Plant Physiol. (1977) 59, 849-853

Pyruvate Dehydrogenase Complex from Higher Plant Mitochondria and Proplastids: Kinetics[1]

Received for publication August 23, 1976 and in revised form January 26, 1977

PAUL THOMPSON, E. ELLEN REID, C. RICHARD LYTTLE,[2] AND DAVID T. DENNIS
Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6

ABSTRACT

A steady-state kinetic analysis has been performed on the pyruvate dehydrogenase complex from pea (*Pisum sativum* L.) mitochondria and castor bean (*Ricinus communis* L.) proplastids. Substrate interaction kinetics for all substrates gave parallel lines consistent with a multisite ping-pong mechanism. Product inhibition studies showed uncompetitive inhibition between acetyl-CoA and pyruvate and competitive inhibition between NADH and NAD^+, both of which are also consistent with this mechanism. In the mitochondrial complex, acetyl-CoA showed noncompetitive inhibition *versus* CoA which suggests that the intermediate complex is kinetically important in the lipoamide transacetylase component of this complex. In contrast, the proplastid complex showed competitive inhibition in this interaction. NADH is a noncompetitive inhibitor *versus* CoA in both complexes indicating that these complexes, like the mammalian complex, may have protein-protein interactions between the second and third enzymes of the complex. Since NADH also shows noncompetitive inhibition *versus* pyruvate, this interaction may extend to all components of the complex. Acetyl-CoA shows noncompetitive inhibition *versus* NAD^+ which may also be a result of interaction between the second and third enzymes of the complex. The limiting Michaelis constants for substrates and the inhibitor constants for both complexes were determined.

The pyruvate dehydrogenase complex consists of three enzymes: pyruvate dehydrogenase (pyruvate lipoate oxidoreductase, EC 1.2.4.1), lipoate transacetylase (acetyl-CoA dihydrolipoate S-acetyl transferase, EC 2.3.1.12), and dihydrolipoamide dehydrogenase (NADH lipoamide oxidoreductase, EC 1.6.4.3). The reactions of these three enzymes are shown in the accompanying paper (11) which also gives an account of the purification and preliminary characteristics of the pea mitochondrial complex and isolation of the proplastid complex. The reactions of the complex occur at three distinct sites on the complex located on three different proteins. The nature of the reactions at each of these sites suggests that the over-all mechanism should be ping pong. A rate equation for a three-site ping-pong mechanism has been derived by Cleland (4) and from this it was predicted that the pyruvate dehydrogenase complex should show initial substrate velocity patterns which are parallel when plotted in double reciprocal form, regardless of which substrate is varied at fixed levels of a second. Such a pattern has been demonstrated for the pyruvate dehydrogenase complexes from bovine kidney (16) and pig heart (7). The products of each enzyme component should be competitive with the substrate for that enzyme unless the central complexes are kinetically important, in which case they will be noncompetitive. This prediction has been confirmed for acetyl-CoA and NADH in the bovine kidney and pig heart complexes (7, 16). Other product inhibition patterns should be uncompetitive except for the interaction of one product and the substrate of the subsequent enzyme when it will be noncompetitive, if the reaction at that site is random sequential. In the mammalian complexes (7, 16), acetyl-CoA and NADH are both uncompetitive against pyruvate but acetyl-CoA and NADH are noncompetitive against NAD^+ and CoA, respectively. This may indicate that these products can act as dead end inhibitors, or the binding of products may sterically hinder the binding of substrates on the adjacent enzyme. A third possibility is that there may be protein-protein interactions between the second and third enzymes of the complex (7, 16).

The steady-state kinetic analyses to determine the reaction mechanism of the plant complex have not been described. The regulatory properties of the enzyme from potato tubers have been described and some preliminary kinetics performed (5). Since some of the interactions between products and substrates are other than those predicted by theory, it is important that further kinetics be performed. Also, a steady-state kinetic analysis may be used to determine whether the mitochondrial enzyme and the enzyme from proplastids (11) are significantly different.

