BIOLOGIA PLANTARUM (PRAHA)

30 (5) : 362—372, 1988

Pyruvate Kinase, an Enzyme Subject to Regulation in *Dioscorea alata*

U. OLUOHA

Department of Biochemistry, University of Benin, Benin City, Nigeria

Abstract. Different species of yam tubers were examined for the presence of pyruvate kinase and phosphatase activities. Pyruvate kinase was purified 25 fold with a yield of 50 %, using ammonium sulphate precipitation and ion exchange chromatography on DEAE-Sephadex. Partially purified enzyme showed normal Michaelis-Menten kinetics. However, pyruvate kinase from crude extract of dormant yam tuber showed slight sigmoid response towards phosphoenolpyruvate and magnesium and to a certain extent ADP. The enzyme is activated by AMP and inhibited by ATP and citrate in both crude and partially purified preparations. Further studies on the effect of energy charge on the enzyme strongly suggest that pyruvate kinase from *D. alata* is a regulatory enzyme. No evidence was found for the presence of more than one pyruvate kinase in germinating *D. alata* tuber. With the exception of *D. dumentorum*, all the other three species of yams studied contain very little or no detectable phosphatase activity during dormancy. However, phosphatase activity increased during germination in all the species, thus excluding the use of sprouting yam tubers for kinetic study of pyruvate kinase.

Pyruvate kinase (EC 2.7.1.40, ATP-pyruvate 2-O-phosphotransferase) from a variety of tissues (CARMINATTI *et al.* 1968, POGSON 1968, BAILLEY and WALKER 1969, IBSEN *et al.* 1971) and organisms (HUNSLEY and SUELTER 1969, CORNISH and JOHNSON 1971, LIAN and ATKINSON 1971) seems to be a regulatory enzyme. It shows sigmoid saturation curve towards phosphoenolpyruvate (PEP) and is activated by fructose-1,6-bisphosphate in eucaryotes and AMP in procaryotes. However, some investigators (CARMINATTI *et al.* 1971, MUSTAFA and HOCHACHKA 1971) have described pyruvate kinases which do not conform to the above generalization. Although pyruvate kinase has been identified in yam tubers, its kinetic properties have not been studied in detail. The activation of pyruvate kinase by mono-valent and di-valent cations in relation to plant nutrition has been reported (MILLER and EVANS 1957, EVANS 1963). However, investigations to date (DUGGLEBY and DENNIS 1973, TURNER and TURNER 1980) indicate that plant pyruvate kinase does not show sigmoid kinetics towards phosphoenolpyruvate and is not activated by fructose-1,6-bisphosphate. Since neither of these properties is common to all pyruvate kinase, it has become necessary to re-examine the yam enzyme.

Received October 28, 1987; accepted February 19, 1988

Most plant tissues contain phosphatase (EC 3.1.3.2, orthophosphoric-monoester phosphohydrolase) which interferes with pyruvate kinase assay (DUGGLEBY and DENNIS 1973). Therefore a source of enzyme in yam tuber has to be found that contains little phosphatase activity. Secondly, since yams are mainly carbohydrate storing organs, it will be instructive to know if regulatory properties can be ascribed to pyruvate kinase from this source.

The aim of this investigation is to study in detail the kinetic and regulatory properties of pyruvate kinase from *D. alata* and to see if the enzyme can respond to energy charge.

MATERIALS AND METHODS

Materials

The yam species used in this study were obtained from the author's experimental farm in Benin City, Nigeria. In the growth experiment, uncut seed yam tubers were grown in mounds of loamy soil exposed to sufficient sunshine. At intervals the tubers were removed, washed with distilled water and various tissues extracted. All reagents and chemicals were of analytical grade and were products of Sigma.

Enzyme Assay

Pyruvate kinase activity was assayed at 30 °C, using the method of PON and BONDAR (1967). The standard assay mixture contained (final concentrations) 60 mM Tris/HCl buffer pH 7.5; 80 mM KCl; 8 mM $MgSO_4$; 0.2 cm^3 of partially purified enzyme; 0.2 mM ADP; 0.3 mM PEP in a total assay volume of 2.50 cm^3. The reaction was initiated by the addition of PEP and the rate of decrease in absorbance of PEP at 230 nm was continuously followed using Unicam SP 1800 double beam spectrophotometer coupled to a linear chart recorder. Phosphatase activity was similarly determined except for the omission of ADP in the reaction mixture.

