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Some Catalytic and Regulatory Properties of Pyruvate Kinase from the Spadix and Retractor Muscles of *Nautilus pompilius*[1]

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ABSTRACT: Pyruvate kinase was partially purified from the spadix and retractor muscles of *Nautilus pompilius*. In both cases, the enzyme was activated by magnesium and potassium ions with similar affinities (apparent K_a values were 0.63 ± 0.04 mM and 5.8 ± 0.4 mM, respectively, for the enzyme from the spadix; and 0.77 ± 0.06 mM and 6.7 ± 0.8 mM, respectively, for the enzyme from the retractor muscle). The enzymes showed normal hyperbolic saturation kinetics for the substrates adenosine 5′-diphosphate and phosphoenolpyruvate, and the apparent K_m values were identical when measured at saturating concentrations of the cosubstrate (apparent K_m values were 0.28 ± 0.01 mM and 0.063 ± 0.005 mM, respectively, for the spadix). Adenosine 5′-triphosphate, alanine, and citrate were found to be inhibitors. The enzyme from the spadix was more susceptible to inhibition by alanine than that from the retractor muscle. For the latter enzyme, inhibition by alanine was noncompetitive with respect to phosphoenolpyruvate, but the inhibition was nonlinear; it also decreased the affinity for Mg^{2+}. For the enzyme from the spadix, inhibition by alanine changed the saturation kinetics for phosphoenolpyruvate to sigmoidal form. The affinity for Mg^{2+} was also decreased by alanine. For both enzymes, fructose-1, 6-bisphosphate at a concentration of 0.05 mM partially reversed the inhibition by alanine, but not that by adenosine 5′-triphosphate. The sigmoidal kinetics observed for phosphoenolpyruvate could also be reversed by increasing the concentration of Mg^{2+}. In general, the properties were found to be similar to those of other pyruvate kinases from the mantle muscle of squid and octopus, except for the observation of inhibition by alanine. These regulatory properties are discussed with respect to potential control of glycolytic flux during muscle activity.

CEPHALOPODS ARE SOME OF the most active marine invertebrates but are among the least studied at a biochemical level. Recently, several workers have shown that energy metabolism in the mantle muscle is based on the catabolism of carbohydrates rather than on the oxidation of fat, since enzymes of the glycolytic pathways are found in high activity whereas those of fatty acid oxidation are low (Ballantyne, Hochachka, and Mommsen 1981; Hochachka et al. 1975). Anaerobic or hypoxic conditions lead to the accumulation of octopine from the glycogen reserves (Greishaber and Gäde 1976; Hochachka, Fields, and Hartline 1977) because octopine dehydrogenase (EC 1.5.1.11) is used to maintain redox balance in the cytosol (Ballantyne et al. 1981; Fields, Baldwin, and Hochachka 1976; Gäde 1980; Robin and van Thoai 1961).

Flux through the glycolytic pathway is controlled at the level of phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40) (Scrutton and Utter 1968; Williamson 1965, 1966). A wide range of metabolites have been shown to affect phosphofructokinase (Mansour 1972, Newsholme 1971, Tornheim and Lowenstein 1976), and the

