	K_m [mM]	V_{max} [μmol min^{-1} mg^{-1} (protein)]	K_i [mM]
NONE	2.44	625	–
5.0 mM ATP	3.44	476.6	4.88
2 mM ADP	4.0	625	3.13
2 mM ADP-glucose	5.0	625	1.19
5 mM Na_2SO_4	4.0	513	7.10
0.6 mM AMP	1.61	1196.0	–
5.0 mM $MgCl_2$	1.85	741.0	–
5.0 mM $CaCl_2$	1.98	664.0	–

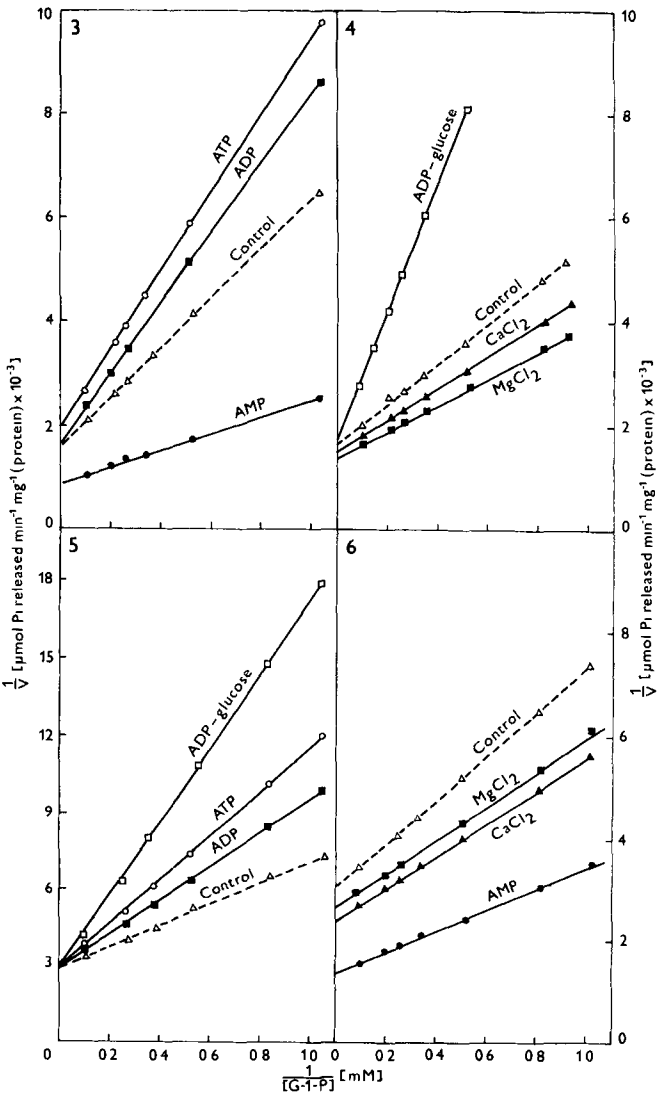

Fig. 3. Effects of nucleotides on DC_1 activity. Enzyme activity was determined in the direction of starch synthesis by measuring P, released in the presence and absence of 5 mM ATP, 2 mM ADP, AMP (0.6 mM), using 5 mg soluble starch. Glucose-1-phosphate concentration was varied as indicated.

Fig. 4. Effects of ADP-glucose and metal salts on DC_1 activity. Phosphorylase activity was assayed in the presence and absence of $MgCl_2$ (5 mM) $CaCl_2$ (5 mM) and ADP-glucose (2 mM) as in Fig. 3.

Fig. 5. Effects of ATP, ADP, and ADP-glucose on DC_2 from *D. cayenensis* tuber. Phosphorylase activity was assayed in the presence and absence of ATP (5 mM), ADP-glucose (2mM) and ADP (2 mM). All other conditions are as in Fig. 3.

