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FUNCTIONAL CHANGES ASSOCIATED WITH THE SEQUENTIAL TRANSFORMATION OF L_4' INTO L_4 PYRUVATE KINASE

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Summary

The functional changes, associated with the sequential transformation of L_4' into L_4 pyruvate kinase (ATP:pyruvate 2-*O*-phosphotransferase, EC 2.7.1.40) were studied. L_4' enzyme from human erythrocytes shows strong hysteretic behaviour: the initial rate of the enzyme preincubated with an unsaturating concentration of phospho*enol*pyruvate is much higher than of the enzyme preincubated with ADP, at the same phospho*enol*pyruvate concentration, although the 'final activity' (the activity of the linear part of the reaction progress curve) was the same in both cases. This phenomenon was observed both in the presence and absence of fructose 1,6-diphosphate. High concentrations of both Mg^{2+}_{free} and $MgATP^{2-}$ diminish the difference in initial rate, between the ADP and phospho*enol*pyruvate preincubated enzymes: Mg^{2+}_{free} by stabilizing the phospho*enol*pyruvate-induced form; $ATPMg^{2-}$ by stabilizing the ADP-induced form. The magnitude of the difference in initial rates of the ADP- or phospho*enol*pyruvate-preincubated enzyme is a function of both substrates. L_4 pyruvate kinase(either from human liver or trypsin treated L_4' enzyme) does not, or to a very slight extent, show such behaviour. $L_2'L_2$ pyruvate kinase shows behaviour intermediate between L_4' and L_4 enzymes.

A model is proposed to describe the kinetic behaviour of L_4' and L_4 enzymes.

Introduction

In previous work [1,2], the 'sequential transformation hypothesis' for the L-type pyruvate kinase (ATP:pyruvate 2-*O*-phosphotransferase, EC 2.7.1.40) forms in man was proposed: the L-type subunit is synthesized as an active precursor L', which can be proteolytically degraded into L. In the liver

proteolytic degradation would be rapid and only the homotetramer of L, L_4, is found. The major form in erythroblasts and reticulocytes is L_4'. Maturation of the cell is accompanied by the appearance of $L_2'L_2$, as the major form in the old erythrocyte. L_4' shows Michaelis-Menten kinetics, the transformation into L_4 causes the appearance of positive homotropic interactions and an increase in affinity for phospho*enol*pyruvate.

We proposed hysteretic behaviour for L_4' pyruvate kinase: the enzyme would exist in at least three interconvertible forms: R, T and T'. The R form would be stabilized by phospho*enol*pyruvate, the T form by ADP. T' would arise from T by a slow phospho*enol*pyruvate-induced process (hysteretic response). T' would have a higher affinity for phospho*enol*pyruvate than T and an equal affinity for ADP (unpublished data). This model is partly consistent with the data of Badwey and Westhead [3], who showed that phospho*enol*pyruvate induces a slow interconversion of human erythrocyte pyruvate kinase to a more active form.

In this work we investigate the functional changes, in terms of hysteretic behaviour and affinity for substrates and ligands, associated with the sequential transformation of L_4' into L_4 pyruvate kinase.

Materials and Methods

Phospho*enol*pyruvate (tricyclohexylammonium salt); ADP (disodium salt); ATP (disodium salt); NADH (disodium salt) and lactate dehydrogenase (rabbit muscle) were purchased from Boehringer, Mannheim, F.R.G. Bovine serum albumin was from Sigma, St. Louis, U.S.A. Trypsin-TCPK was from Merck, F.R.G. All reagents were of the highest purity available.

L_4' and $L_2'L_2$ pyruvate kinase were purified from fresh human erythrocytes [1], L_4 pyruvate kinase from human liver, taken within 12 h after death [4], as described previously. Specific activities for L_4' and $L_2'L_2$ were 15 and 200 IU/mg, and 360 IU/mg for L_4. Enzymes were stored at $(NH_4)_2SO_4$ precipitates at −80° C. For kinetic studies, enzymes were dissolved in 0.01 M Tris-HCl, pH 8.0 (25° C); 0.05 M KCl; 1 mM $MgCl_2$; 1 mM dithiothreitol; 0.1 mM diisopropylphosphofluoridate; 2 mM ϵ-aminocaproic acid; 0.5 M sucrose and 1 mg/ml bovine serum albumin. Enzymes were dialysed extensively against this buffer (not containing albumin) at 4° C, and diluted to approx. 0.1 IU/ml before use in kinetic experiments. Enzyme activity was measured at 25° C in the coupled lactate dehydrogenase assay, as described by Bücher and Pfleiderer [5]. The reaction mixture contained in a final volume of 1 ml: 0.01 M Tris-HCl, pH 8.0, at 25° C; 0.05 M KCl; 1 mM dithiothreitol; 0.1 mM NADH and 10 IU lactate dehydrogenase, dialysed against the buffer mentioned above. Upless otherwise indicated in the text, the Mg^{2+}_{free} concentration was kept at 1 mM with $MgCl_2$, using a equilibrium constant of $K = 5.5 \cdot 10^{-3}$ M for the Mg-PEP$^-$ complex (PEP, phospho*enol*pyruvate) [6], $K = 3.39 \cdot 10^{-4}$ M for the MgADP$^-$ complex [7] and $K = 2.51 \cdot 10^{-5}$ M for the $MgATP^{2-}$ complex [7]. Enzyme was preincubated in the cuvet for 5 min, containing one of both substrates, and the reaction was initiated with the addition of the other one. Decrease in absorbance at 340 nm was recorded on a Beckman Acta recording spectrophotometer. Initial activity was calculated by drawing the asymptote through