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physiological implications of these have been discussed by Newsholme and Start (1973). Pyruvate kinase from the muscle of most mammals is inhibited by adenosine 5′-triphosphate (ATP) (Cardenas and Dyson 1973, Tanaka et al. 1967), but on the other hand, the enzyme from the heart of the turtle, the muscle of some fish, and some bivalve mollusks is inhibited by alanine and activated by fructose-1, 6-bisphosphate (FDP) (Bannister and Anastasi 1976, Fields et al. 1978, Holwerda and de Zwaan 1973, Mustafa and Hochachka 1971, Randall and Anderson 1975, Somero and Hochachka 1968, Storey and Hochachka 1974). These properties may be correlated with the necessity to function under prolonged hypoxia (Fields et al. 1978, Hochachka and Somero 1973). Few studies have been performed on the control of glycolysis in cephalopods. Storey and Storey (1978) examined the changes on the concentrations of glycolytic intermediates in the mantle muscle of *Loligo pealeii*, and concluded that phosphofructokinase was rate-limiting. The catalytic properties of phosphofructokinase from the mantle of the squid *Symplectoteuthis oualaniensis* and the cuttlefish *Sepia officinalis* have been determined, and the results indicate that the regulatory properties are similar to those reported for the enzyme isolated from the muscles of a variety of animals (Storey 1981, Storey and Hochachka 1975*a*). Pyruvate kinase has been studied in the muscle of three cephalopods, *Symplectoteuthis oualaniensis*, *Octopus cyanea*, and *Sepia officinalis*. The enzymes were shown to have similar K_m (Michaelis constant) values for adenosine 5′-diphosphate (ADP) and phosphoenolpyruvate (PEP), and to be inhibited by ATP and citrate (Guderley et al. 1976, Storey 1981, Storey and Hochachka 1975*b*). The pyruvate kinase from *Sepia officinalis* was also activated by FDP (Storey 1981). Pyruvate kinase occurs in high activity in the spadix and retractor muscles of the chambered nautilus, being approximately equal to the activity of octopine dehydrogenase (Hochachka, French, and Meredith 1978). This is surprising since in *Symplectoteuthis oualaniensis* and *O. cyanea* the activity of octopine dehydrogenase is much higher than that of pyruvate kinase (Fields et al. 1976, Hochachka et al. 1975). Activities of citrate synthetase in the spadix and retractor muscle suggest that the retractor muscle has a higher aerobic capacity than does the spadix, but the activities of octopine dehydrogenase and arginine kinase are comparable in these tissues (Hochachka et al. 1978). The spadix muscle can function under hypoxic conditions that lead to the accumulation of octopine (Hochachka et al. 1977), indicating a high capacity for anaerobic glycolysis. The high activity of pyruvate kinase in these tissues suggested that the enzyme from the spadix of *Nautilus pompilius* might have some catalytic and regulatory features that differ from those exhibited by pyruvate kinase from the mantle muscle of other cephalopods. This study was undertaken to determine whether the catalytic and regulatory properties of pyruvate kinase in the retractor and spadix muscles of *Nautilus* are comparable to those reported for other cephalopod muscles. The data indicated that the affinities for the substrates were similar and comparable to other cephalopods. Unlike other cephalopods, the enzymes from the spadix and retractor muscles were inhibited by alanine, this inhibition being partially reversed by FDP. Also, the enzyme from the spadix was more susceptible to alanine inhibition than was that from the retractor muscle.

MATERIALS AND METHODS

Chambered nautilus were obtained from local fishermen in the Tanon Strait, Republic of the Philippines, and kept in running seawater on board the R/V *Alpha Helix*. The spadix muscle was dissected from freshly killed animals, frozen, flown to Seattle, and kept frozen at −20°C until required. All biochemicals were purchased from Sigma Chemical Co., St. Louis, Missouri; all other chemicals were of reagent grade.

Enzyme Assay

Pyruvate kinase catalyzes the reaction

$$\text{Phosphoenolpyruvate} + \text{ADP} \rightarrow \text{Pyruvate} + \text{ATP}$$

It was assayed by the method of Bucher and Pfleiderer (1955). Pyruvate formation was followed by coupling with lactate dehydrogenase and measuring the rate of oxidation of reduced nicotinamide adenine dinucleotide (NADH) by decrease in A_{340}. All assays were performed at 25°C with a Varian Superscan 1BE spectrophotometer equipped with a thermostated cell holder. The temperature was maintained by a Haake FK circulating water bath. All assays were performed at 25°C. Standard assay conditions were 4 mM adenosine 5′-diphosphate (ADP), 2 mM phosphoenolpyruvate (PEP), 10 mM $MgSO_4$, 100 mM KCl, 0.2 mM NADH, and 5 units of beef heart lactate dehydrogenase in 100 mM imidazole-HCl, pH 7.0.

Protein Assay

Protein concentration was determined by measuring the optical density at 260 and 280 nm (Layne 1957).

