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THE PURIFICATION AND KINETIC CHARACTERIZATION OF EEL WHITE MUSCLE PYRUVATE KINASE

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Abstract—1. A stable, homogeneous preparation of pyruvate kinase from white muscle of the American eel, *Anguilla rostrata* with a specific activity of 350 units/mg has been obtained.

2. The enzyme has a pH optimum in the range 6.3–6.5 and requires Mg^{2+} and K^+ for maximum activity.

3. Eel muscle pyruvate kinase exhibits slight co-operativity in the binding of the substrate phosphoenol-pyruvate. It is activated by fructose-1,6-bisphosphate in a pH dependent manner and is inhibited by both alanine and phenylalanine. These properties are very similar to the properties of the mammalian M_2 isozyme.

INTRODUCTION

Pyruvate kinase (ATP:pyruvate 2-*O*-phosphotransferase, EC 2.7.1.40) is an enzyme which has been found to have a variety of tissue and species specific forms. Four isozymes have been found in mammals. Type M_1, found in adult muscle, and the widely distributed type M_2 are structurally related, but the M_2 type is regulated by fructose-1,6-bisphosphate whereas the M_1 type is not (Saheki *et al.*, 1982). The L type, with allosteric properties, is the major form found in liver and has kinetic and structural properties similar to the R type found only in erythrocytes (Hall and Cottam, 1978).

Less information about the structure and properties of pyruvate kinase from the tissues of species other than mammals is available. Isozymes from chicken tissues appear to have similar properties to the isozymes from mammals (Guderley and Cardenas, 1980). However, tissue specific pyruvate kinases from a variety of types of fish have different kinetic properties from the enzymes of corresponding mammalian tissues (Walton and Cowey, 1982). Fish muscle isozymes, unlike the mammalian M_1 enzyme, are activated by fructose-1,6-bisphosphate. The liver enzyme from fish is not activated by fructose-1,6-bisphosphate whereas the mammalian L type isozyme is. Little is known of the structural basis or physiological significance of these differences.

Preliminary studies on an ammonium sulphate fraction have indicated that a pyruvate kinase isozyme from the white muscle of the American eel (*Anguilla rostrata*) has allosteric properties with respect to activation by fructose-1, 6-bisphosphate (Moon and Hulbert, 1980b) as has been observed for the muscle enzyme of other types of fish.

In the present study pyruvate kinase was isolated in an homogeneous, stable form and in high yield from eel white muscle. The effects of pH, monovalent cations, and the amino acids alanine and phenylalanine on the activity of the purified enzyme have been examined. Effects of the presence of the activator, fructose-1,6-bisphosphate, and of the variation of temperature and pH on the affinity for the substrate phosphoenolpyruvate have also been determined. Comparison of these kinetic properties of eel muscle pyruvate kinase determined in this study with the properties of other pyruvate kinases reported in the literature indicate that the eel muscle enzyme has many features in common with the mammalian non-muscle M_2 isozyme.

EXPERIMENTAL PROCEDURES

Materials

The tri-monocyclohexylammonium salt of phospho-enol-pyruvate, the cyclohexylamine salt of the reduced form of β-nicotinamide adenine dinucleotide, the di(cyclohexylammonium) salt of adenosine 5′-diphosphate, the tetracyclohexylammonium salt of D-fructose-1,6-bisphosphate, bovine serum albumin and rabbit muscle lactic dehydrogenase Type V were obtained from Sigma Chemical Co., St. Louis, Missouri. Sephadex G-25 (coarse) and Sephacryl S-300 were products from Pharmacia Fine Chemicals, Montreal, Canada. Whatman DE-32 and CM-23 advanced ion exchange celluloses were obtained from Mandel Scientific Co., Montreal, Canada. The white muscle of mature bronze American eels, *Anguilla rostrata*, which had been captured and maintained as previously described (Moon and Hubert, 1980a), was used as the source of the enzyme.