MATERIALS AND METHODS

Preparation and Assay of Pyruvate Dehydrogenase Complexes. The mitochondrial complex was purified either to the pelleted fraction or glycerol gradient stage (11). Proplastids were isolated on discontinuous gradients (10). Assays were performed as described in the accompanying paper (11). In all cases, care was taken to ensure that true initial rate measurements were made. Saturating concentrations of substrates were determined from preliminary kinetics and for the complex from proplastids they were: 2.5 mM NAD^+, 90 μM CoA, 3 mM pyruvate, 0.5 mM TPP,[3] 20 mM $MgCl_2$, and 2.4 mM cysteine HCl. Saturating concentrations used for the mitochondrial complex were: 1 mM $MgCl_2$, 1 mM NAD^+, 130 μM CoA, 1 mM pyruvate, 0.5 mM TPP, 1 mM cysteine HCl. All biochemicals were purchased from the Sigma Chemical Co. All other chemicals were of analytical grade.

Analysis of Kinetic Data. Kinetic data were analyzed by using a nonlinear regression computer program following the methodology of Duggleby and Dennis (6). All of the substrate interaction data were fitted to both of the following equations:

$$v = \frac{V_{max}}{1 + \frac{K_A}{[A]} + \frac{K_B}{[B]} + \frac{K_{AB}}{[A][B]}} \quad (I)$$

[1] This research was supported by a grant from the National Research Council of Canada.

[2] Present address: University of Chicago, Ben May Laboratories, Chicago, Ill.

[3] Abbreviation: TPP: thiamine pyrophosphate.

$$v = \frac{V_{max}}{1 + \frac{K_A}{[A]} + \frac{K_B}{[B]}} \quad \text{(II)}$$

where A and B are the varied substrates, K_A and K_B are the respective limiting Michaelis constants, V_{max} is the maximum velocity, and K_{AB} is an interaction term. A preliminary kinetic analysis was used to determine the useful range of substrate concentrations. The third substrate in the reaction mixture was held constant at saturating concentrations as determined by the preliminary analysis.

All data from inhibition studies were fitted to the equation for noncompetitive inhibition (III). In addition, the data were either fitted to the equation for competitive inhibition (IV) or uncompetitive inhibition (V) as determined by inspection of the double reciprocal plots.

$$v = \frac{V'_{max}}{1 + \frac{K'_A}{[A]} + \frac{K'_A[I]}{K_{IS}[A]} + \frac{[I]}{K_{II}}} \quad \text{(III)}$$

$$v = \frac{V'_{max}}{1 + \frac{K'_A}{[A]} + \frac{K'_A[I]}{K_{IS}[A]}} \quad \text{(IV)}$$

$$v = \frac{V'_{max}}{1 + \frac{K'_A}{[A]} + \frac{[I]}{K_{II}}} \quad \text{(V)}$$

In these equations, A is the variable substrate, I is the inhibitor, K'_A and V'_{max} are the Michaelis constant and maximum velocity, respectively, in the presence of inhibitor, and K_{IS} and K_{II} are the slope and intercept inhibition constants, respectively.

In the method of data analysis described by Duggleby and Dennis (6), replicates of individual rates are not performed since it appears to be more advantageous to increase the number of data points rather than perform replicates. It is also assumed that the measured velocity, v, is normally distributed around the predicted velocity, $\bar{v}$, and the variance in initial velocities is constant. Furthermore, the errors in substrate and inhibitor concentrations are assumed to be constant at all concentrations. Since replicates are not performed, no estimate of pure error can be made. However, when the data were plotted as double reciprocal plots, straight lines were obtained, showing that the complex obeys normal Michaelis kinetics. The data must, therefore, fit the complete equations for substrate interactions (I) and for product inhibition interactions (III). The residual sum of squares (RSS_c), which results when the data are fitted to the complete equations, therefore gives a measurement of the pure error of the data. When the data are fitted to the reduced equations (II, IV, and V), the residual sum of squares (RSS_r) is due both to pure error and lack of fit to the equations. The residual sum of squares (RSS_{lf}) due to lack of fit to the equation can be estimated from

$$RSS_{lf} = RSS_r - RSS_c$$

The significance of the lack of fit to the reduced equations can be determined by a variance ratio (F) test

$$F = \frac{RSS_{lf}\,(n - p)}{RSS_c} \quad \text{(VI)}$$

Where n is the number of assays and p (the number of parameters in the complete equations) is 4. In all cases, the null hypothesis is used, *i.e.* the reduced equation is chosen if a significantly better fit is not found with the complete equation, *i.e.* if the residual sum of squares due to the lack of fit to the reduced equation is not significant as compared with the error in the data. Using this analysis, the significance of the K_{AB}, $\frac{[I]}{K_{II}}$ and $\frac{K'_A[I]}{K_{IS}[A]}$ terms in the complete equations (I and III) can be evaluated by comparison of the experimentally determined F value with tables of F values, at a probability of 0.99.