Enzyme Preparation

Small portions of the muscle were thawed and weighed. They were homogenized for 3 min in a Sorval Omnimixer set at maximum speed in 5 vol ice-cold 100 mM Tris-HCl buffer, pH 7.5, containing 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 30,000 × *g* for 20 min, and the pellet was discarded. Ammonium sulfate was slowly added to 40% saturation, and the solution was stirred for 1 hr at 0°C. The suspension was centrifuged as above, and the pellet was discarded. The supernatant was further treated with ammonium sulfate to 60% saturation as above, then centrifuged, and the supernatant was discarded. The pellet was dissolved in 20 mM potassium phosphate, pH 6.5, containing 10 mM 2-mercaptoethanol, dialyzed against 1 liter of the same buffer, and then applied to a 5 × 1.5-cm column of phosphocellulose that had been previously equilibrated with the same buffer. The enzyme was eluted with a linear gradient from 0 to 300 mM KCl in 20 mM potassium phosphate, pH 6.5. It elutes as two peaks, one of which represented about 20% of the total activity and is found in the void volume. The other elutes approximately midway through the gradient. The tubes containing peak activity of the enzyme were pooled, dialyzed against 20 mM potassium phosphate, pH 7.0, containing 10 mM 2-mercaptoethanol, and this partially purified preparation was used for all kinetic studies. No significant difference in the catalytic properties of the two peaks of pyruvate kinase was found, and all the data presented are from the larger peak. The specific activities of these preparations were 30 units/mg protein for the enzyme from the spadix and 12 units/mg protein for the enzyme from the retractor muscle.

Kinetic Analysis

The K_m values were estimated by the method of Wilkinson (1961) or by plotting log $[v/(V - v)]$ versus log S (where v is the initial velocity, V is the maximal velocity, and S is the concentration of substrate). The effects of inhibitors were determined by means of double reciprocal plots; inhibition constants were calculated from these and also by the method of Dixon (1953). All kinetic constants were determined at least twice.

RESULTS AND DISCUSSION

Effects of pH, Cation Requirements, and Substrate Affinities

The pH profile for the enzyme showed an optimum between pH 6.8 and 7.2 when assayed with the standard concentrations of substrates and cofactors. This is similar to the pH optimum of pyruvate kinase from other cephalopods (Guderley et al. 1976, Storey and Hochachka 1975*b*), but lower than that of bivalves (Livingstone and Bayne 1974, Mustafa and Hochachka 1971, de Zwaan and Holwerda 1972).

All previously studied pyruvate kinases have an absolute requirement for a divalent cation that can be met by Mg^{2+} or Mn^{2+}, and they are also activated by K^+ or NH_4^+ (Kayne 1973). Pyruvate kinase from the retractor and spadix adhered to this pattern, with saturation kinetics of K^+ and Mg^{2+} following

hyperbolic patterns. The apparent K_a (activation constant) values for K^+ were 5.8 ± 0.4 mM and 6.7 ± 0.8 mM for the spadix and retractor, respectively; the values for Mg^{2+} were 0.63 ± 0.04 mM and 0.77 ± 0.06 mM, respectively. The enzyme was also activated by Mn^{2+}, but the initial velocities were about one-third those obtained with Mg^{2+}, so Mn^{2+} was not studied further. These results are very similar to those obtained from other cephalopods and other organisms (Guderley et al. 1976; Kayne 1973; Munday, Giles, and Poat 1980; Storey and Hochachka 1975*b*).

The enzymes showed normal Michaelis–Menten saturation kinetics for ADP and phosphoenolpyruvate. The enzyme from the retractor muscle had K_m values for phosphoenolpyruvate and ADP of 0.06 ± 0.006 mM and 0.28 ± 0.01 mM, respectively. These did not change when the concentration of the cosubstrate was altered; a similar situation is found in pyruvate kinase from the muscle of other cephalopods (Guderley et al. 1976, Storey 1981, Storey and Hochachka 1975*b*). In contrast to this, the apparent K_m values for phosphoenolpyruvate and ADP of the enzyme from the spadix did change as the concentration of the cosubstrate was altered; therefore, the K_m values were calculated from replots of the initial velocity data (Cleland 1971). These were 0.074 mM (range 0.065–0.09, five determinations) for phosphoenolpyruvate and 0.33 mM (range 0.27–0.41, five determinations) for ADP. These results suggest that the reaction mechanism for the pyruvate kinase from the spadix is different from the random order binding found in the enzyme from mammalian muscles (Kayne 1973). The data are inadequate to unequivocally determine the reaction mechanism for the spadix enzyme. On the other hand, the data are equivocal for the retractor enzyme, which may or may not have a random binding mechanism. For both enzymes, the K_m values for ADP are similar to those reported for *Sepia officinalis*, *Symplectoteuthis oualaniensis*, and *Octopus cyanea*, but the apparent K_m for phosphoenolpyruvate is lower than that found in *Symplectoteuthis oualaniensis* (0.15 mM) and *O. cyanea* (0.25 mM) (Guderley et al. 1976, Storey and Hochachka 1975*b*), resembling the values reported for pyruvate