Analytical procedures

Pyruvate kinase activity was determined using a modification of a coupled assay (Bücher and Pleiderer, 1955). The standard assay contained 2.0 mM ADP, 8 mM $MgCl_2$, 45 mM KCl, 0.15 mM β-NADH, 2–4 units of dialyzed lactic dehydrogenase, 2.5 mM PEP and 50 mM imidazole, pH 6.3 in a cuvette volume of 2.5 ml. Cuvette temperatures were controlled by coupling the jacketed cuvette holder to a Neslab water bath. Assays were conducted at 30°C unless otherwise indicated and initial reaction rates were linear for at least 3 min. One unit of activity is defined as the amount of enzyme which produced 1 μmol of pyruvate per min under these conditions. To determine the effects of various modifiers of pyruvate kinase activity, concentrated enzyme solutions were preincubated with these substances at 4°C for 5 min prior to dilution into the assay buffer containing the modifiers. Double reciprocal plots were used to determine maximum velocities (V_{max}) and substrate or activator concentrations required to achieve one-half maximum velocity (apparent K_m and apparent K_a

respectively). Hill plots were used to determine Hill coefficients. Each kinetic experiment was performed in triplicate with a very small variation in the kinetic parameters obtained (less than 5%).

Protein was measured by a modified Biuret procedure using bovine serum albumin as the protein standard (Itzhaki and Gill, 1964). For solutions of the purified enzyme, protein concentration, expressed in mg/ml, was determined using an absorption coefficient ($A^{0.1\%}_{280nm}$) value of 0.69 cm^{-1}.

Analytical SDS–polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (1970). Electrophoresis was carried out at pH 9.3 on 7.0% (w/v) polyacrylamide gels under denaturing conditions by adding 8 M urea to the separating and stacking gel solutions of Davis (1964). Similarly electrophoresis was carried out at pH 3.8 on 7.0% (w/v) polyacrylamide gels under denaturing conditions by adding 8 M urea to the separating and stacking gel solutions of Williams and Reisfeld (1964). Carboxymethylated pyruvate kinase was dissolved in sample buffer containing 8 M urea before application to the urea gels. Pyruvate kinase was carboxymethylated according to the method of Anderson and Randall (1975). Protein was located on SDS-containing gels by staining with Coomassie Blue and on urea gels by staining with Amido Black.

Enzyme purification

All procedures were carried out at 4°C. Frozen eel white muscle (approx. 500 g) was ground in a meat grinder and extracted for 30 min with two volumes of a solution containing 10 mM potassium phosphate, 2 mM EDTA, pH 5.8, 10% (v/v) glycerol and 0.1 mM DTT. The suspension was then strained through cheesecloth and the muscle was re-extracted with two vol. of 5 mM EDTA buffer, pH 5.8 containing 0.15 M KCl and 0.1 mM DTT. The second suspension was strained through cheesecloth and the two extracts were pooled and strained again. After adjustment of the pH of the extract to pH 5.4 by the dropwise addition of 5 M acetic acid, the extract was centrifuged at 9000 ***g*** for 15 min in a Beckman JA-21 centrifuge.

The supernatant of the pH 5.4 treatment was brought to 40% saturation by the gradual addition of solid $(NH_4)_2SO_4$ (243 g/l of initial solution) and after stirring for 30 min, the solution was centrifuged at 15,000 ***g*** for 15 min. The supernatant was brought to 75% saturation by the addition of solid $(NH_4)_2SO_4$ (245 g/l of supernatant) and after 30 min of stirring of the solution was centrifuged at 15,000 ***g*** for 15 min. The precipitate was dissolved in 15 ml of 5 mM potassium phosphate buffer, pH 5.8 containing 30% (v/v) glycerol and 0.1 mM DTT (Buffer A) and desalted by passage through a column (5 cm × 80 cm) of Sephadex G-25 equilibrated with this buffer.