Analytical Methods

Protein was determined using protein-dye binding method described by BRADFORD (1976). Bovine serum albumin was used as protein standard. Potassium was estimated by flame emission spectroscopy at 760 nm using Unican SP 90A.

Extraction of Pyruvate Kinase from Yam Tubers

10 g of peeled yam tubers were removed from the middle region, washed in cold distilled water and homogenized in 100 cm^3 of 0.1 M Tris/HCl buffer pH 7.5, containing 50 % v/v glycerol and EDTA (1.0 mM). The homogenate was filtered through several layers of muslin and the extract centrifuged at 34 000 *g* for 15 min at 4 °C. The extract was placed in cellophane dialysis tubing and concentrated by immersing the tubing in polyethyleneglycol powder (2.0 g of the powder per cm^3 of extract) at 4 °C. All subsequent procedures including centrifugation were carried out at 4 °C. All other yam tissues were similarly extracted using 1 g of tissue to 5 cm^3 of buffer and the crude extracts were used without further treatment.

Partial Purification of *D. alata* Pyruvate Kinase

The supernatant fraction of the concentrated extract was brought to 54 % saturation with $(NH_4)_2SO_4$ with constant stirring and left at 0 °C for 10 min. The mixture was centrifuged at 35 000 *g* for 15 min and the precipitate discarded. The resulting supernatant was brought to 70 % saturation with $(NH_4)SO_4$ and left for 30 min. The mixture was centrifuged as before for 40 min. The precipitated protein was dissolved in 10.0 cm^3 of 0.03 M TES buffer pH 7.5 and dialyzed overnight against 4 changes of 0.01 M Tris/HCl buffer pH 7.5, containing 50 % v/v glycerol. The dialysized protein was centrifuged and the supernatant (10.0 cm^3) was applied to 3 × 20 cm column of DEAE-Sephadex A-50 which has been equilibrated with 0.01 M Tris/HCl buffer pH 7.5, containing 50 % v/v glycerol. The enzyme solution was washed into the column with an equal volume of this buffer and then eluted with a linear gradient formed between this buffer and 0.5 M KCl in the same buffer. A flow rate of 0.5 cm^3 per min was maintained and 5.0 cm^3 fractions were collected. The most active fractions were pooled, concentrated and later centrifuged. The supernatant was used as a partially purified enzyme.

Effect of pH Activity of Pyruvate Kinase from *D. alata*

A crude extract of sprouting *D. alata* was assayed at various pH values using Tris/MES buffer.

Effect of AMP, ATP and Citrate on Kinetics of Partially Purified Enzyme

Activity of pyruvate kinase was assayed at various concentrations of PEP and ADP with addition of fixed concentrations of AMP (1.0 mM), ATP (3.0 mM) and citrate (2.0 mM).

Determination of Nucleotides

ATP was determined by coupling it to hexokinase/glucose-6-phosphate dehydrogenase system and measuring the increase in absorbance of NADPH at 340 nm (KACHMAR and DONALD 1976). ADP was determined by coupling it to pyruvate kinase reaction and measuring the decrease in absorbance of phosphoenolpyruvate at 230 nm as described under enzyme assay. AMP was calculated by assuming equilibrium constant, $(([AMP] \times [ATP])/[ADP]^2)$ of 0.8 (ATKINSON 1968).

Effect of Energy Charge on Partially Purified Pyruvate Kinase Activity

Mixtures of AMP and ATP with appropriate energy charge and total adenylate concentration of 5 mM were incubated with 22 mM $MgCl_2$ and 20 units of rabbit muscle adenylate kinase per cm^3, in 50 mM TES buffer, adjusted to pH 7.5 with Tris, for 10 min to establish equilibrium. Appropriate amounts of these mixtures were added to assay mixture containing 60 mM Tris/TES buffer pH 7.5, 9 mM $MgCl_2$, 50 mM KCl, 0.1 mM PEP and 0.2 cm^3 of partially purified enzyme to give total adenylate concentration of 0.2 mM, 1.0 mM and 2.0 mM and the enzyme activity determined.

Factors Affecting Energy Charge Response of Pyruvate Kinase from *D. alata*

The same method as above was employed but using various ADP, AMP and ATP concentrations calculated as described by ATKINSON (1968) assuming

equilibrium constant (([AMP] × ATP])/[ADP]²)) of 0.8 and total adenylate concentration of 1.0 mM. Various concentrations were added to the assay and enzyme activity was determined.

Kinetics of Partially Purified Enzyme in Respect to Mg^{2+}

Enzyme activity was determined at various magnesium ion concentrations using the assay method.