RESULTS

The data from the kinetic studies are presented as double reciprocal plots and all data are shown fitted by the computer program to the model chosen using the F test. Each line is not plotted independently, but is the best fit to all of the data in one experiment. Some of the assays of the mitochondrial complex were performed using the complex purified as far as the glycerol gradient (11), whereas other assays were performed with enzyme which had been sedimented by high speed centrifugation (11). The type of preparation used is indicated in each figure.

This report describes a comparison of the kinetics of the pea mitochondrial pyruvate dehydrogenase complex and the castor bean proplastid complex. A more valid comparison between the complexes of the two organelles would be obtained if the organelles were from one source. However, so far it has been very difficult to obtain mitochondria from developing castor beans which were free from proplastids although a pure proplastid fraction can be obtained. On the other hand, no pyruvate dehydrogenase complex has been found in any other fraction except the mitochondrial fraction when homogenates from etiolated peas have fractionated on a discontinuous sucrose density gradient. In order to be sure, therefore, that pure fractions are being used, the organelles were separated from the two sources.

Substrate Interaction Kinetics. The kinetic constants for the substrates for both complexes are presented in Table I. In all cases, the complete equation (I) did not give a significantly better fit to the data at the 99% confidence level than the reduced equation (II), so that the K_{AB} term is not significant and the double reciprocal plots are parallel. This is consistent with a multisite ping-pong mechanism (4). An example of substrate interaction kinetics is presented in Figure 1. It should be noted

Table I

Substrate Kinetic Constants for the Pyruvate Dehydrogenase Complex From Developing Castor Bean Proplastids and Pea Mitochondria

Substrates Varied		Kinetic Constants[a] (From Equation II)			
		Proplastids		Mitochondria	
A	B	K_A[b]	K_B[c]	K_A[b]	K_B[c]
		μM			
Pyruvate	CoA	53	4.6	72	4
NAD^+	CoA	100	5.7	238	6
Pyruvate	NAD^+	47	100	74	307

[a] A standard deviation was calculated for all constants and was 10% or less of the values.

[b] The limiting Michaelis constant for substrate A

[c] The limiting Michaelis constant for substrate B

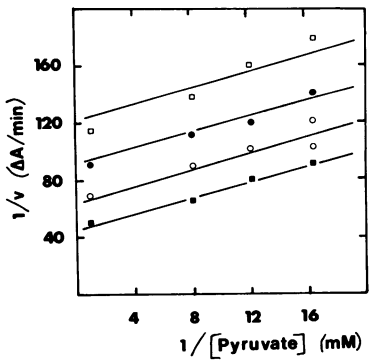


FIG. 1. Kinetics of the pea mitochondrial pyruvate dehydrogenase complex with respect to pyruvate at 1 mM (■), 0.4 mM (○), 0.2 mM (●) and 0.13 mM (□) NAD^+. The enzyme was purified to the glycerol gradient stage. Data are shown fitted to equation II ($K_{AB} = 0$).

that the limiting Michaelis constants for the substrates calculated from different substrate interactions are very similar, suggesting that different preparations give similar kinetics. The data from the mammalian complexes also have parallel double reciprocal plots (7, 16).