CM-23 cellulose, equilibrated with Buffer A, was added batchwise to the protein solution (approx. 650 ml). After each addition of CM-23 cellulose (approx. 20 ml of swollen ion exchanger per addition), the solution was stirred and the ion exchanger was allowed to settle before the supernatant fraction was assayed for pyruvate kinase activity. The procedure was stopped when the pyruvate kinase activity in the supernatant fraction was reduced to 95% of the initial activity observed prior to the addition of the ion exchanger. The mixture was centrifuged at 9000 ***g*** for 15 min and the supernatant fraction containing the pyruvate kinase activity was collected. The CM-23 cellulose was washed with an equal volume of Buffer A and the washings were combined with the supernatant fraction. After the pH was adjusted to pH 7.8 by the dropwise addition of 5 M KOH, the solution was applied to a column (2.5 cm × 40 cm) of DE-32 cellulose equilibrated with 5 mM potassium phosphate buffer, pH 7.8 containing 30% (v/v) glycerol and 0.1 mM DTT (Buffer B). The column was washed with two volumes of Buffer B and then with two volumes of Buffer B containing 0.04 M KCl. The pyruvate kinase activity was eluted with a linear gradient formed from 400 ml of Buffer B containing 0.04 M KCl and 400 ml of Buffer B containing 0.12 M KCl. Pyruvate kinase activity was eluted as a single peak. The fractions containing this activity were concentrated by ultrafiltration in an Amicon cell with an XM-50 membrane.

Ten millilitre portions of the concentrated DE-32 cellulose eluate containing approx. 100 mg of protein were applied to a column (2.5 cm × 80 cm) of Sephacryl S-300 equilibrated with 50 mM potassium phosphate buffer, pH 7.5 containing 30% (v/v) glycerol and 0.1 mM DTT. The column was developed with this buffer and the fractions containing pyruvate kinase specific activities greater than or equal to 320 units/mg were pooled and stored in this buffer at 4°C.

RESULTS

A typical preparation of pyruvate kinase from eel white muscle is summarized in Table 1. An overall purification of twenty-fold was routinely obtained. The major step contributing to this purification was chromatography on DE-32 cellulose carried out as illustrated in Fig. 1. In this step and during subsequent chromatography on Sephacryl S-300, it was necessary to include 30% (v/v) glycerol to the buffers to prevent loss of activity. The purified enzyme gave one band accounting for 95% of the protein when electrophoresis was carried out under reducing and non-reducing conditions on SDS–polyacrylamide gels. Electrophoresis on polyacrylamide gels under anionic and cationic conditions in the presence of 8 M urea also indicated that one polypeptide component accounted for at least 95% of the protein.

Kinetic properties of the purified enzyme were investigated at 30°C in order to compare these results to those of other pyruvate kinase isozymes. Figure 2 shows the effect of pH on enzyme activity. The enzyme was more active in phosphate buffer than it was in the two other buffers examined, but in all cases a rather broad profile was obtained with the maximum activity occurring between pH 6.0 and pH 6.5. At pH 6.3 and 30°C, the maximum velocity exhibited by eel pyruvate kinase was constant for a range of imidazole–HCl concentrations between 0.025 and 0.250 M. Therefore, 0.05 M imidazole–HCl was em-

Table 1. Summary of the purification of pyruvate kinase from 500 g of eel white muscle. Enzyme and protein assays were carried out as described in the text

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)
1. Supernatant of pH 5.4 treatment	16,200	280,000	17.2	100
2. 40–75% saturation $(NH_4)_2SO_4$ fraction	9050	239,000	26.4	85
3. CM–cellulose supernatant	5900	203,000	34.4	73
4. DEAE–cellulose eluate	320	90,000	281	32
5. Sephacryl S-300 eluate	180	63,000	350	23

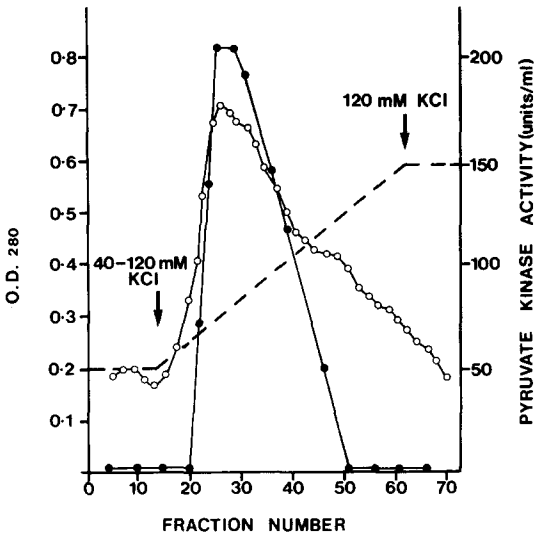

Fig. 1. DEAE-cellulose chromatography of eel white muscle pyruvate kinase. Partially purified enzyme was applied to the column and eluted using a KCl gradient as described in the text. The 16.5 ml fractions containing the pyruvate kinase activity were pooled for further purification.

ployed as the assay buffer for the subsequent kinetic experiments.