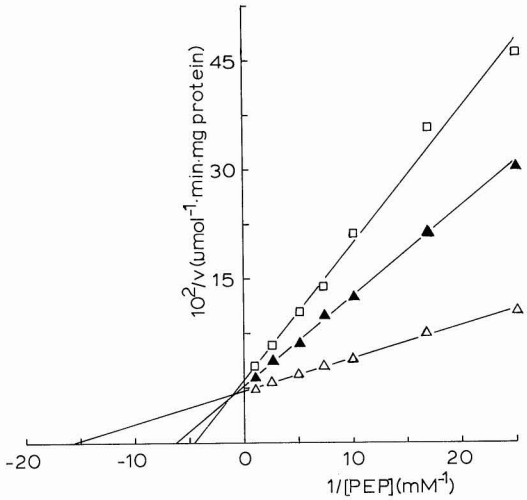

FIGURE 1. Double reciprocal plot showing the pattern of inhibition by ATP with respect to phosphoenolpyruvate (PEP) for pyruvate kinase from the spadix. LEGEND: △, 0 mM MgATP; ▲, 2.5 mM MgATP; □, 5.0 mM MgATP. OTHER CONDITIONS: 2 mM ADP, 10 mM $MgSO_4$, 100 mM KCl, 100 mM imidazole HCl, pH 7.0, 25°C.

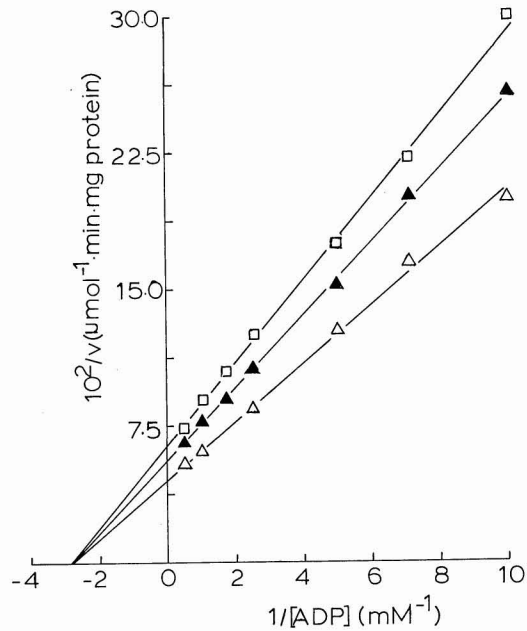

FIGURE 2. Double reciprocal plot showing the pattern of inhibition by ATP with respect to ADP for pyruvate kinase from the spadix. LEGEND: △, 0 mM MgATP; ▲, 2.5 mM MgATP; □, 5.0 mM MgATP. OTHER CONDITIONS: 1.0 mM phosphoenolpyruvate, 10 mM $MgSO_4$, 100 mM KCl, 100 mM imidazole HCl, pH 7.0, 25°C.

kinase from the mantle muscle of *Sepia officinalis* (0.07 mM) (Storey 1981) and from the adductor muscle of bivalves (Holwerda and de Zwaan 1973, Mustafa and Hochachka 1971, de Zwaan and Holwerda 1972).