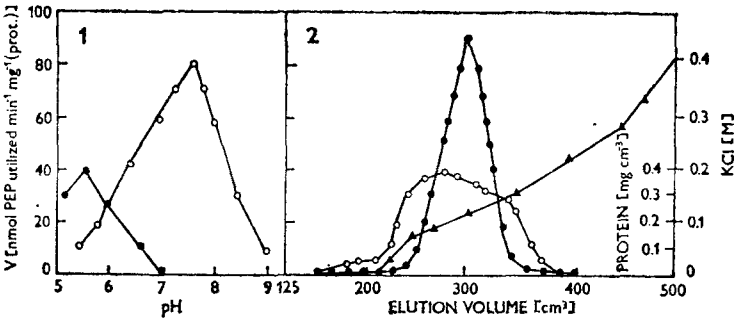

Fig. 1. Effect of pH on *D. alata* pyruvate kinase. — Crude extract of sprouting *D. alata* pyruvate kinase obtained, as described under materials and methods, was assayed for pyruvate kinase and phosphatase activities at various pH values using Tris/MES buffer. — Pyruvate kinase ○ phosphatase ●.

Fig. 2. Chromatography of *D. alata* pyruvate kinase on DEAE-Sephadex. — 10 cm^3 dialyzed extract of *D. alata* pyruvate kinase was applied on DEAE-Sephadex A-50 column, (3.0 × 20 cm). The flow rate was maintained at 0.5 cm^3 min^{-1} and 5 cm^3 fractions were collected. Pyruvate kinase activity, protein content, K^+ concentration were determined as described under materials and methods. The KCl gradient was varied from 0.0 to 0.5 M. — Pyruvate kinase ●, protein ○, KCl ▲.

RESULTS

Source of Pyruvate Kinase

Extracts of a number of yam tissues were examined for pyruvate kinase and phosphatase activities (Table 1). There is very little or no phosphatase activity detected in ungerminated yam tubers with the exception of *D. dumentorum*. During germination, the activities of both enzymes increased. This rapid increase in phosphatase activity excludes the use of sprouting yam tuber for detailed kinetic study of pyruvate kinase. The highest phosphatase activity occurred in the leaf tissues except *D. rotundata* with the highest enzyme content in the extract from the stem. Chromatography of extract from 70 % $(NH_4)_2SO_4$ precipitation on DEAE-Sephadex ion exchanger gave a single peak of pyruvate kinase activity (Fig. 2). However, this procedure did not give a satisfactory separation of pyruvate kinase and phosphatase from the extract of sprouting *D. alata* but pyruvate kinase eluted in the same position as that from dormant yam tuber. This indicates that the increase in pyruvate kinase activity during germination did not involve the synthesis of a different enzyme. Therefore extracts from dormant yam tuber were used as a source of pyruvate kinase for kinetic studies.

Kinetic Studies

The pH of maximum activity of pyruvate kinase and phosphatase has to be 7.5 and 5.5 respectively (Fig. 1). Phosphatase activity is very low above pH 6.5 but increased as pH is lowered. This indicates that *D. alata* contains an acid phosphatase with no activity at pH 7.5.

Preliminary studies of the kinetics of pyruvate kinase from crude extract of water yam displayed curved reciprocal plots when concentrations of PEP, ADP and magnesium ions were varied (Fig. 7A, B, C, respectively)

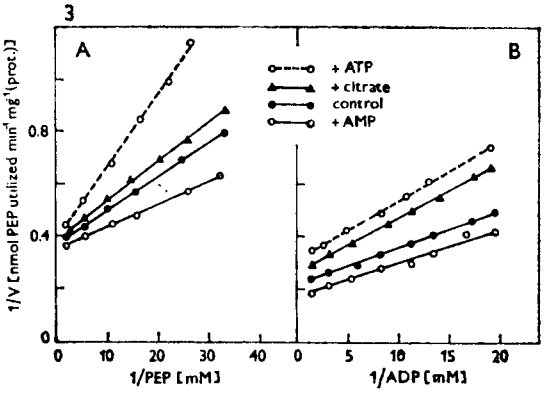

Fig. 3. Effect of AMP, citrate and ATP on the kinetics of pyruvate kinase from *D. alata*. — The enzyme was purified and assayed, as described under materials and methods, section using 50 mM Tris/TES buffer pH 7.5, 0.1 mM PEP and 0.1 mM ADP. Additions to the assay were: — None ●, AMP (1.0 mM) ○ (full line), 2.0 mM citrate ▲, 3.0 mM ATP ○ (dashed line). — A: Assay mixture contained 0.1 mM ADP at various values of PEP. — B: Assay mixture contained 0.1 mM PEP and ADP concentrations as indicated. The data were plotted in double reciprocal form.