the origin of the reaction progress curve on the chart. 'Final activity' is calculated from the linear phase of the reaction progress curve. Final activities were only calculated when less than 5% of the substrates had been converted. For this reason, enzyme activities as low as 10^{-5} IU/ml were sometimes used. The affinity for phospho*enol*pyruvate was determined with ADP at 3 mM and with phospho*enol*pyruvate concentration ranging from 0.1 to 10 mM in the absence of fructose 1,6-bisphosphate and 0.02—10.0 mM in the presence of this compound (0.1 mM). The affinity for ADP was determined with ADP concentrations in the range of 0.1—3.0 mM, and phospho*enol*pyruvate at 4 mM.

Results

Initial observations

L_4' pyruvate kinase from human erythrocytes showed different initial rates, when the enzyme was preincubated with either phospho*enol*pyruvate or ADP. Upon preincubation of the enzyme with phospho*enol*pyruvate at an unsaturating concentration (1 mM), and initiating the reaction with ADP at a saturating concentration (3 mM), the reaction progress curve showed initially a rapid decrease in A_{340}, followed by a lower linear decrease (which we refer to as 'final activity'). On the other hand, the enzyme preincubated with ADP (3 mM) showed initially a slow decrease in A_{340}, followed by a more rapid linear decrease ('final activity'). Final activities were always the same for enzymes preincubated with either ADP or phospho*enol*pyruvate, although

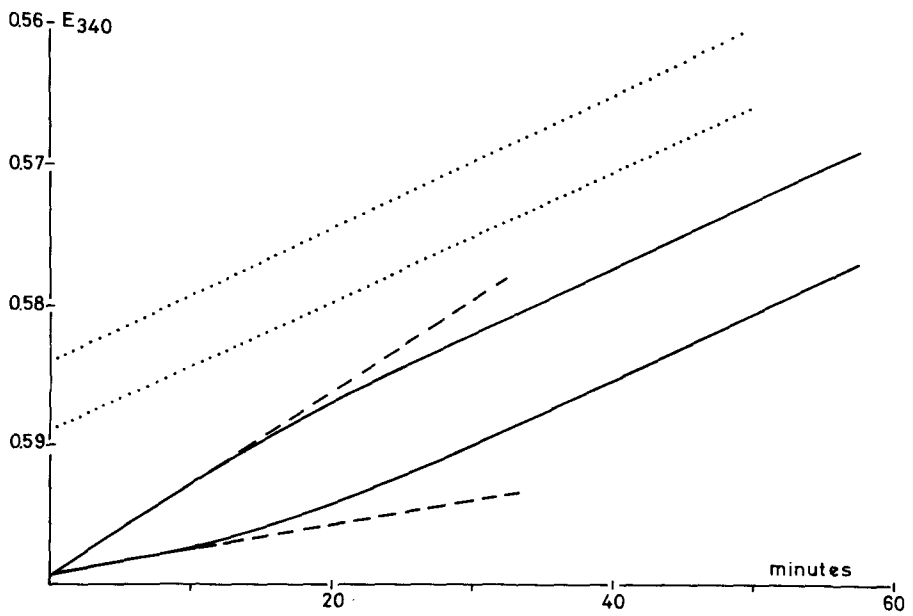

Fig. 1. Reaction progress curves of L_4' and L_4 pyruvate kinase. Upper drawn line: L_4' enzyme preincubated with phospho*enol*pyruvate (1.0 mM); reaction initiated with ADP (3.0 mM). Lower drawn line: L_4' enzyme preincubated with ADP (3 mM); reaction initiated with phospho*enol*pyruvate (1.0 mM). Upper stippled line: L_4 enzyme preincubated with phospho*enol*pyruvate (1.0 mM); reaction initiated with ADP (3.0 mM). Lower stippled line: L_4 enzyme preincubated with ADP (0.3 mM); reaction initiated with phospho*enol*pyruvate (1.0 mM).