Regulation by Metabolites

Like other pyruvate kinases, the enzyme was inhibited by MgATP and citrate. Phosphoarginine at a concentration of 20 mM had no effect on the enzymes, unlike pyruvate kinase from the mantle of *Octopus cyanea* and *Sepia officinalis* (Guderley et al. 1976, Storey 1981). In the case of the spadix enzyme, inhibition by ATP was noncompetitive with respect to ADP and competitive with respect to phosphoenolpyruvate (Figures 1, 2), the apparent K_i (inhibition constant) value being 1.5 mM (conditions: 100 mM KCl, 10 mM $MgS0_4$, 2 mM ADP). In the case of the retractor enzyme, the pattern of inhibition by ATP was mixed noncompetitive with respect to both phosphoenolpyruvate and ADP, the apparent K_i value being 3 mM (conditions: 100 mM KCl, 10 mM $MgSO_4$, 2 mM ADP). The inhibition by citrate was noncompetitive with respect to both substrates, but the apparent K_i value was found to be between 20 and 25 mM

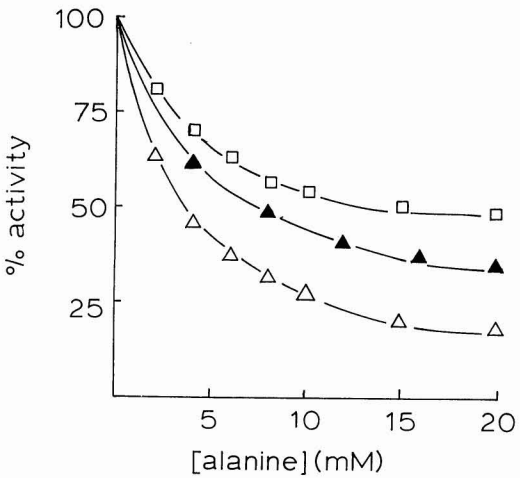

FIGURE 3. Effect of alanine on the activity of pyruvate kinase from the spadix at varying concentrations of $MgSO_4$. LEGEND: ▵, 5 mM $MgSO_4$; ▲, 10 mM $MgSO_4$; □, 15 mM $MgSO_4$. OTHER CONDITIONS: 0.4 mM phosphoenolpyruvate, 2.0 mM ADP, 100 mM KCl, 100 mM imidazole HCl, pH 7.0, 25°C.

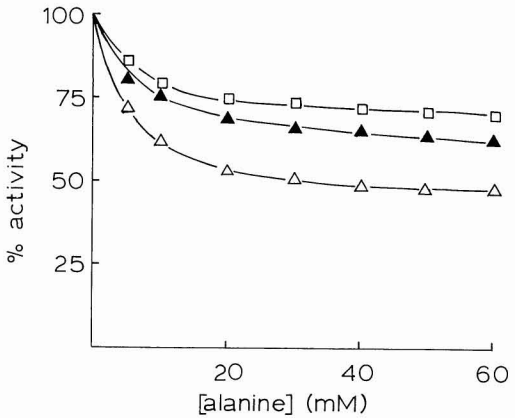

FIGURE 4. Effect of alanine on the activity of pyruvate kinase from the retractor muscle at varying concentration of $MgSO_4$. LEGEND: ▵, 5 mM $MgSO_4$; ▲, 10 mM $MgSO_4$; □, 15 mM $MgSO_4$. OTHER CONDITIONS: 0.4 mM phosphoenolpyruvate, 2.0 mM ADP, 100 mM KCl, 100 mM imidazole HCl, pH 7.0, 25°C.

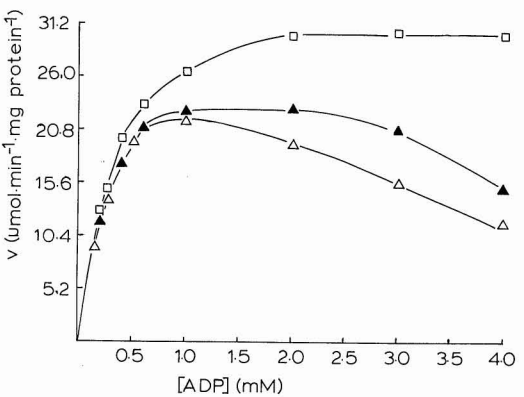

FIGURE 5. Effect of alanine on the saturation kinetics of ADP for pyruvate kinase from the spadix muscle. LEGEND: □, 0 mM alanine; ▲, 10 mM alanine; ▵, 20 mM alanine. OTHER CONDITIONS: 1.0 mM phosphoenolpyruvate, 10 mM $MgSO_4$, 100 mM KCl, 100 mM imidazole HCl, pH 7.0, 25°C.