Product Inhibition Kinetics. The values of the kinetic constants for the product inhibition data derived from the equation chosen by the statistical analysis are given in Table II. It should be noted that the limiting Michaelis constants determined from product inhibition studies are very similar to those found in substrate interaction kinetics (Table I). A comparison of the inhibition patterns found in the proplastid enzyme in comparison to those from bovine kidney and pig heart is shown in Table III

The inhibition when pyruvate was the substrate and acetyl-CoA the inhibitor was uncompetitive, and the inhibition when NAD^+ was the substrate and NADH the inhibitor was competitive. Both of these inhibition patterns are the same as the predicted patterns from the rate equation (4). These data are the same as those described for the mammalian complexes (7, 16). The data for interaction between pyruvate and acetyl-CoA in the potato tuber complex (5) have been plotted as noncompetitive inhibition, but it is not possible to determine if the data are significantly different from uncompetitive inhibition. Competitive inhibition has been reported for this interaction for the *Escherichia coli* complex (12) but only under certain conditions (2). This enzyme may not obey normal kinetics under all substrate conditions (2). NADH appears to be competitive with NAD^+ in most systems (5, 8, 17).

When NAD^+ was the substrate and acetyl-CoA the inhibitor, and when CoA was the substrate and NADH the inhibitor, the patterns observed were noncompetitive (Figs. 2 and 3). A similar pattern was found in the mammalian complexes (7, 16). In the case of these complexes, it was suggested that this might be due to protein-protein interactions between the second and third enzymes of the complex. To support this hypothesis, a direct effect of acetyl-CoA and CoA on the third enzyme has been demonstrated, when the third enzyme is part of the complex (16).

In both of the plant complexes when pyruvate was the substrate and NADH was the inhibitor, noncompetitive inhibition was also found (Figs. 4 and 5). It is possible that NADH is a dead end inhibitor at the first enzyme of the complex or that binding of NADH causes steric hindrance to the binding of pyruvate, although this is unlikely due to the distance apart of the first and third enzymes, if the organization of the plant complexes is similar to that from other sources. Inasmuch as there is some evidence that protein-protein interactions are op-

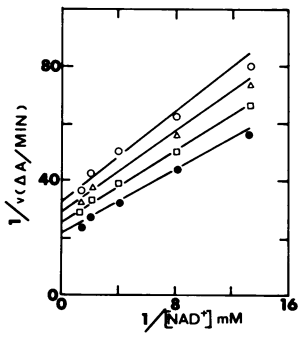


FIG. 2. Kinetics of the pyruvate dehydrogenase complex from castor bean endosperm proplastids with respect to NAD^+ in the presence of 0 (●), 167 μM (□), 333 μM (△), and 500 μM (○) of the product acetyl-CoA.

TABLE II

Substrate Kinetic Constants and Product Inhibition Constants for the Pyruvate Dehydrogenase Complex from Developing Castor Bean Proplastids and Pea Mitochondria

Substrate	Acetyl-CoA as Inhibitor			NADH as Inhibitor		
	Kinetic Constant[a]	Value		Kinetic Constant[a]	Value	
		Proplastids	Mitochondria		Proplastids	Mitochondria
		uM			uM	
Pyruvate	K_A	62	74	K_A	57	73
	K_{II}	370	236	K_{IS}	570	106
				K_{II}	330	261
CoA	K_A	6	2	K_A	6	7
	K_{IS}	23	14	K_{IS}	130	81
	K_{II}		472	K_{II}	890	144
NAD^+	K_A	130	209	K_A	110	168
	K_{IS}	1100	257	K_{IS}	27	17
	K_{II}	1000	298			

[a] The standard deviation was evaluated and found to be approximately 10% in all cases.

TABLE III

Product Inhibition Patterns Observed from the Castor Bean Proplastids and Pea, Kidney and Heart Mitochondrial Pyruvate Dehydrogenase Complexes

Inhibitor	Substrate	Inhibition Pattern Observed			
		Castor Bean Proplastid	Pea Mitochondria	Kidney[a] Mitochondria	Heart[b] Mitochondria
Acetyl-CoA	Pyruvate	Uncompetitive	Uncompetitive	Uncompetitive	Uncompetitive
Acetyl-CoA	Coenzyme A	Competitive	Non-competitive	Competitive	Competitive
Acetyl-CoA	NAD^+	Non-competitive	Non-competitive	Non-competitive	Non-competitive
NADH	Pyruvate	Non-competitive	Non-competitive	Uncompetitive	Uncompetitive
NADH	Coenzyme A	Non-competitive	Non-competitive	Non-competitive	Non-competitive
NADH	NAD^+	Competitive	Competitive	Competitive	Competitive