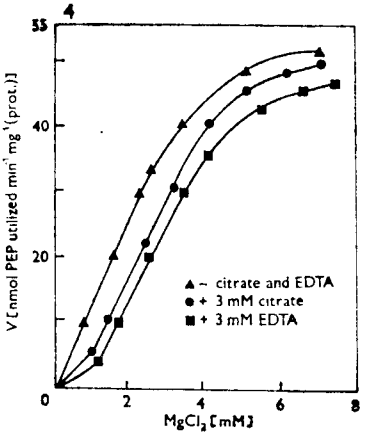

Fig. 4. Kinetics of purified enzyme with respect to magnesium. — The activity of the purified enzyme was determined at various magnesium ion concentrations using the assay method. — Enzyme activity assayed in the absence of citrate and EDTA (▲); enzyme activity determined in the presence of 3 mM citrate (●); enzyme activity assayed in the presence of 3 mM EDTA (■).

TABLE 1

Pyruvate kinase and phosphatase content of various yam tissues. The yam tubers were grown as described under materials and methods section. The crude extracts of various tissues were assayed for pyruvate kinase and phosphatase activities. Enzyme activity is expressed as nmoles of PEP utilized per min per mg protein. Each value represents the average of 4 determinations ± standard error of mean

Yam species	Tissues	Period of germination [weeks]	PK activity	Phosphatase activity
D. rotundata	*Dormant tuber	0	100 ± 5.0	ND
	Germinated tuber	4	150 ± 4.0	10.0 ± 0.6
	Roots	4	170 ± 6.2	40.0 ± 1.0
	Stem	4	180 ± 7.5	60.0 ± 2.0
	Leaves	4	170 ± 4.5	50.0 ± 2.5
D. alata	*Dormant tuber	0	80 ± 2.0	ND
	Germinated tuber	4	120 ± 3.0	20.0 ± 0.8
	Roots	4	110 ± 1.2	50.0 ± 1.5
	Stem	4	100 ± 3.6	44.0 ± 1.8
	Leaves	4	180 ± 5.0	90.0 ± 3.0
D. cayenensis	*Dormant tuber	0	100 ± 2.5	1.5 ± 0 2
	Germinated tuber	4	150 ± 5.2	10.0 ± 0.7
	Roots	4	120 ± 6.0	25.0 ± 0.4
	Stem	4	140 ± 3.2	60.0 ± 0.7
	Leaves	4	140 ± 2.1	30.0 ± 2.1
D. dumentorum	*Dormant tuber	0	90 ± 2.0	35.0 ± 1.6
	Germinated tuber	4	120 ± 3.2	40.0 ± 2.1
	Stem	4	100 ± 2.0	60.0 ± 3.0
	Roots	4	110 ± 1.1	55.0 ± 2.5
	Leaves	4	130 ± 1.6	65.0 ± 2.8

ND = not detectable

* Stored for 4 months after harvesting in a well-aerated place at a temperature of 28 to 30 °C.

but the partially purified enzyme did not show the same effect (Figs. 3A, B, and 4). Pretreatments of the crude enzyme with various compounds such as EDTA, ATP, citrate, 2-mercaptoethanol, $MgCl_2$ and alanine or assaying the enzyme activity under various conditions of pH and temperature did not change the sigmoid character of the enzyme. *D. alata* pyruvate kinase is inhibited by trisodium citrate, ATP and activated by AMP (Fig. 3). Since these inhibitions and activation were first observed in crude extracts, the enzyme was partially purified. Fig. 2 shows that the enzyme eluted as a single peak between 0.075 and 0.175 M potassium chloride. No phosphatase activity was detectable. The eluted enzyme was activated by AMP and inhibited by citrate, both effects being constant across the peak activity.

The partially purified enzyme showed normal kinetics towards PEP and ADP, Fig. 3A and B respectively, while effects of ATP, AMP and citrate on the kinetics of the enzyme are also shown in Fig. 3. The values of kinetic constants reported here were extrapolated from the double reciprocal plots in Fig. 3.