for both enzymes; therefore, it is unlikely that this has any physiological relevance, and it was not studied further. Both enzymes were inhibited by alanine (Figures 3, 4), with the spadix enzyme being inhibited to a greater extent. It was found that the degree of inhibition by alanine was changed by altering the concentration of Mg^{2+}; inhibition was more pronounced at lower concentrations and more reduced at higher concentrations

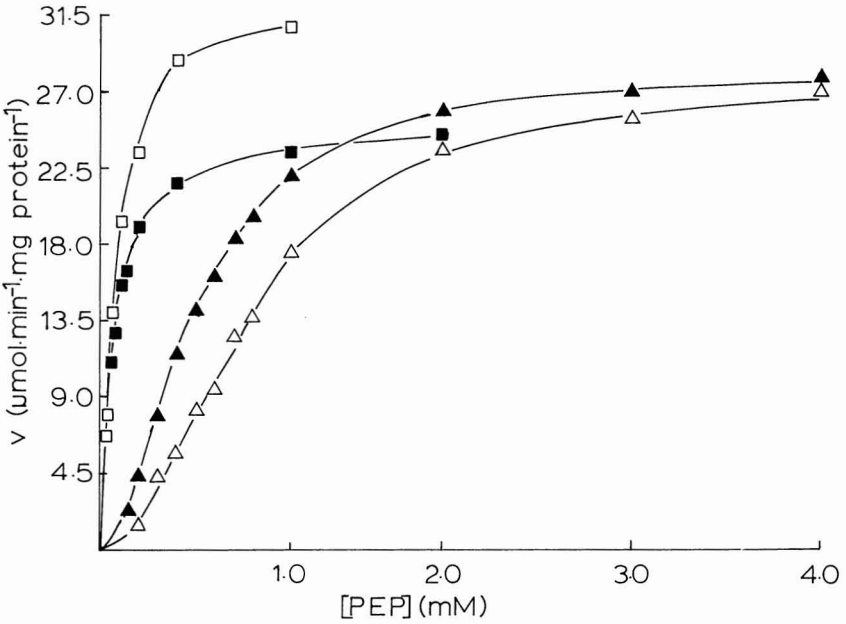

FIGURE 6. Effect of alanine on the saturation kinetics of phosphoenolpyruvate (PEP) for pyruvate kinase from the spadix muscle. LEGEND: □, 0 mM alanine; ▲, 2.5 mM alanine; △, 5.0 mM alanine; ■, 10 mM alanine + 0.05 mM FDP. OTHER CONDITIONS: 2.0 mM ADP, 5 mM $MgSO_4$, 100 mM KCl, 100 mM imidazole HCl, pH 7.0, 25°C.

(Figures 3, 4). In the presence of alanine, the retractor muscle pyruvate kinase displayed normal saturation kinetics for phosphoenolpyruvate, but the saturation kinetics for ADP were anomalous with apparent substrate inhibition at high concentrations of ADP. The spadix enzyme showed similar saturation kinetics for ADP in the presence of alanine, but the saturation kinetics for phosphoenolpyruvate were changed to a sigmoidal pattern (Figures 5, 6). Alanine decreased the apparent affinity for phosphoenolpyruvate of both enzymes; 50 mM alanine increases the apparent K_m about fourfold in the case of the retractor enzyme, and 10 mM alanine increases the apparent $S_{0.5}$ about tenfold in the case of the spadix enzyme (Table 1). Adenosine 5′-diphosphate has been shown to chelate Mg^{2+} (Cumme, Horn, and Achilles 1973); therefore, it is possible that the apparent inhibition by high concentrations of ADP was due to a combination of chelation of free Mg^{2+} by ADP and a decrease in the affinity of the enzyme for Mg^{2+}. As shown in Table 2, alanine profoundly decreases the apparent affinity for Mg^{2+} for both enzymes. The enzyme from the retractor muscle retained normal hyperbolic saturation kinetics in the presence of alanine, but the enzyme from the spadix had sigmoidal saturation kinetics in the presence of alanine (Figure 7). When the data for inhibition by alanine were plotted according to Dixon (1953), it was found that the inhibition was nonlinear; therefore, no inhibition constants for alanine were obtained for the enzymes. Fructose-1, 6-bisphosphate partially reverses the inhibition by alanine (Tables 1, 2), and in the case of the spadix enzyme, it changes the saturation curves for Mg^{2+} and phosphoenolpyruvate to hyperbolic form (Figures 6, 7). In the presence of 40 mM alanine the apparent K_a for FDP was 0.002–0.003 mM for the spadix enzyme (conditions: 2 mM ADP, 0.4 mM phosphoenolpyruvate, 100 mM KCl, 10 mM $MgSO_4$), and the effect was maximal at a concentration of 0.05 mM. In the absence of alanine, FDP had no effect on the enzymes, unlike the pyruvate kinase from *Sepia officinalis* (Storey 1981).