[a] Data from reference 3
[b] Data from reference 4

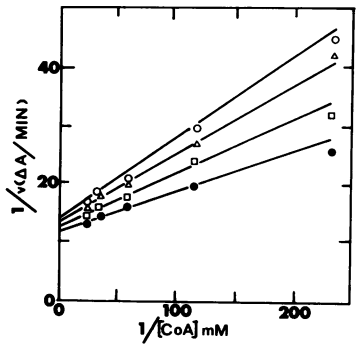


FIG. 3. Kinetics of the pyruvate dehydrogenase complex from castor bean endosperm proplastids with respect to CoA in the presence of 0 (●), 47 μM (□), 94 μM (△) and 140 μM (○) of the product NADH.

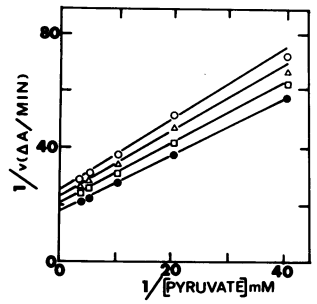


FIG. 4. Kinetics of the pyruvate dehydrogenase complex from castor bean endosperm proplastids with respect to pyruvate in the presence of 0 (●), 47 μM (□), 94 μM (△) and 140 μM (○) of the product NADH.

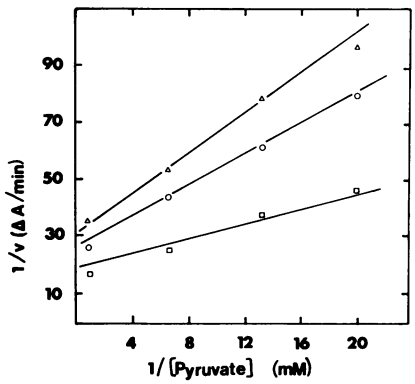


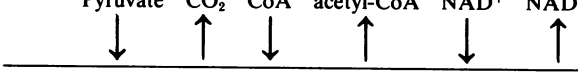
FIG. 5. Kinetics of the pea mitochondrial pyruvate dehydrogenase complex with respect to pyruvate at 0 (□), 197 μM (○) and 170 μM (△) NADH. The complex was purified to the high speed pellet stage. Data shown fitted to equation III.

erating between the second and third enzymes of the bovine complex (16), it is possible that in the plant complexes this interaction extends throughout the complex and involves all three enzymes. Such large conformational changes might be of importance when considering the regulation of the complexes.

The interaction between the inhibitor, acetyl-CoA, and substrate CoA was noncompetitive for the mitochondrial complex (Fig. 6), since the *F* value determined from the residual sum of squares is much higher than the *F* value from statistical tables. This indicates that noncompetitive inhibition gives a better fit to the data than competitive inhibition. This is in contrast to the competitive inhibition found in the proplastid complexes (Fig. 7). Competitive inhibition for this interaction has also been suggested in the bovine (16) and rat kidney (3) and pig heart (7) and brain (14) and *Neurospora* (8) complexes. Even though the intercept effect in the pea mitochondrial complex is small, there is a statistically significant difference between this complex and the castor bean proplastid enzyme. This difference could indicate differences between the complexes. The data from the pea mitochondrial complex indicate that the central complex in the second reaction of the complex is kinetically significant. The ability to detect the central complex may be a function of the assay conditions employed.

DISCUSSION

The data for the substrate interaction kinetics of both plant pyruvate dehydrogenase complexes are consistent with a multisite ping-pong mechanism and can be diagrammed as follows:



This reaction mechanism is to be expected with an enzyme complex such as the pyruvate dehydrogenase complex which consists of three enzymes catalyzing three consecutive reactions. This reaction mechanism is the same as that described for bovine kidney (16) and pig heart complexes (7).