TABLE I

RELATIVE INITIAL RATES (%)

The activities of the enzymes, preincubated with the highest phospho-enolpyruvate (PEP) concentration (1 mM in the absence of fructose-1,6-P_2 (FDP); 0.1 mM in the presence (0.1 mM of this compound), in the absence of $MgATP^{2-}$ are taken as the 100% value. Unless otherwise indicated, the values are averages of at least three determinations. $L_4(T)$ is obtained by tryptic treatment of L_4' enzyme.

	Preincubated without $MgATP^{2-}$				Preincubated in the presence of 5 mM $MgATP^{2-}$		
	L_4'	$L_2'L_2$	L_4	$L_4(T)$	L_4'	$L_2'L_2$	L_4
Preincubated PEP 1.0 mM start ADP 3.0 mM	100	100	100	100	43	50	62
Preincubated ADP 3.0 mM start PEP 1.0 mM	23 (29—19, n = 8)	42 (52—37, n = 6)	84 (91—73, n = 5)	100	8	29	58
Preincubated PEP 0.2 mM start ADP 3.0 mM	18	37	39	31	3	5	4
Preincubated ADP 3.0 mM start PEP 0.2 mM	3	11	33	26	4	4	2
Preincubated PEP 0.1 mM, FDP 0.1 mM start ADP 3.0 mM	100	100	100	100	10	28	87
Preincubated ADP 3.0 mM, FDP 0.1 mM start PEP 0.1 mM	16 (31—7, n = 5)	42 (49—41, n = 4)	95 (100—91, n = 5)	101	7	27	87
Preincubated PEP 0.01 mM, FDP 0.1 mM start ADP 3.0 mM	4	15	30	32	3	5	15
Preincubated ADP 3.0 mM, FDP 0.1 mM start PEP 0.01 mM	3	11	29	29	3	4	16

initial activities differ substantially. L_4 from human liver (or trypsin-treated L_4' from human erythrocytes) hardly showed such behaviour. Progress curves of reactions started with either ADP or phospho*enol*pyruvate were almost straight lines, and yielded almost identical initial rates (Fig. 1). $L_2'L_2$ from human erythrocytes showed behaviour intermediate between L_4' and L_4. In the presence of saturating concentrations of the allosteric effector fructose 1,6-diphosphate (0.1 mM) a similar phenomenon was observed (Table I, left half).

Influence of Mg^{2+}_{free}

The experiments described above were performed at $Mg^{2+}_{free} = 1$ mM. We studied the influence of Mg^{2+}_{free} upon the hysteretic behaviour of the different forms of L-type pyruvate kinase from man, both in the presence of saturating concentrations fructose 1,6-diphosphate (0.1 mM), and in the absence of this compound. Figs. 2 and 3 show the influence of increasing Mg^{2+}_{free} concentrations upon the response on either one of the substrates. In the absence of fructose 1,6-diphosphate, the (initial rate)$^{-1}$ versus Mg^{2+}_{free} plots of L_4 pyruvate kinase almost overlapped: both at high and at low Mg^{2+}_{free} concentrations, preincubation with either ADP or phospho*enol*pyruvate yielded equal initial rates (Fig. 2C). L_4' pyruvate kinase however, yielded very different initial rates at low Mg^{2+}_{free} concentrations (Fig. 2A). The difference between initial rates of the