Apparent K_a values and the effects on V_{max} of four monovalent cations K^+, NH_4^+, Na^+ and Li^+ were determined at 30°C in 0.05 M imidazole–HCl, pH 6.3. Although the K_a for both K^+ (4.5 mM and NH_4 (4.9 mM)) was similar, the V_{max} of the enzyme was 1.4 times as great in the presence of saturating concentrations of K^+. The enzyme has a much lower affinity for Na^+ and Li^+ and these cations only activate the enzyme to a small degree. Under the same conditions and at saturating levels of K^+, the apparent K_a for Mg^{2+} was determined to be

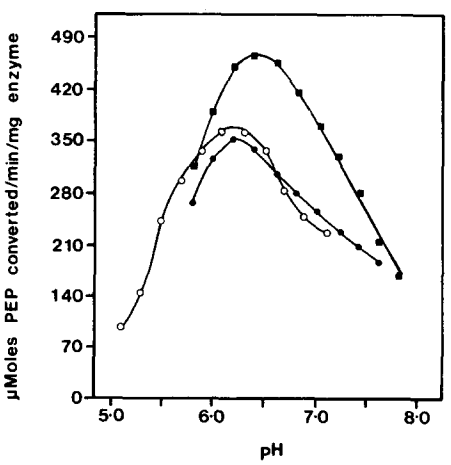

Fig. 2. Effect of pH on the activity of purified eel white muscle pyruvate kinase. The enzyme was assayed at 30°C in the presence of 50 mM buffer containing 2.5 mM PEP, 2.0 mM ADP, 8.0 mM $MgCl_2$, 45 mM KCl, 0.15 mM-β NADH and 3 units of dialyzed lactic dehydrogenase. ○——○, MES (2[N-Morpholino] ethanesulphonic acid); ●——●, imidazole; and ■——■, potassium phosphate.

Table 2. Effect of pH and FDP on the affinity of eel pyruvate kinase for PEP. The assays were carried out at 30°C in the presence of 2.0 mM ADP, 8.0 mM $MgCl_2$, 45 mM KCl, 0.15 mM β-NADH and 3 units of dialyzed lactic dehydrogenase. At pH 6.3 and pH 7.2 the buffer used was 50 mM imidazole–HCl. At pH 8.0, the buffer was 50 mM Tris–HCl. The concentration of FDP when present was 0.5 mM. Apparent K_m and V_{max} values were calculated from Lineweaver–Burk plots

pH	FDP	Apparent K_m PEP (mM)	V_{max}
6.3	–	0.09	350
	+	0.06	350
7.2	–	0.28	220
	+	0.10	355
8.0	–	0.66	85
	+	0.33	163

0.84 mM. The concentration of Mg^{2+} reported here represents the total Mg^{2+} in the assay medium and therefore includes Mg^{2+} bound to PEP, ADP and fructose-1,6-bisphosphate as well as free Mg^{2+}. For all the kinetic experiments performed, the calculated free Mg^{2+} concentration was saturating and non-inhibitory.

The affinity of the enzyme for the substrate PEP at saturating levels of Mg^{2+} and K^+ and at 30°C was investigated as a function of hydrogen ion concentration in the presence and absence of FDP. In Table 2 it can be seen that in the absence of FDP the apparent K_m for PEP increases with increasing pH and that the V_{max} decreases. When FDP is present, the effects of increasing pH on the apparent K_m and the V_{max} are moderated. The effects of hydrogen ion concentration on kinetic parameters of eel muscle pyruvate kinase are complex. Lineweaver–Burk plots generated from data obtained at pH 6.3 in the presence and absence of FDP and at pH 7.2 and 8.0 in the presence of FDP were linear. However, at higher pH values, biphasic Lineweaver–Burk plots and Hill plots were generated from data obtained in the absence of FDP. Figure 3 illustrates the plots of data obtained at pH 8.0 in the presence and absence of FDP. The apparent K_m value given in Table 2 in the absence of FDP was calculated from data obtained at low concentrations of PEP. At higher concentrations, a second apparent K_m of 2.40 mM was calculated. Two Hill coefficients (n_H) could also be calculated from the data. For PEP concentrations less than 0.5 mM an n_H of 1.0 was calculated while at PEP concentrations greater than 1.5 mM a value of 1.4 was calculated. Similarly, at pH 7.2 there was an increase in the Hill coefficient from 1.47 to 2.40 at high substrate concentrations in the absence of FDP.