Km and V_{max} obtained for PEP (Fig. 3A) are 34 μM and 28 nmol min^{-1}

mg^{-1}, respectively. In the presence of ATP, Km (74 μM) increased, while V_{max} (28 nmol min^{-1} mg^{-1} protein) remained unchanged. This indicates that ATP is a competitive inhibitor with Ki of 2.5 mM. Citrate inhibition is non-competitive as shown by a decrease in V_{max} (25 nmol min^{-1} mg^{-1} protein) and an unaltered Km (34 μM). The Ki (16 mM) is very high. In the presence of AMP, V_{max} (31 nmol min^{-1} mg^{-1} protein) increased while Km (23 μM) decreased.

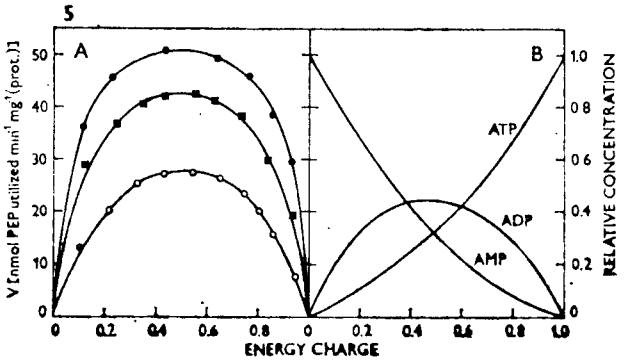

Fig. 5. Effect of energy charge on partially purified pyruvate kinase activity. — A: Mixture of AMP and ATP with appropriate energy charge and total adenylate concentration of 5 mM were incubated with 22 mM $MgCl_2$ and 20 units of rabbit muscle adenylate kinase per cm^3 in 50 mM TES buffer adjusted to pH 7.5 with Tris. Appropriate amounts of these mixtures were added to assay mixtures containing 60 mM Tris/TES buffer pH 7.5, 9 mM $MgCl_2$, 60 mM KCl, 0.1 mM PEP, and 0.2 cm^3 of purified enzyme to give total adenylate concentration of 0.2 mM ○, 1.0 mM ■, 2.0 mM ●, and enzyme activity determined. — B: Calculated relative concentrations of AMP, ADP, and ATP are plotted against energy charge. Calculations were made by assuming an equilibrium constant (([ATP] [AMP])/[ADP]²)) of 0.8 (Atkinson 1968).

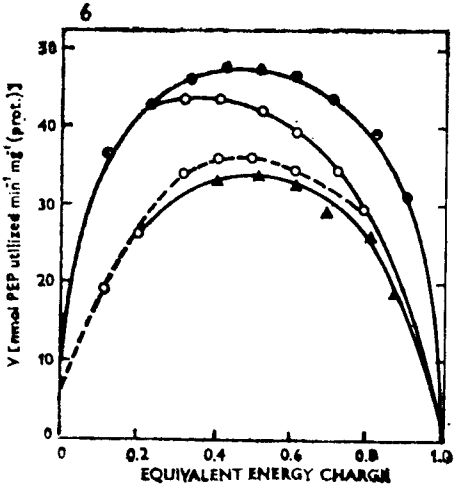

Fig. 6. Factors affecting the energy charge response of partially purified *D. alata* pyruvate kinase. — Methods were as in Fig. 5A, using AMP, ADP and ATP concentrations calculated as in Fig. 5B, assuming total adenylate concentration of 1.0 mM. Addition to the assay mixtures were: — ADP ○ (dashed line), AMP, ADP and ATP ○ (full line), ATP and ADP ▲, ADP and AMP ⊙.

Km and V_{max} computed for ADP (Fig. 3B) are respectively, 70 μM and 50 nmol min^{-1} mg^{-1} protein). ATP inhibition of the enzyme is shown by an increase in Km (77 μM) and a decrease in V_{max} (33 nmol min^{-1} mg^{-1} protein). In the presence of citrate, Km (80 μM) increased while V_{max} (38 nmol min^{-1} mg^{-1} protein) decreased. This indicates that ATP and citrate are mixed-type inhibitors with respect to ADP. The presence of AMP lowered the Km (64 μM) and increased the V_{max} (55 nmol min^{-1} protein).