The above reaction mechanism indicates that there are three independent active sites. The interaction between acetyl-CoA and pyruvate and between NADH and NAD^+ are as would be predicted by the above reaction mechanism. As will be discussed in the accompanying paper (15), and as was the case in the potato (5), pig heart (3), and *E. coli* (13) complexes, the interaction between NAD^+ and NADH is a major control mechanism. The interactions between the product-substrate pairs, NADH-CoA, acetyl-CoA-NAD^+, and NADH-pyruvate are noncompetitive. According to the theoretical mechanism developed by

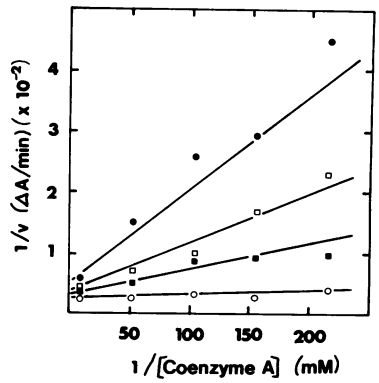


FIG. 6. Kinetics of the pea mitochondrial pyruvate dehydrogenase complex with respect to CoA at 0 (○), 89 μM (■), 177 μM (□) and 354 μM (●) acetyl-CoA. The complex was purified to the high speed pellet stage. Data are shown fitted to equation III.

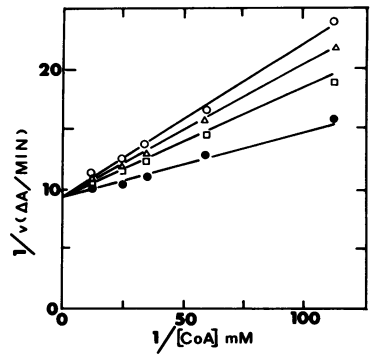


FIG. 7. Kinetics of the pyruvate dehydrogenase complex from castor bean endosperm proplastids with respect to CoA in the presence of 0 (●), 17 μM (□), 25 μM (△), and 33 μM (○) of the product acetyl-CoA.

Cleland (4), the interaction between acetyl-CoA and NAD^+ might be noncompetitive if the reaction mechanism of the dihydrolipoyl dehydrogenase is random sequential. It is also possible that there is dead end inhibition by the products in this complex. In the bovine kidney complex, it was suggested that there is protein-protein interaction between the second and third enzymes in the complex (16) and such a mechanism could exist in plants. The noncompetitive inhibition between NADH and pyruvate might indicate that the protein-protein interactions can also include all three enzymes of the complex such that binding of NADH affects the binding of pyruvate. This interaction would be exerted through a considerable distance when the size of the complex is considered. The $s_{20,w}$ value of the pea complex is 47.5 (11), and since this is probably a partially dissociated complex, it must be considered a minimum value. The lipoate transacetylase component acts as a core to bind the other enzymes (8). Obviously, if protein-protein interactions are important, the occupation of a small number of active sites in the third enzyme of the complex must cause conformational changes in a large number of other subunits.

In the pea mitochondrial complex, the interaction between acetyl-CoA and CoA is also noncompetitive. The type of interactions between this product and substrates will depend on the reaction mechanism for this particular enzyme component and could be either competitive or noncompetitive depending on whether the central complex is kinetically important (4). By the use of lipoamide, TPP, and hydroxyethyl-TPP, the reaction mechanism of each component could be studied. However, since the lipoic acid is normally covalently bound to an ϵ-amino group in the lipoate transacetylase (9), and TPP and hydroxyethyl-TPP are also tightly bound, the TPP and lipoic acid components are not in equilibrium with the external medium and this will affect the interpretation of the kinetics considerably. Kinetic analysis of the individual enzymes may yield results, therefore, which have little bearing on the normal reaction mechanism of these enzyme components while in the complex. The proplastid complex shows a competitive interaction. This may be due to a different mechanism in the second enzyme or the fact that the assay did not detect a small intercept effect.