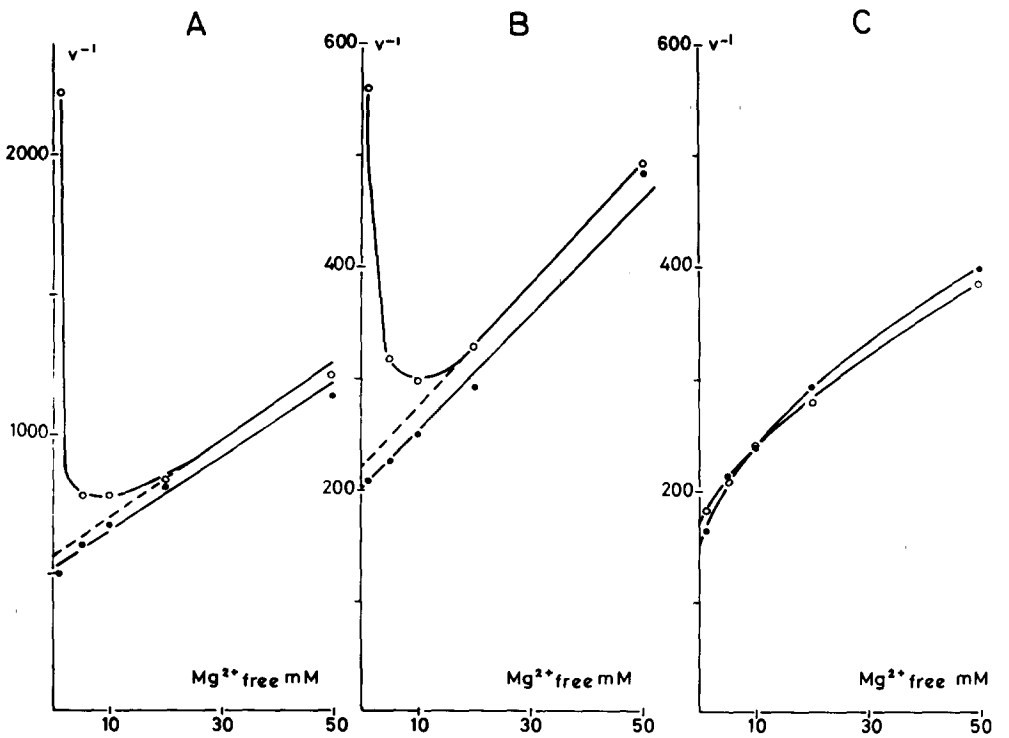

Fig. 2. Dixon plots (initial rate)$^{-1}$ versus [Mg^{2+}_{free}] of L_4' (A); $L_2'L_2$ (B), and L_4 (C) pyruvate kinase. ●———●, enzymes preincubated with phospho*enol*pyruvate (1.0 mM); reaction initiated with ADP (3.0 mM); ○———○, enzymes preincubated with ADP (3.0 mM); reaction initiated with phospho*enol*pyruvate (1.0 mM).

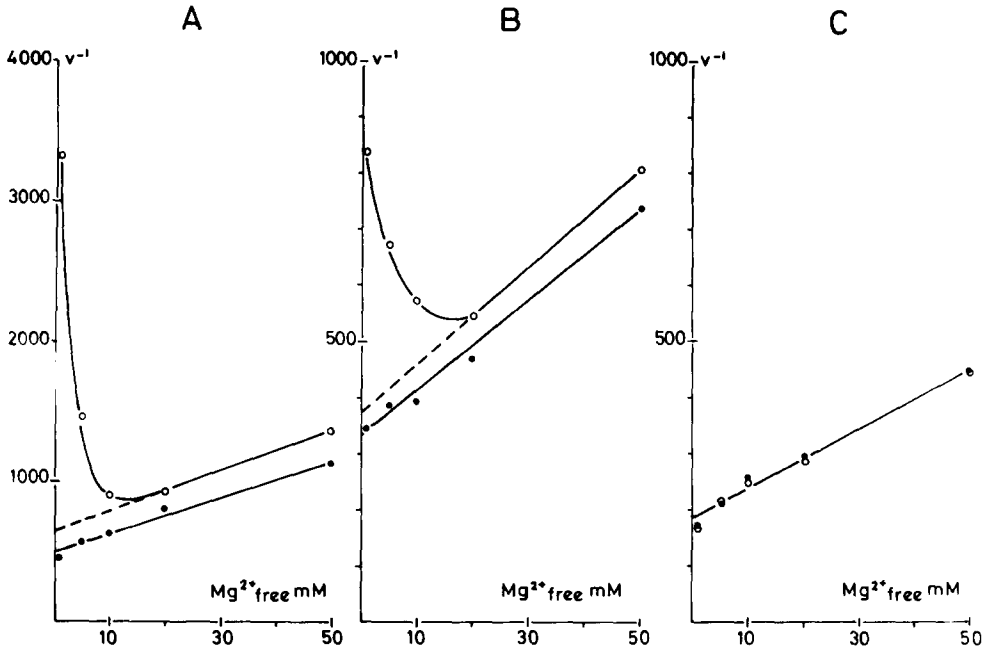

Fig. 3. Dixon plots (initial rate)$^{-1}$ versus [Mg^{2+}_{free}] of L'_4 (A); L'_2L_2 (B), and L_4 (C) pyruvate kinase in the presence of fructose 1,6-diphosphate. ●——●, enzymes preincubated with phospho*enol*pyruvate (0.1 mM); reaction initiated with ADP (3.0 mM); ○——○, enzymes preincubated with ADP (3.0 mM); reaction initiated with phospho*enol*pyruvate (0.1 mM).

phospho*enol*pyruvate or ADP-preincubated enzyme becomes smaller upon increasing Mg^{2+}_{free}. At Mg^{2+}_{free} = 20 or 50 mM the difference is approximately 15%. Again, L'_2L_2 showed behaviour intermediate between L'_4 and L_4 (Fig. 2B).

In the presence of saturating concentrations of fructose 1,6-diphosphate (0.1 mM) Mg^{2+}_{free} has a similar effect upon the initial rates of the phospho*enol*-pyruvate or ADP-preincubated enzymes (Fig. 3). For L_4 pyruvate kinase no difference is observed at either high or low Mg^{2+}_{free} concentrations (Fig. 3C); for L'_4 the difference that is observed at Mg^{2+}_{free} = 1 mM becomes smaller upon increasing Mg^{2+}_{free} (Fig. 3A). L'_2L_2 pyruvate kinase shows behaviour intermediate between L'_4 and L_4 enzymes (Fig. 3B).