Fig. 6. Effects of AMP, $MgCl_2$ and $CaCl_2$ on DC_2. Enzyme activity was assayed as in Fig. 3. in the presence and absence of $MgCl_2$ (5 mM), $CaCl_2$ (5 mM) and AMP (0.6 mM).

Table 3
Kinetic constants of phosphorylase fraction DC_2 from *D. cayenensis*. Enzyme activity was assayed as described under Material and Methods in the direction of starch synthesis. The Pi released was estimated as detailed under assay method. The kinetic constants reported here were extrapolated from double reciprocal plots in Figs 5 and 6.

Addition to the assay	K_m [mM]	V_{max} [μmol Pi min^{-1} mg^{-1} (protein)]	K_i [mM]
NONE	1.51	344.5	–
5 mM ATP	3.12	344.5	4.5
2 mM ADP	2.43	344.5	3.27
2 mM ADP-glucose	2.89	344.5	2.19
5 mM Na_2SO_4	2.19	243.0	11.10
0.6 mM AMP	1.18	769.2	–
5 mM $MgCl_2$	1.10	400.0	–
5 mM CaCl	1.10	425.5	–

and ADP-glucose are competitive inhibitors of the enzyme (Fig 5 and 6). AMP, magnesium and calcium are activators of DC_2 and this is shown by decreased K_m and increased V_{max} in their presence (Fig 6 and Table 3).

The effect of sodium sulphate on the activities of DC_1 and DC_2 are shown in Fig 7. This shows clearly that sodium sulphate is an inhibitor of the two enzyme forms.

In the presence of the salt, the K_m obtained for both enzyme forms increased while the V_{max} decreased (Tables 2 and 3) with K_i values of 7.1 mM (DC_1) and 11.1 mM for DC_2, indicating that sodium sulphate is mixed-type inhibitor.

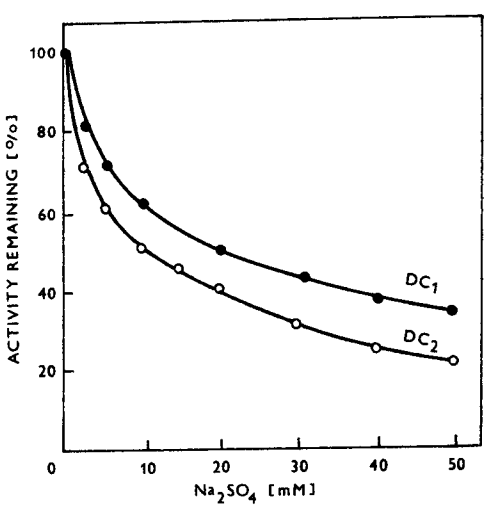

Fig. 7. Effect of sodium sulphate on DC_1 and DC_2. Enzyme activity was determined as in Fig. 3, using 0.1 cm^3 of each enzyme fraction, 5 mg soluble starch and 10 mM glucose-1-phosphate. Na_2SO_4 concentration was varied as indicated.

Phosphorylase Activity in Direction of Phosphorolysis

In the direction of degradation, ATP, ADP ADP-glucose and sodium sulphate were inhibitors of DC_1 and DC_2 and the type of inhibitions were similar to those found for DC_1 and DC_2 in the synthetic direction. AMP, calcium and magnesium activated both enzyme forms. DC_1 was, as in the synthetic direction more sensitive to activation than DC_2. However, the K_m found using P_i were 3.0 mM and 2.1 mM for DC_1 and DC_2 respectively. The K_m found for DC_1 using starch and glycogen were respectively 4 mg and 7 mg while K_m for DC_2 were 6 mg and 8 mg respectively.