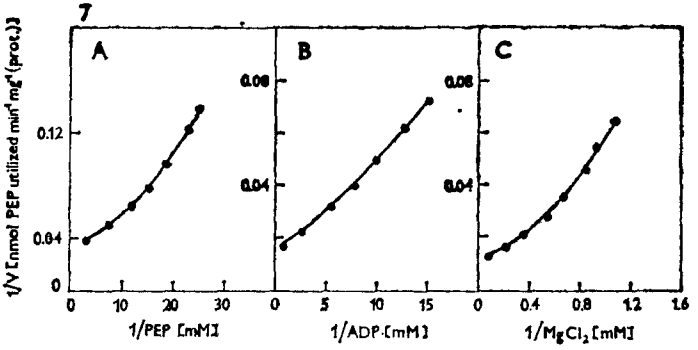

Fig. 7. Kinetics of crude pyruvate kinase from *D. alata* in respect to PEP, ADP and magnesium ions. — Enzyme activity was determined as described under enzyme assay. Assay mixture contained; A: 0.2 mM ADP, 8 mM $MgCl_2$ and PEP; B: 0.1 mM PEP, 8.0 mM $MgCl_2$ and ADP; C: 0.1 mM PEP, 0.2 mM ADP and $MgCl_2$.

Concentrations as indicated. All the data were plotted in double reciprocal form.

Assaying the enzyme activity in the presence of ATP, citrate and AMP did not alter the shape of the curve to sigmoidal (Fig. 3A,B). However, in the absence of citrate, the enzyme showed normal kinetics towards magnesium (Fig. 4) with Km of 5.0 mM, but the presence of 3 mM citrate caused some sigmoidicity (Fig. 4). Fig. 4 also showed the same effect when EDTA was added in place of citrate.

Partially purified enzyme like pyruvate kinase from other sources is inhibited by ATP (Fig. 3). This inhibition coupled with the activation of the enzyme by AMP should cause the enzyme to respond to an energy charge as has been reported for the enzyme from *Azobacter vinelandii* (LIAN and ATKINSON 1971). The effect of an energy charge on *D. alata* pyruvate kinase at three adenylate concentrations is shown in Fig. 5. The concentration of ADP appears to be the main factor determining the shape of the curves. To assess the effect of each nucleotide on the energy charge mixtures on the curves, the composition of energy charge mixtures was calculated and assays were performed using the calculated concentrations of ADP in the presence and absence of each of the two nucleotides. The result is shown in Fig. 6. Clearly ADP concentration determines the general appearance of the curves. AMP activation is evident over most of the curves. However, the effect is most pronounced at low energy charge where the concentration of AMP is the greatest. ATP inhibition is appreciable above an energy charge of 0.3. In the range of 0.7 to 0.95 where most of the cells seem to operate, the two effects compensate for each other.

DISCUSSION

The problem encountered in the study of pyruvate kinase from higher plants is the presence of phosphatase activity in plant extracts which interferes with the assay. Of the yam tissues examined only dormant yam tubers were found to show little or no phosphatase activity under the condition of the experiment. The germinated yam tubers contain substantial phosphatase activity with pH optimum of 5.5. However, pH optimum for yam phosphatase activity is too far removed from that of pyruvate kinase (Fig. 1) for it to cause much interference. Another problem is the low pyruvate kinase content of *D. alata* which necessitated the concentration of the crude extract. The extracts were prepared in the presence of 50 % v/v glycerol since plant pyruvate kinase has been shown to be less stable in the absence of glycerol (DUGGLEBY and DENNIS 1973).

Pyruvate kinase from crude extract of *D. alata* showed some activity but addition of KCl greatly increased the activity. However, the presence of extraneous mono-valent cations such as Na^+ and NH_4^+ made it difficult to determine if mono-valent cation requirement is absolute as it is reported for other eucaryotic pyruvate kinase (MILLER and EVANS 1957, HUNSLEY and SUELTER 1969).

Crude pyruvate kinase showed sigmoid kinetics towards PEP, ADP and magnesium (Fig. 7). However, assaying the enzyme activity after pretreatment with various compounds and under different conditions of pH and temperature failed to change the sigmoid kinetics. It is possible that the deviation from normal kinetics is an artifact of the assay of crude preparation. It is also possible that the enzyme from this source is a regulatory enzyme *in vivo* which has been modified on extraction.

It has been reported that adipose tissue pyruvate kinase showed normal kinetics when extracted using imidazole buffer, but pretreatment with various compounds converted it to a form that showed sigmoid kinetics (POGSON 1968). This regulatory form of the enzyme is characterized by a high ratio between activities observed at high and low PEP concentrations. This criterion was used to assess if the yam pyruvate kinase could be converted from non-regulatory to regulatory form. No change was observed after pretreatment of *D. alata* enzyme with EDTA, ATP, citrate, 2-mercaptoethanol, $MgCl_2$ and alanine. Pyruvate kinase from various sources show regulatory properties only under certain conditions of pH (ROZENGURT *et al.* 1969) and temperature (SOMERO 1969). When the yam enzyme was assayed over a range of pH values from 5 to 8.5 and a range of temperature from 20 °C to 40 °C no marked change in kinetics towards PEP and ADP was noticed.