At higher pH values the affinity of the enzyme for PEP also depended upon the temperature. At pH 7.2, the apparent K_m for PEP in the absence of FDP was 0.12 mM at 18°C compared to 0.28 mM at 30°C. In the presence of FDP it was also decreased to 0.06 mM at 18°C compared to 0.10 mM at 30°C. However, at pH 6.3, the apparent K_m for PEP was constant over the temperature range 13°–30°C in the presence and absence of FDP.

At the pH optimum of 6.3 and at 30°C no effect of the substrates ADP and PEP on the binding of the other to the enzyme was observed. The apparent K_m for ADP was 0.35 mM in a range of PEP concen-

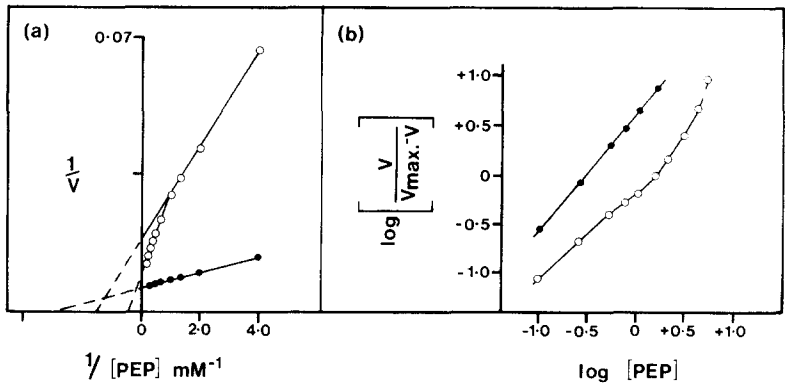

Fig. 3. Lineweaver–Burk (A) and Hill plots (B) of the data obtained from measuring initial velocity as a function of PEP concentration at pH 8.0 and 30°C using purified eel muscle pyruvate kinase. ○——○, in the absence of FDP; ●——●, in the presence of 0.5 mM FDP.

trations from 0.125 mM to 2.5 mM. Similarly, the apparent K_m for PEP of 0.09 mM was constant between ADP concentrations of 0.1 to 2.0 mM.

Alanine and phenylalanine have been shown to inhibit a variety of pyruvate kinase isozymes and the effects of these amino acids on the activity of the eel muscle enzyme were therefore examined. At pH 7.2 and 30°C, both amino acids inhibit the enzyme at low concentrations of PEP. When the concentration of PEP was 0.25 mM, the activity of the enzyme decreased to 50% of the activity of a control in the presence of 4 mM alanine and to 10% of the activity of a control in the presence of 4 mM phenylalanine. Figure 4 shows the effect of PEP and FDP on the phenylalanine inhibition. The presence of phenylalanine decreases the affinity of the enzyme for PEP. At a phenylalanine concentration of 2 mM the apparent K_m for PEP increased from 0.28 mM to 0.67 mM with no effect on V_{max}. When FDP was present, 2 mM phenylalanine increased the apparent K_m for PEP from 0.10 mM to 0.23 mM and the V_{max} decreased by 25%.

DISCUSSION

The purification procedure described allows the preparation of pyruvate kinase from the white muscle of the American eel in pure form, as judged by polyacrylamide gel electrophoresis. The yield is high and the preparation is stable in glycerol. When 30% (v/v) glycerol was present the purified enzyme was stable at 4°C for at least 6 months. The specific activity was comparable to that of other pyruvate kinase preparations from skeletal muscle (Hall and Cottam, 1978).