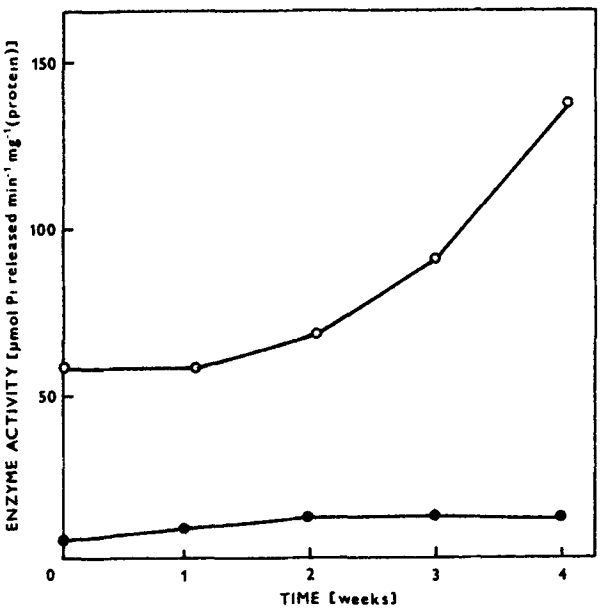

Fig. 8. Phosphorylase activity during sprouting and yam tuber formation. Phosphorylasee activity was determined in direction of starch synthesis at intervals of one week. Pi released was determined as described under materials and methods.
Activity during sprouting O—O—O
Activity during tuber formation ●—●—●

DISCUSSION

We refer to the two forms of the enzyme obtained as DC_1 and DC_2 but refrain from referring to them as isoenzymes, since the primary structures were not known (Stitt and Steup 1985). The results shown in Figs 2A and 2B compare the elution behaviours of the two enzyme forms chromatographed separately on the same DEAE-Sephadex column. No complete separation was possible. However, ammonium sulphate gradient solubilization effectively separated the two forms. A non specific phosphatase has been separated from phosphorylase in this work.

It has been reported that phosphorylase could synthesize polyglucan without a primer (Tsai and Nelson 1968, 1969, Kamogawa *et al.* 1968). However, subsequent studies have shown that the ability of phosphorylase to synthesize polyglucan in the absence of a primer was due to presence of contaminating primer (Kamogawa *et al.* 1968, Khanna *et al.* 1971, Gerbrandy and Doorgeest 1972, Lee and Braun 1973, Burr and Nelson 1975). Recent studies demonstrated that phosphorylase from white yam tuber (*D. rotundata*) could synthesize polyglucan in the absence of added primer, after removal of possible contaminating primer (Oluoha 1989). In this study, the two forms of phosphorylase from *D. cayenensis* tuber showed an absolute requirement for a primer and did not function in its absence even when up to 40 mM glucose-1-phosphate was used as the only substrate. Similar results have been reported for isoenzymes of phosphorylase from banana fruits (Singh and Aanwal 1976).

Phosphorylase activity from *D. cayenensis* increased several times during sprouting but decreased to a very low level during tuber formation, the active period of starch synthesis (Fig 8). This may indicate that *D. cayenensis* tuber phosphorylases do not play a major role in starch biosynthesis. Similar results have been obtained with phosphorylase I from developing and germinating pea seeds (Matheson and Richardson 1976) and white yam tuber phosphorylase during tuber ontogenesis (Oluoha 1989). These enzymes have been said to function mainly as starch degrading enzymes. The findings that phosphorylase from *D. cayenensis* tuber showed absolute primer requirement and inhibited by ADP-glucose and ADP, the substrate and product of starch synthetase respectively, strongly support the view that the primary role of the yam enzyme is degradative.

The optimum pH found for DC_1 and DC_2 are 6.3 and 6.5 respectively. The pH optimum (6.3) for DC_1 was similar to white yam tuber enzyme. The use of different buffer systems did not alter the pH optima obtained in this study. Both enzymes exhibited the same optimum temperature (40 °C) and above this the enzyme became prograssively inactivated. Higher temperature optima, 45 °C and 50 °C have been found for phosphorylase isoenzymes from banana fruits (Singh and Sanwal 1976) and mistletoe leaves (Khanna *et al.* 1971).