It has been found in this study that pyruvate kinase from *D. alata* is inhibited by trisodium citrate (Fig. 3). The enzyme in the extracts of cotton seed also showed this inhibition (DUGGLEBY and DENNIS 1973). However, the commercially obtained rabbit muscle pyruvate kinase (Sigma type II) did not show this effect. This citrate inhibition seems to be a property of the yam enzyme and also that of higher plants rather than the artifact of the assay system. Pyruvate kinase from *D. alata* is activated by AMP and inhibited by ATP. No effect was observed on the addition of up to 4 mM fructose-1,6-bisphosphate, a characteristic activator of pyruvate kinase

from several sources (BAILLEY and WALKER 1969, HUNSLEY and SUELTER 1969, LIAN and ATKINSON 1971).

The crude enzyme is inhibited by citrate and ATP and activated by AMP, which indicates that it may possess regulatory properties. After partial purification of the enzyme, these effects of ATP, citrate and AMP were still seen (Fig. 3) but the enzyme showed normal kinetics toward PEP and ADP. The kinetics of partially purified enzyme towards magnesium ions are hyperbolic but the addition of citrate gave a sigmoid saturation curve (Fig. 4). However, a similar effect was seen on addition of EDTA (Fig. 4). Therefore the sigmoid shape indicates magnesium chelation. The shape of the energy charge response curve is typical of enzyme involved in the regulation of the energy-generating pathway (ATKINSON 1968). This is mainly an effect of ADP concentration.

In conversion of fat and other metabolic intermediates into carbohydrate, PEP is the main branch point intermediate that can be utilized for energy generation or gluconeogenesis. Pyruvate kinase should therefore be subject to regulation. The regulation will be expected to be similar to that shown by phosphofructokinase which is inhibited by ATP, citrate and PEP and is activated by phosphate (KELLY and TURNER 1971). It has been shown that regulatory enzymes from ATP-generating sequence are highly active at low levels of energy charge and decrease sharply in activity as the charge increases above the value of 0.75 (ATKINSON 1968, LIAN and ATKINSON 1971). This regulatory pattern in is fact found for pyruvate kinase from *D. alata* (Figs. 5 and 6). The yam enzyme responds sharply to adenylate energy charge with a decrease in activity at high energy charge value (Fig. 5) and increase in activity at low energy charge as expected of a regulatory enzyme of an ATP-generating sequence. However, the enzyme exhibited zero activity at the extremes of the energy charge scale (Figs. 5 and 6). This is because ADP is the substrate of the enzyme and hence the reaction is necessarily zero at the charge value of 0, and 1 where the adenylate pool does not contain ADP.

From the results presented here, it can be concluded that *D. alata* pyruvate kinase is a regulatory enzyme.

REFERENCES

ATKINSON, D. E.: The energy charge of adenylate pool as a regulatory parameter: Interaction with feed back modifier. — Biochemistry **7** : 4030—4034, 1968.

BAILEY, E., WALKER, P. R.: Comparison of properties of pyruvate kinase of the fat body and flight muscle of the adult male desert locust. — Biochem. J. **111** : 359—364, 1969.

BRADFORD, M. M.: A rapid and sensitive method for the quantitation of microgramme quantities of protein utilizing the principle of protein-dye binding. — Anal. Biochem. **72** : 248—254, 1976.

CARMINATTI, H., JIMENEZ DE ASUA, L., LEIDERMAN, B., ROZENGURT, E.: Allosteric properties of skeletal muscle pyruvate kinase. — J. biol. Chem. **246** : 7284—7288, 1971.

CARMINATTI, H., JIMENEZ DE ASUA, L., RECONDO, E., PASSERON, S., ROZENGURT, E.: Some kinetic properties of liver pyruvate kinase (Type L). — J. biol. Chem. **243** : 3051— 3056, 1968.

CORNISH, A. S., JOHNSON, E. J.: Regulation of pyruvate kinase from *Thiobacillus neopolitanus*. — Arch. Biochem. Biophys. **142** : 584—590, 1971.

EVANS, H. J.: Effect of potassium and other univalent cations on activity of pyruvate kinase in *Pisum sativum*. — Plant Physiol. **38** : 397—402, 1963.

DUGGLEBY, R. G., DENNIS, D. T.: The characterization and regulatory properties of pyruvate kinase from cotton seed. — Arch. Biochem. Biophys. **155** : 270—277, 1973.