The activity of the purified enzyme was found to be maximal between pH 6.0 and pH 6.5 in three different buffers. A previous study of a partially purified pyruvate kinase from eel white muscle indicated a pH optimum close to pH 6.0 and a considerably narrower activity as a function of pH profile (Moon and Hulbert, 1980a). However, the very low specific activity of the enzyme used in these studies suggests that it may have undergone considerable denaturation which could have altered its kinetic properties. A pH optimum in the range 6.0 to 6.5 is considerably lower than that observed for the enzyme isolated from the muscle of a variety of mammalian species (Cardenas *et al.*, 1973; Baranowska and Baranowski, 1975; Berglund and Humble, 1979) and the river sturgeon (Randall and Anderson, 1975). However, it is similar to the values obtained for the enzyme isolated from the muscle of several fish species (Johnston, 1975; Fields *et al.*, 1978; Guppy and Hochachka, 1979) and for some mammalian liver (Hall and Cottam, 1978) and M_2-type pyruvate kinases (Schering *et al.*, 1982). It therefore seems clear that the pH optimum of pyruvate kinase isozymes is not constant for a given tissue or organ, but varies with the requirements of individual species.

The effects of activators, pH, temperature and inhibitors on substrate affinities and maximum activities exhibited by purified eel white muscle pyruvate kinase are complex. Of the four monovalent cations tested, K^+ is the best activator of the enzyme fol-

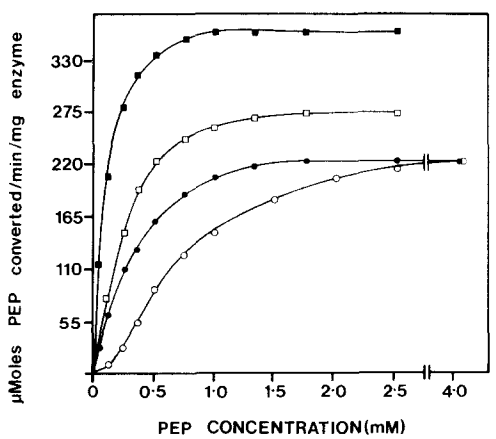

Fig. 4. The effect of phenylalanine on the affinity of purified eel muscle pyruvate kinase for PEP. ●——●, in the absence of FDP and phenylalanine; ○——○, in the absence of FDP and the presence of 2.0 mM phenylalanine; □——□, in the presence of 0.5 mM FDP and 2.0 mM phenylalanine; ■——■, in the presence of 0.5 mM FDP and the absence of phenylalanine.

lowed by NH_4^+ then Na^+ and lastly Li^+. This is similar to the manner in which rabbit muscle pyruvate kinase responds to monovalent cation activators (Kayne, 1971).

Eel pyruvate kinase is activated by fructose-1,6-bisphosphate in a complex and pH-dependent manner. At all pH values examined, the affinity of the enzyme for the substrate PEP was increased in the presence of FDP, but an increase in the V_{max} due to the presence of FDP was only observed at pH values greater than 6.3. This was particularly evident at pH 8.0 where the enzyme exhibited two apparent K_m values for PEP in the absence of FDP but in its presence only the lower K_m value was observed and there was a 1.9-fold increase in the maximum velocity. An identical finding was reported for the M_2 isozyme but not the M_1 isozyme of the pig (Berglund and Humble, 1979). In some cases, the biphasic nature of a Lineweaver–Burk plot can be attributed to the phenomenon of negative co-operativity (Dixon and Webb, 1979). However in this instance, Hill plots of the data obtained at pH 8.0 indicated that the enzyme exhibited no cooperativity, either negative or positive, in binding the substrate PEP for concentrations less than 0.5 mM while slightly positive cooperative binding kinetics prevailed for PEP concentrations greater than 1.0 mM. Similar complex Hill plots exhibiting inflection points between two linear sections have been described for the M_2 and L type pyruvate kinases of rat liver (Van Berkel *et al.*, 1973, 1977; Van Berkel, 1974). Such kinetic behavior can be attributed to the presence of 2 kinetically different forms of the same enzyme which exhibit different affinities for the substrate PEP.