The regulation of pyruvate kinase by

TABLE 1

EFFECTS OF ALANINE AND FDP ON THE AFFINITY FOR PHOSPHOENOLPYRUVATE OF PYRUVATE KINASE FROM THE SPADIX AND RETRACTOR MUSCLES

	RETRACTOR (K_m, mM ± SE)	SPADIX ($S_{0.5}$, mM)	h*
Control	0.060 ± 0.006	0.063	1.0
0.05 mM FDP	0.057 ± 0.009	0.061	1.0
50 mM alanine	0.28 ± 0.02		
50 mM alanine + 0.05 mM FDP	0.046 ± 0.005		
5 mM alanine		0.40	1.6
10 mM alanine		0.49	1.6
10 mM alanine + 0.05 mM FDP		0.066	1.0

CONDITIONS: 2.0 mM ADP, 10 mM $MgSO_4$, 100 mM KCl, 100 mM imidazole HCl, pH 7.0, 25°C.
*Hill constant.

TABLE 2

EFFECTS OF ALANINE AND FDP ON THE AFFINITY FOR Mg^{2+} OF PYRUVATE KINASE FROM THE SPADIX AND RETRACTOR MUSCLES

	RETRACTOR (K_m, mM ± SE)	SPADIX ($S_{0.5}$, mM)	h*
Control	0.77 ± 0.06	0.63	1.0
0.05 mM FDP	0.80 ± 0.08	0.62	1.0
50 mM alanine	3.72 ± 0.17		
50 mM alanine + 0.05 mM FDP	0.69 ± 0.08		
5 mM alanine		5.5	1.7
10 mM alanine		7.8	1.7
10 mM alanine + 0.05 mM FDP		0.78	1.0

CONDITIONS: 2.0 mM ADP, 1.0 mM phosphoenolpyruvate, 100 mM KCl, 100 mM imidazole HCl, pH 7.0, 25°C.
*Hill constant.

alanine and FDP is typical of the enzyme found in vertebrate liver (Cardenas and Dyson 1973, Kayne 1973, Seubert and Schoner 1971, Tanaka et al. 1967), the hepatopancreas of octopus or crustacea (Giles, Poat, and Munday 1976, 1977; Guderley et al. 1976), and the mantle tissue of bivalves (Livingstone and Bayne 1974, Mustafa and Hochachka 1971). In these tissues, inhibition by alanine is considered to play a role in preventing futile cycling at this level during gluconeogenesis. There is no evidence as yet that the muscles of bivalves or cephalopods have any capability for gluconeogenesis as has been recently shown for the skeletal muscles of humans (Hermansen and Vaage 1977), although this possibility cannot be discounted as an explanation for the regulatory properties of pyruvate kinase from the spadix. Another role for alanine inhibition of pyruvate kinase has been proposed in the case of bivalve mollusks. These organisms produce alanine, succinate, and volatile fatty acids during anoxia, and it has been suggested that alanine inhibits pyruvate kinase after the early stage of anoxia, channelling phosphoenolpyruvate toward succinate (Hochachka and Mustafa, 1972; Hochachka, Fields, and Mustafa 1973; de Zwaan, Kluytmans, and Zandee 1976). The available evidence indicates that octopine is the major end product of glycolysis in the spadix (Hochachka et al. 1977) and retractor muscle (Hochachka, unpublished observations). This does not require any special regulatory properties of pyruvate kinase, least of all inhibition of the enzyme, so another explanation must be sought. A third possibility is that this method of regulating pyruvate kinase makes it very responsive to changes in glycolytic flux