HUNSLEY, J. R., SUELTER, G. H.: Yeast pyruvate kinase II: Kinetic properties. — J. biol. Chem. **244** : 4819—4822, 1969.

Ibsen, K. H., Schiller, K. W., Hass, T. A.: Interconvertible kinetic and physical forms of human erythrocyte pyruvate kinase. — J. biol. Chem. **246** : 1233—1240, 1971.

Kachmar, J. P., Donald, V. M.: Determination of erythrocyte glucose-6-phosphate dehydrogenase activity. — In: Tiezt, N. W. (ed.): Fundamentals of Clinical Chemistry. Pp. 670—672. Saunders, Philadelphia 1976.

Kelly, G. J., Turner, J. F.: Co-operativity in pea seed phosphofructokinase. — Biochim. biophys. Acta **242** : 559—565, 1971.

Lian, C. I., Atkinson, D. E.: Regulation at the phosphoenolpyruvate branch point in *Azotobacter vinelandii* pyruvate kinase. — J. Bacteriol. **106** : 37—44, 1971.

Miller, C., Evans, H. J.: The influence of salts on pyruvate kinase from tissue of higher plants. — Plant Physiol. **32** : 346—354, 1957.

Mustafa, T., Hochachka, P. W.: Catalytic properties of pyruvate kinase in tissue of marine bivalve. — J. biol. Chem. **246** : 3196—3203, 1971.

Pogson, C. I.: Adipose-tissue pyruvate kinase, properties and interconversion of two active forms. — Biochem. J. **110** : 67—77, 1968.

Pon, N. G., Bondar, R. J. L.: A direct spectrophotometric assay for pyruvate kinase. — Anal. Biochem. **19** : 272—279, 1967.

Rozengurt, E., Jimenez de Asua, L., Carminatti, H.: Some kinetic properties of liver pyruvate kinase (Type L) II, effect of pH on its allosteric behaviour. — J. biol. Chem. **244** : 3142—3147, 1969.

Somero, G. N.: Pyruvate kinase variants of the Alaska king-crab: evidence for a temperature dependent interconversion between two forms having distinct and adoptive kinetic properties. — Biochem. J. **114** : 237—241, 1969.

Turner, J. P., Turner, D. H.: Regulation of glycolysis and pentose phosphate pathway. — In: Stumf, P. K., Conn, E. E. (ed.): Biochemistry of Plants. Vol 2. Pp. 279—316. Academic Press, New York—London 1980.

BOOK REVIEW

Harris, K. F. (ed.): Current Topics in Vector Research, Vol. 3. — Springer Verlag, New York—Berlin—Heidelberg—London—Paris—Tokyo 1987. 263 pp. Hardcover DM 136.—.

The subject of the third volume of Current Topics in Vector Research series (first two volumes were issued in 1983 and 1984 by Praeger Publishers) is divided into 8 independent chapters written by 12 specialists.

The first two chapters are devoted to the ecology of arboviruses and their vectors in Australia and to the ecology of tropical virus disease dengue. The third chapter shows approaches to the protection of cultures against virus diseases transmitted by homopterous insects on the basis of mathematical models of plant and pest population development. In the fourth chapter the system of aphid trapping, its methods and tools are described and importance of this system for the prognosis of outbreaks of economically important viruses on both national and international scales is demonstrated. The fifth chapter is a survey of nepoviruses of the Americas complementing to some extent the reviews of other authors which mostly deal with nepoviruses of Europe. The ecology, epidemiology and control of nepoviruses are included, the questions connected with replication of nepoviruses being discussed in the sixth chapter. The following chapter is devoted to the viruses transmitted mechanically, by nematodes, fungi or insects in the soil, and the last chapter shows the possibilities of virus and MLO detection in plants and vectors using immunoelectronomicroscopic methods, describing the main techniques of ISEM, their use, both advantages and disadvantages. The text of each chapter is, according to its subject, supplied by tables, figures, schemes or photos. All chapters are well written with the use of a reasonable amount of literature sources. The subject index is included.

The survey of the chapters indicates that the subject of the book is rather heterogeneous, analogically to the previous two volumes of the Current Topics in Vector Research series. A certain shortcoming of this series seems to be the fact that the logically connected chapters are included in various volumes of the series issued during four years.

Regardless of this notice the book is valuable and helpful not only to virologists, but also to entomologists, nematodologists and, of course, to specialists in plant protection research.

P. Ryšánek (*Praha*)