The relative *Km* values for the three substrates are the same in the two complexes. The *Km* for CoA is much lower than the *Km* for pyruvate and NAD^+ and has a similar value in both complexes. The *Km* for CoA is low in complexes from most sources (5, 14, 18) although that from pig heart is higher (7). These constants are not, however, limiting *Km* values. The *Km* for NAD^+ in the proplastid complexes is approximately twice that found for the potato tuber complex (5), and that from the mitochondrion five times that found for the potato tuber complex. The difference between this *Km* value in the proplastid and mitochondrial complexes may indicate a difference in the complexes. The inhibitor constant for NADH in the mitochondrial enzyme is approximately 10% of the value for the *Km* for NAD^+, indicating that NADH is bound tightly. The ratio between the *Km* values for substrate and product is important in regulation (1), and in this case regulation of the complex by product can be anticipated. The inhibitor constant for NADH in the proplastid constant is higher than in the mitochondrial complex and since the *Km* for NAD^+ is lower, less effective regulation could be anticipated. In general, all of the inhibitor constants for the proplastid complex are higher than those for the mitochondrial complex, whereas the *Km* values for substrates are lower. These differences may indicate differences in the complexes from these sources.

LITERATURE CITED

1. Atkinson DE, PJ Roach, JS Schwedes 1975 Metabolite concentrations and concentration ratios in metabolic regulation. Adv Enzyme Reg 13: 393-411
2. Bisswanger H, U Henning 1971 Regulatory properties of the pyruvate-dehydrogenase complex from *Escherichia coli*: positive and negative cooperativity. Eur J Biochem 24: 376-384
3. Bremer J 1969 Pyruvate dehydrogenase, substrate specificity and product inhibition. Eur J Biochem 8: 535-540
4. Cleland WW 1973 Derivation of rate equations for multisite Ping-Pong mechanisms with Ping-Pong reactions at one or more sites. J Biol Chem 248: 8353-8355
5. Crompton M, GG Laties 1971 The regulation function of pyruvate dehydrogenase. Arch Biochem Biophys 143: 143-150
6. Duggleby RG, DT Dennis 1974 Nicotinamide adenine dinucleotide-specific glyceraldehyde 3-phosphate dehydrogenase from *Pisum sativum*. J Biol Chem 249: 167-174
7. Hamada M, K Koike, Y Nakaula, M Koike, T. Hashimoto 1975 A kinetic study of the α-keto acid dehydrogenase complexes from pig heart mitochondria. J Biochem 77: 1047-1056
8. Harding RW, DF Caroline, RP Wagner 1970 The pyruvate dehydrogenase complex from the mitochondrial fraction of *Neurospora crassa*. Arch Biochem Biophys 138: 653-661
9. Nawa H, WT Brady, M. Koike, LJ Reed 1960 Studies on the nature of protein-bound lipoic acid. J Am Chem Soc 82: 896-903
10. Reid EE, CR Lyttle, DT Canvin DT Dennis 1975 Pyruvate dehydrogenase complex activity in proplastids and mitochondria of developing castor bean endosperm. Biochem Biophys Res Commun 62: 42-47
11. Reid EE, P Thompson, CR Lyttle, DT Dennis 1977 Pyruvate dehydrogenase complex from higher plant mitochondria and proplastids. Plant Physiol 59: 842-848
12. Schwartz ER, LO Old, LJ Reed 1968 Regulatory properties of pyruvate dehydrogenase from *Escherichia coli*. Biochem Biophys Res Commun 31: 495-500
13. Shen LC, DE Atkinson 1970 Regulation of pyruvate dehydrogenase from *Escherichia coli:* interactions of adenylate energy charge and other regulatory parameters. J Biol Chem 245: 5974-5978
14. Siess E, J Wittman, O Wieland 1971 Interconversion and kinetic properties of pyruvate dehydrogenase from brain. Hoppe-Seylers Z Physiol Chem 352: 447-452
15. Thompson P, EE Reid, CR Lyttle, DT Dennis 1977 Pyruvate dehydrogenase complex from higher plant mitochondria and proplastids: regulation. Plant Physiol 59: 854-858
16. Tsai CS, MW Burgett, LJ Reed 1973 α-Keto acid dehydrogenase complexes. XX. A kinetic study of the pyruvate dehydrogenase complex from bovine heart. J Biol Chem 248: 8348-8352
17. Wais U, U Gillman, J Ullrich 1973 Isolation and characterisation of pyruvate dehydrogenase complex from brewers' yeast. Hoppe-Seylers Z Physiol Chem 354: 1378-1388
18. Wieland OH, U Hartmann, EA Siess 1972 *Neurospora crassa*. Pyruvate dehydrogenase: interconversion by phosphorylation and dephosphorylation. FEBS Lett 27: 240-244