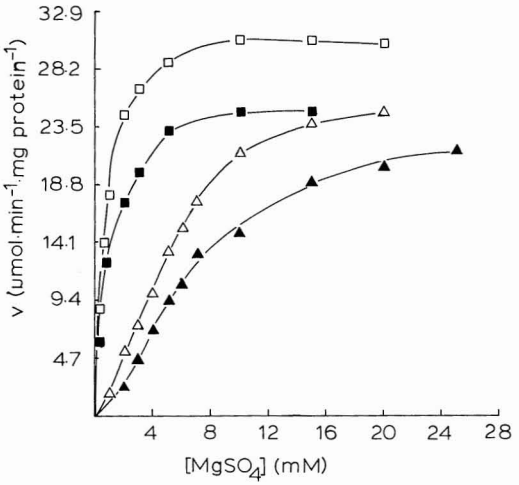

FIGURE 7. Effect of alanine and FDP on the saturation kinetics of $MgSO_4$ for pyruvate kinase from the spadix muscle. LEGEND: □, 0 mM alanine, or 0 mM alanine + 0.05 mM FDP; △, 5 mM alanine; ▲, 10 mM alanine; ■, 10 mM alanine + 0.05 mM FDP. OTHER CONDITIONS: 2.0 mM ADP, 1.0 mM phosphoenolpyruvate, 100 mM KCl, 100 mM imidazole HCl, pH 7.0, 25°C.

mediated by changes in the concentration of FDP (Munday et al. 1980). In this context, the amount of alanine would change little and provide a constant background of low enzyme activity. Any increase in the concentration of FDP that might occur when glycolytic flux increases (Williamson 1966) would activate pyruvate kinase, bringing about a close couple between phosphofructokinase and pyruvate kinase (Hochachka and Somero 1973). In this context, it is interesting to note that the activity of citrate synthetase is higher in the retractor muscle than in the spadix, and the retractor also has a higher mitochondrial content than the spadix (Hochachka et al. 1978). This suggests that the retractor muscle may have a greater capacity for aerobic metabolism, and that the spadix may rely extensively on anaerobic metabolism during activity. Thus, the spadix may experience a greater change in glycolytic flux than the retractor muscle, and this may be correlated with the increased sensitivity to alanine. This may be a partial explanation of the occurrence of the phenomenon of alanine inhibition of pyruvate kinase in the muscle of some vertebrates such as the porpoise (Hochachka and Storey 1975), the turtle (Storey and Hochachka 1974), and various fishes (Bannister and Anastasi 1976, Fields et al. 1978, Randall and Anderson 1975). The turtle and the porpoise are capable of withstanding prolonged anoxic periods in a dive (Hochachka and Storey 1975), but it is uncertain whether all the fish studied could be exposed to prolonged hypoxic stresses or short bursts of high activity dependent on anaerobic metabolism. In all these cases, the thesis of Munday et al. (1980) is a potential explanation of the phenomenon of alanine inhibition of muscle pyruvate kinase.

The effect of alanine on the affinity of the enzyme for Mg^{2+} is intriguing, but little can be said about the physiological relevance of this phenomenon. The concentration of free Mg^{2+} in the spadix is unknown; in frog sartorius and gastrocnemius muscle the free Mg^{2+} has been estimated to be about 0.6 mM (Gupta and Moore 1980), and there is no evidence as yet that the concentration of free Mg^{2+} varies as physiological status is changed (Gupta and Moore 1980). Hence, it is uncertain whether the pyruvate kinase from the spadix or retractor muscle is regulated directly by changes in the concentration of Mg^{2+}. For complete comprehension of the variations of glycolytic control in cephalopods, further studies on the energy metabolism of the muscle will be necessary.

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