Varying Mg^{2+}_{free} at phospho*enol*pyruvate$_{total}$ = constant yields increasing Mg-PEP$^-$/PEP^{3-} ratios. Because the true substrates of human L-type pyruvate kinase are unknown up to now, a conclusion about a $K_{i(app)}$ for Mg^{2+}_{free} cannot be drawn from the Dixon plots described above. However, L'_4, L'_2L_2 and L_4 seem to be equally inhibited by Mg^{2+}_{free}, both in the presence and absence of fructose 1,6-diphosphate (Figs. 2 and 3).

Influence of MgATP^{2-}

At Mg^{2+}_{free} = 1 mM, preincubation of L'_4 and L'_2L_2 pyruvate kinase with MgATP^{2-} diminishes the difference in initial rate of the enzymes preincubated with either ADP or phospho*enol*pyruvate (at low phospho*enol*pyruvate concentrations). This phenomenon is observed both in the absence and presence of fructose 1,6-diphosphate (0.1 mM) (Table I). So the enzymes preincubated

with low concentrations of phospho*enol*pyruvate are much more inhibited by $MgATP^{2-}$ than the enzymes preincubated with ADP. This suggests that $MgATP^{2-}$ induces the same less active conformation as ADP. In the presence of 0.1 mM 1,6-diphosphate, L_4 pyruvate kinase from human liver preincubated with phospho*enol*pyruvate (0.1 mM) is only 15% inhibited by 5 mM $MgATP^{2-}$. However L'_4 preincubated with phospho*enol*pyruvate is inhibited for 90% under that conditions. In the absence of fructose 1,6-diphosphate, L'_4 is slightly more inhibited by $MgATP^{2-}$ than L_4 (Table I).

Influence of phosphoenolpyruvate

As reported in our preliminary communication [2] the magnitude of the difference in initial rates of the ADP or phospho*enol*pyruvate-preincubated enzymes, is a function of the concentrations of both substrates. As saturating ADP concentrations (3 mM) we studied the difference in initial rate of the ADP or phospho*enol*pyruvate-preincubated enzymes as a function of the phospho*enol*pyruvate concentration (0.02—10 mM), both in the presence and absence of fructose 1,6-diphosphate (Figs. 4 and 5).

In the presence of fructose 1,6-diphosphate (0.1 mM). L_4 pyruvate kinase (either from human liver or trypsin-treated L'_4 pyruvate kinase) shows no difference at all in initial rate of the ADP or phospho*enol*pyruvate-preincubated enzymes, at all phospho*enol*pyruvate concentrations tested: the *v* versus phospho*enol*pyruvate plots are hyperboles and totally overlap (Fig. 4C). However, L'_4 pyruvate kinase shows a marked difference in initial rate of ADP or phos-

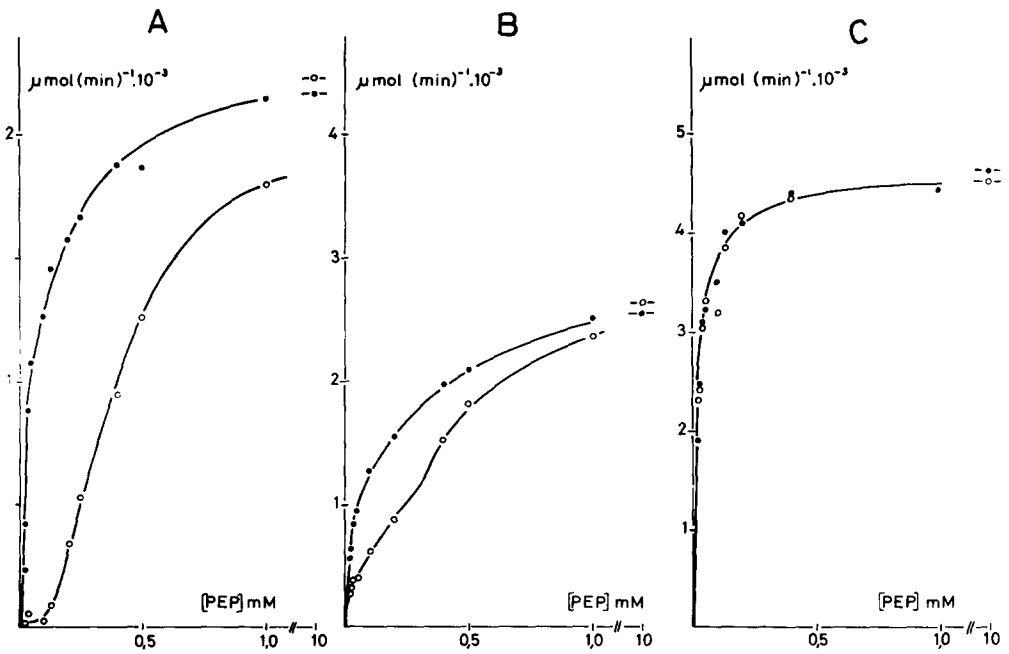

Fig. 4. Initial rate versus phospho*enol*pyruvate plots of L'_4 (A); L'_2L_2 (B) and L_4 (C) pyruvate kinase. ADP, 3 mM. Fructose 1,6-diphosphate, 0.1 mM ●———●, enzyme preincubated with phospho*enol*pyruvate; ○———○, enzyme preincubated with ADP.