It has been found that ATP and AMP had no effect on isoenzymes A and C from banana but inhibitet fraction B (Singh and Sanwal 1976). However ATP and AMP have been reported to activate phosphorylase from mistletoe leaves (Khanna *et al.* 1971). Although the two nucleotides were found to inhibit one of the isoenzymes of maize endosperm (Tsai and Nelson 1969) and developing barley grains (Baxter and Duffus 1973), Lee and Braun (1973) reported that ATP has no effect on sweet maize phosphorylase.

In this study ADP, ATP and ADP-glucose inhibited the two forms of phosphorylase from *D. cayenensis*, while AMP activated them. Similar results

have been reported for *D. rotundata* phosphorylase (Oluoha 1989). In fact ATP, ADP and ADP-glucose were competitive inhibitors of DC_2 (Figs 5 and 6), while ATP is a mixed-type competitive/non-competitive inhibitor for DC_1 (Table 2). ADP and ADP-glucose were found to be competitive inhibitors of DC_1 (Figs 3 and 4).

Although 10 mM magnesium has been found to activate maize phosphorylase 2-fold (Tsai and Nelson 1968), Burr and Nelson (1975) found magnesium inhibitory to purified maize phosphorylase. However, it has been reported that magnesium had no effect on phosphorylase from mistletoe leaves (Khanna *et al.* 1971), banana fruits (Singh and Sanwal 1976) and sweet maize (Lee and Braun 1973). A recent report indicated that calcium and magnesium uncompetitively inhibited phosphorylase from *D. rotundata* tuber (Oluoha 1989). In this study, magnesium and calcium activated the two forms of phosphorylase from *D. cayenensis* tuber. V_{max} found for DC_1 in the presence of AMP, magnesium and calcium were 1175, 741 and 664.4 μmol min^{-1} mg^{-1} protein respectively (Table 2), while the V_{max} found for DC_2 were 769.2, 400 and 425.5 μmol min^{-1} mg^{-1} protein respectively (Table 3). These results indicate that DC_1 is more sensitive to activation than DC_2.

Sodium sulphate at high concentration (0.7 M) has been reported to activate liver phosphorylase (Appleman et al. 1966), while Bur and Nelson (1975) used it to activate inactivate maize phosphorylase treated with glucoamylase. However, it has been found that at sodium sulphate concentrations above 12.5 mM, phosphorylase from *D. rotundata* was inhibited (Oluoha 1989). In this study, it was observed that the two forms of phosphorylase from *D. cayenensis* tuber were inhibited by Na_2SO_4 (Fig 7). DC_2 is more sensitive to this inhibition than DC_1. 10 mM Na_2SO_4 is required for 50 % inhibition of DC_2 while DC_1 needed 20 mM for 50 % inhibition.

K_m obtained for DC_1 and DC_2 in the direction of degradation were respectively 3.0 mM and 2.1 mM Pi in the presence of 10 mg soluble starch. These values are low when compared to 4.2 mM found for maize enzyme (Burr and Nelson 1975), 10 mM for white yam tuber enzyme (Oluoha 1989) and 14 mM reported for pea seed enzymes (Matheson and Richardson 1976). These low K_m values indicate high affinity of the enzymes for inorganic phosphate. The K_m obtained using soluble starch and glycogen were 4 mg and 6 mg for DC_1 respectively, while K_m values for DC_2 using the same substrate were 6 mg and 8 mg respectively. Similar results have been reported for *D. rotundata* phosphorylase (Oluoha 1989) and pea seed enzyme (Matheson and Richardson 1976).

In conclusion, two forms of phosphorylase have been isolated from *D. cayenensis* tuber with different K_m, V_{max} and optimum pH. The two forms exhibited different degrees of sensitivity to inhibitors and activatots and showed absolute requirement for primer. There are no unusual properties which indicate

that the enzyme forms play any significant role in biosynthesis of starch in *D. cayenensis* tuber. Strong inhibition by ADP-glucose and ADP and absolute requirement for a primer show that the primary function of the phosphorylase is degradative.

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