In mammals, pyruvate kinase isozymes can be distinguished on the basis of their affinities for the substrate PEP and their response to FDP at saturating concentrations of ADP, K^+ and Mg^{2+}. The enzyme from skeletal muscle shows hyperbolic binding kinetics and is not influenced by the presence of FDP (Hall and Cottam, 1978). The enzyme from liver shows that pronounced co-operative effects in the binding of PEP and FDP greatly increases the affinity of the enzyme for this substrate (Hall and Cottam, 1978). The binding of PEP to M_2-type isozymes is much less co-operative than is the binding of this substrate to the mammalian liver isozyme, however it is facilitated by the presence of FDP and the effect of FDP increases with increasing pH (Van Berkel *et al.*, 1973; Berglund and Humble, 1979; Ibsen *et al.*, 1981). Therefore with respect to its affinity for PEP and its response to FDP, pyruvate kinase from eel muscle most resembles the mammalian M_2-type isozyme. Comparison with data obtained for other species indicates that, with the exception of birds and mammals, most muscle pyruvate kinases exhibit increased affinities for the substrate PEP in the presence of FDP (Zammit *et al.*, 1978). Although there is considerable variation in the details of this process between species, this observation suggests that the more primitive forms of the enzyme are FDP-sensitive and the development of FDP-insensitivity represents a more recently evolved property of the muscle enzyme.

In common with what has been reported for other poikilothermic animals (Somero and Hochachka, 1968; Randall and Anderson, 1975; Guppy and Hockachka, 1979), the apparent K_m for PEP of eel pyruvate kinase was found to decrease with decreasing temperature, although the effect was pH -dependent. This is in contrast to a previous report using a partially purified eel white muscle pyruvate kinase in which the apparent K_m for PEP was observed to increase with decreasing temperature (Moon and Hulbert, 1980a). The low specific activity of this preparation or its contamination by impurities may account for the discrepancy.

Eel pyruvate kinases behaves in a fashion more similar to mammalian isozymes than to other fish pyruvate kinases with respect to its affinity for one substrate in the presence of varying concentrations of the second substrate. The kinetic data obtained in the present study is consistent with random substrate binding for ADP and PEP as has been reported for mammalian M_1 (Ainsworth and Macfarlane, 1973) and M_2-type pyruvate kinases (Jiménez de Asua *et al.*, 1971; Walker and Potter, 1973). In contrast, it has been reported that decreasing the concentration of one substrate increases the affinity of the muscle enzyme of several types of fish for the second substrate (Fields *et al.*, 1978; Guderley and Cardenas, 1980).

Alanine and phenylalanine both inhibited pyruvate kinase from eel white muscle and the effect is only partially reversed by the addition of FDP. The effects of amino acids on pyruvate kinase isozymes from other species are variable. For example, alanine inhibits L-type pyruvate kinase from rats (Imamura *et al.*, 1972) but has no effect on the bovine liver isozyme (Cardenas *et al.*, 1975). Pyruvate kinase from frog skeletal muscle is not inhibited by alanine or phenylalanine but the enzyme from frog heart is inhibited by both amino acids (Flanders *et al.*, 1971). Only the mammalian M_2-type isozyme has consistently been shown to be sensitive to inhibition by both alanine and phenylalanine and this inhibition is only partially reversed by the presence of FDP (Schering *et al.*, 1982).

The purified enzyme from eel muscle therefore has kinetic properties similar to mammalian M_2 pyruvate kinase with respect to pH dependent FDP activation and inhibition by the amino acids alanine and phenylalanine. This is presumably reflected in structural similarities between the eel muscle enzyme and the mammalian M_2 isozyme. At present, the mammalian M_1 and M_2 isozymes are thought to be the products of a single gene from which two distinct mRNA molecules can be made (Hance *et al.*, 1982; Noguchi and Tanaka, 1982). The mechanism, structural consequences and physiological role of this process is uncertain. Comparison of the structural and kinetic properties of these isozymes with those of other species may be expected to be of value in determining the origin and function of pyruvate kinase isozymes. Because it can be purified in high yield and, although a muscle enzyme, has kinetic properties similar to the mammalian M_2 isozyme, studies of the structure and function of pyruvate kinase from eel white may be particularly useful in this regard.

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