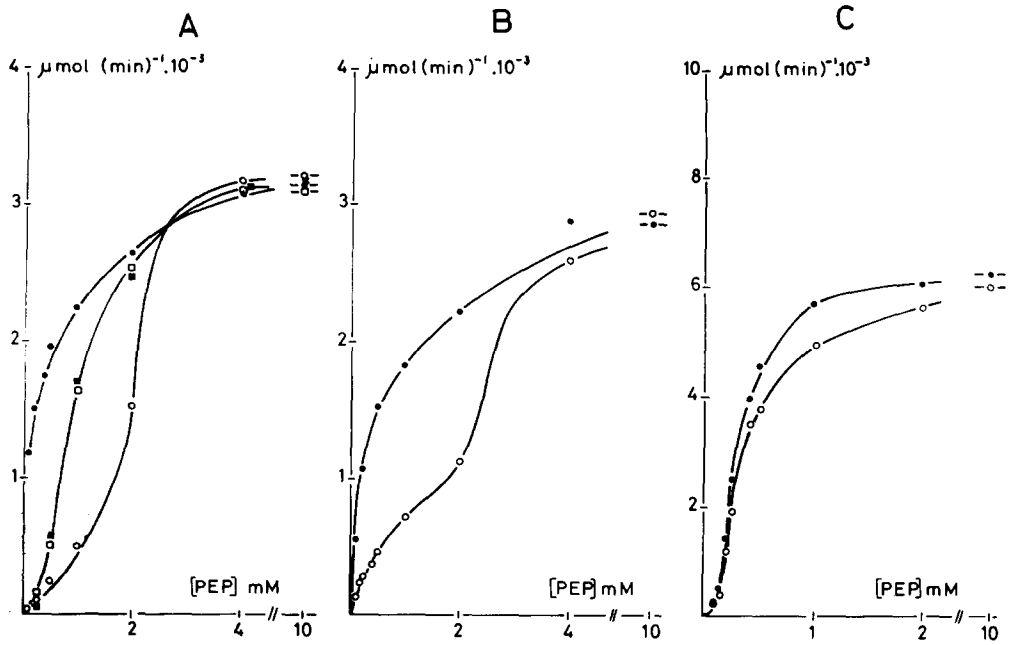

Fig. 5. Activity versus phospho*enol*pyruvate plots of L_4' (A); $L_2'L_2$ (B) and L_4 (C) pyruvate kinase. ADP, 3 mM. •———•, initial rates of enzyme preincubated with phospho*enol*pyruvate; ○———○, initial rates of enzyme preincubated with ADP; □———□, final activities of enzyme preincubated with phospho*enol*pyruvate; ■———■, final activities of enzyme preincubated with ADP.

pho*enol*pyruvate-preincubated enzymes, especially at low phospho*enol*pyruvate concentration. Preincubation of L_4' pyruvate kinase with phospho*enol*pyruvate yields a hyperbolic relationship between initial rate and phospho*enol*pyruvate concentration. However, preincubation with ADP yields a sigmoidal curve (Fig. 4A). $L_2'L_2$ shows behaviour intermediate between L_4' and L_4 enzymes. The plots of $L_2'L_2$ pyruvate kinase show biphasic characteristics (Fig. 4B). When the initial rate of the phospho*enol*pyruvate-preincubated enzymes is taken as a measure, then the sequential transformation of L_4' into L_4 enzyme is accompanied by a slight decrease in $K_{m(app)}$ for phospho*enol*pyruvate (Table II).

In the absence of fructose 1,6-diphosphate. L_4' pyruvate kinase preincubated with phospho*enol*pyruvate shows a hyperbolic relationship between initial rate and phospho*enol*pyruvate concentration. However, the ADP-preincubated enzyme yields a sigmoidal curve (Fig. 5A). In order to determin 'final activities' enzyme activities were always chosen so low, that the linear phase of the reaction progress curve was reached, before 5% of the substrates had been converted. Under these conditions ATP and lactate formation did not influence the reaction rate. The curve of 'final activity' versus phospho*enol*pyruvate concentration, that is the same curve for both the ADP and phospho*enol*pyruvate-preincubated L_4' enzymes, is sigmoidal, and lies intermediate between the two initial rate plots (Fig. 5A). L_4 pyruvate kinase, either from human liver or trypsin-treated L_4' pyruvate kinase, also showed some difference in initial rate of ADP or phospho*enol*pyruvate-preincubated enzymes.

TABLE II

KINETIC PARAMETERS

	L_4'	$L_2'L_2$	L_4	$L_4(T)$
$K_{m(app)}$ PEP (mM)	0.064	biphasic	0.026	0.022
in presence of FDP (0.1 mM)	(0.056—0.069)		(0.023—0.030)	
FDP (0.1 mM)	$n = 4$		$n = 4$	$= 1$
$[S]_{0.5}$ PEP (mM)	0.62	biphasic	0.29	0.21
	(0.40—0.70)		(0.25—0.40)	
	$n = 4$		$n = 4$	$= 1$
$K_{m(app)}$ ADP (mM)	0.23	0.22	0.24	
	(0.33—0.16)		(0.26—0.21)	
	$n = 7$	$n = 1$	$n = 2$	

L_4' and $L_2'L_2$ from human erythrocytes, L_4 from human liver; $L_4(T)$: L_4 obtained by tryptic treatment of L_4'. Kinetic parameters are based upon the initial rates of the phospho-enolpyruvate (PEP)preincubated enzymes. FDP, fructose-1,6-P_2.

However, this difference was much smaller than for L_4' pyruvate kinase, at a corresponding phospho*enol*pyruvate concentration. Both ADP and phospho*enol*pyruvate-preincubated enzymes showed a sigmoidal response of initial rate versus phospho*enol*pyruvate concentration (Fig. 5C). When the initial rate of the phospho*enol*pyruvate-preincubated enzymes in the absence of fructose 1,6-diphosphate is taken as a measure, then the sequential transformation of L_4' into L_4 pyruvate kinase is accompanied by a slight decrease in $[S]_{0.5}$ for phospho*enol*pyruvate (Table II).

Influence of ADP

$K_{m(app)}$ for ADP was determined both in the presence and absence of fructose 1,6-diphosphate, both of ADP and phospho*enol*pyruvate-preincubated enzymes. Preincubation with either phospho*enol*pyruvate or ADP does not influence the $K_{m(app)}$ for ADP at saturating phospho*enol*pyruvate concentrations. Both in the presence and absence of fructose 1,6-diphosphate equal $K_{m(app)}$. ADP were found for the L_4', $L_2'L_2$ and L_4 pyruvate kinase. Values were in the range of 0.16—0.33 mM (Table II). At low phospho*enol*pyruvate concentrations, plots of (initial rate)$^{-1}$ versus (ADP)$^{-1}$, of all three enzyme forms, preincubated with either phospho*enol*pyruvate or ADP, showed strong substrate inhibition patterns (data not shown).

Discussion

In this work we describe the functional changes associated with the sequential transformation of L_4' into L_4 pyruvate kinase. Besides a small increase in the affinity for phospho*enol*pyruvate, this transformation is mainly characterized by a loss of hysteretic behaviour of the enzyme. We proposed a model to describe the hysteretic response of this enzyme towards phospho*enol*pyruvate (unpublished data). L_4' pyruvate kinase would exist in at least three interconvertible forms: R, T and T'. The R form would be stabilized by phospho*enol*pyruvate, the T form by ADP. When the enzyme is in its R form, stabilized by phospho*enol*pyruvate, the equilibrium between R and T, induced by ADP,

is reached slowly. On the other hand, when the enzyme is in its T form, stabilized by ADP, the equilibrium between R and T, induced by phospho*enol*pyruvate, is reached rapidly. The T′ form would arise from T by a slow, phospho*enol*pyruvate-induced process. T′ would have a higher affinity for phospho*enol*pyruvate than T, and an equal affinity for ADP. The mechanism of slow-rapid R—T interconversions, for which a model with several forms intermediate between R and T would be required, is out of the scope of this work. In this work we investigate the behaviour of the enzyme both in the presence of 0.1 mM fructose 1,6-diphosphate, and in the absence of this compound. In order to integrate these data in out previous model, the model has to be extended with a fourth from R*. The R* form could arise from R and is induced by fructose 1,6-diphosphate. R* has a much higher affinity for phospho*enol*pyruvate than R, but has an equal $K_{m(app)}$ ADP. The interconversion between R and R* would be rapid.

Let us consider now, how our data on L_4' pyruvate kinase fit this model. In the absence of fructose 1,6-diphosphate the model is composed of the R, T and T′ forms. Preincubation of the enzyme with phospho*enol*pyruvate induces the R form. Upon initiating the reaction with ADP the enzyme exists in its R form: the plot of initial rate versus phospho*enol*pyruvate is a hyperbole. When the reaction proceeds, the R ⇋ T equilibrium is slowly induced by ADP, and the T ⇋ T′ equilibrium slowly sets. So, the plot of final activity versus phopho*enol*-pyruvate is a sigmoidal curve, due to the presence of R-T and R-T′ pairs (Fig. 5A). Preincubation of L_4' enzyme with ADP induces the T form. Upon initiating the reaction with phospho*enol*pyruvate, the R ⇋ T equilibrium sets rapidly: the plot of initial activity versus phospho*enol*pyruvate concentration is sigmoidal. As the reaction proceeds, phospho*enol*pyruvate induces slowly the T ⇋ T′ transformation. So, the plot of final activity versus phospho*enol*-pyruvate concentration is sigmoidal. Because T′ has a higher affinity for phospho*enol*pyruvate than T, this plot would have a lower $[S]_{0.5}$ for phospho*enol*-pyruvate than the initial rate plot (Fig. 5A). Incubation of the enzyme with $MgATP^{2-}$ would favour the T form of the enzyme. So the enzyme preincubated with low phospho*enol*pyruvate concentrations (R), would be much more inhibited by MgATP than the ADP-preincubated enzyme (T). This is consistent with the data from Table I.

In the presence of fructose 1,6-diphosphate, the model reduces to the R*, T and T′ forms. So the data from Fig. 4A fit in a similar way to the model as described for the enzyme in the absence of fructose 1,6-diphosphate. However, in the presence of fructose 1,6-diphosphate 'final activities' were not determined for technical reasons: we limited ourselves to determine 'final activities' just when less than 5% of the substrates had been converted. At phospho*enol*-pyruvate concentrations as low as 0.02 mM this requirement cannot be easily fulfilled. For L_4 pyruvate kinase from human liver the model would reduce to a simple R ⇋ T model [8]. The R form would be stabilized by phospho*enol*-pyruvate and fructose 1,6-diphosphate, the T form by ATP. The equilibrium between R and T would always be rapidly set. So, initiating the reaction with either ADP or phospho*enol*pyruvate would yield the same initial rate. Such a model has been proposed by Staal et al. for human erythrocyte L-type pyruvate kinase [9].

For L_4 pyruvate kinase the R ⇌ T model predicts that the allosteric inhibition by $MgATP^{2-}$ is stronger in the absence of fructose 1,6-diphosphate than in the presence of this compound: in the presence of fructose 1,6-diphosphate the R ⇌ T equilibrium is always far to the left. 5 mM $MgATP^{2-}$ does not shift it towards the T form, even though the phospho*enol*pyruvate concentration is ten times lower than in the experiment without fructose 1,6-diphosphate. This is consistent with the data from Table I. However, for L_4' pyruvate kinase in the presence of fructose 1,6-diphosphate (0.1 mM) the equilibrium R^* ⇌ T can still be shifted to the right by ADP and ATP. So for L_4' pyruvate kinase the allosteric inhibition by $MgATP^{2-}$ in the presence of fructose 1,6-diphosphate would be much stronger than for L_4 enzyme. This is consistent with the data from Table I. The phospho*enol*pyruvate-preincubated enzymes L_4', $L_2'L_2$ and L_4 (the R forms) each have the same affinity for fructose 1,6-diphosphate. At a phospho*enol*pyruvate concentration of 0.25 mM we measured half-saturation at approx. 0.1 μM (data not shown). This indicates that the difference in allosteric $MgATP^{2-}$ inhibition between L_4' and L_4 enzyme should be due to higher affinity for ATP of the T form of L_4' than of L_4 pyruvate kinase.

Hysteretic transformations have been reported for L-type pyruvate kinase by other authors [3,10—12]. Although our results with L_4' pyruvate kinase differ both quantitatively and qualitatively from the results of Badwey and Westhead [3], in essence our results are the same.

Badwey and Westhead proposed a possible physiological role of the hysteretic transformation in erythrocyte pyruvate kinase [3], consistent with the observed oscillations in the glycolytic rate produced by the oxygenation-deoxygenation cycle [25]. When the erythrocytes move from the lung to the peripheral tissue, the glycolytic flux would increase due to the lower oxygen tension [13—15]. The increase in glycolytic flux would be mainly caused by the binding of 2,3-diphosphoglycerate to deoxyhemoglobin, which relieves the inhibition of phosphofructokinase [16—19], heoxkinase [16,18,20,21] and diphosphoglycerate mutase [22—24]. A lag period in the pyruvate kinase activity would permit a built up of phospho*enol*pyruvate and 2,3-diphosphoglycerate. These high 2,3-diphosphoglycerate levels would facilitate the oxygen release in the tissues. As the red cells traverse the capillaries, the high phospho*enol*pyruvate concentration would slowly transform the pyruvate kinsae to a more active form, and a decrease in the 2,3-diphosphoglycerate concentration to its original level would be the consequence, whereafter the cycle could be repeated.

Pyruvate kinase deficiency is both clinically and biochemically very heterogeneous. There is no correlation between the severity of the disease and the biochemical abnormalities found [26,27]. One might speculate, that hysteretic phenomena described in this paper play an important role in the energy economy of the cell. A defect in this mechanism could impose a metabolic handicap, resulting in a shortened life span of the cell.

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