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KINETIC CHARACTERIZATION OF THE PYRUVATE AND OXOGLUTARATE DEHYDROGENASE COMPLEXES FROM HUMAN HEART

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The Michaelis constant values for the highly purified pyruvate dehydrogenase complex (PDC) from human heart are 25, 13 and 50 μM for pyruvate, CoA and NAD, respectively. Acetyl-CoA produces a competitive inhibition of PDC (K_i = 35 μM) with respect to CoA, whereas NADH produces the same type of inhibition with respect to NAD (K_i = 36 μM). The oxoglutarate dehydrogenase complex (OGDC) from human heart has active sites with two different affinities for 2-oxoglutarate ([S]_0.5 of 30 and 120 μM). ADP (1 mM) decreases the [S]_0.5 values by a half. The inhibition of OGDC (K_i = 81 μM) by succinyl-CoA is of a competitive type with respect to CoA (K_m = 2.5 μM), whereas that of NADH (K_i = 25 μM) is of a mixed type with respect to NAD (K_m = 170 μM).

The pyruvate dehydrogenase (PDC) and oxoglutarate dehydrogenase (OGDC) complexes occupy important metabolic positions, catalysing multistep processes of pyruvate and 2-oxoglutarate oxidative decarboxylation under the influence of five coenzymes: thiamine diphosphate, lipoate, coenzyme A, FAD and NAD [1]. These multienzyme complexes possess universal regulatory mechanisms [1, 2]. Detailed studies on the catalytic and regulatory properties of PDC and OGDC from some sources revealed the presence of species-specific and organ-specific differences between the complexes of different origin [1-7]. In the medical and biological aspects of interest are studies on multienzyme complexes containing human heart 2-oxo acid dehydrogenases and participating in metabolic supply of the myocardium. However, the possibility of the regulation of human heart PDC by reversible phosphorylation has only been described for the crude enzyme preparation [8].
This report presents the main kinetic parameters of highly purified PDC and OGDC isolated from human heart. Certain regulatory pathways for the above multienzyme systems are analysed on the basis of the information obtained.

MATERIALS AND METHODS

PDC and OGDC were isolated and purified from the heart of a 44-year-old man killed in a car accident using the technique applied for purification of the same complexes from bovine heart [9]. The PDC and OGDC preparations obtained had specific activities of 4.8 and 6.0 U/mg protein and on sodium dodecyl sulphate polyacrylamide gel electrophoresis showed a set of subunits typical for the same complexes from other animal sources.

The initial rate of the PDC- and OGDC-catalyzed reactions was recorded by measuring NADH formation at 340 nm and 30°C with a recording spectrophotometer Specord UV/VIS (Carl Zeiss, Jena) using a thermostated cuvette. The reaction medium contained 50 mM potassium phosphate buffer, pH 7.5, 1 mM dithiothreitol, 1 mM MgCl₂, 0.2 mM thiamine diphosphate and different concentrations of pyruvate (for PDC) or 2-oxoglutarate (in the case of OGDC), CoA and NAD. To investigate the inhibitory capacities of the reaction products, either 0.1 mM acetyl-CoA or succinyl-CoA as well as 0.05 mM NADH were used. The reaction was started by the addition of 1 to 3 μg of either the PDC or OGDC enzyme preparation. Kinetic parameters were calculated using the Lineweaver-Burk plot [10].

RESULTS AND DISCUSSION

The kinetic study has shown that the initial rate of the overall reaction of human heart PDC can be described by the Michaelis-Menten equations for each of the three substrates, pyruvate, CoA and NAD. The Michaelis constants, as calculated using the double reciprocal plot (Fig. 1), are equal to 25, 13 and 50 μM for pyruvate, CoA and NAD, respectively. When comparing the $K_m$ values for human heart PDC to similar indices for porcine heart PDC (the most suitable material for the comparison) one can note the following: the $K_m$ values are essentially the same in terms of the data obtained by Randle et al. [11], whereas the Michaelis constant for CoA of PDC from human heart (Fig. 1b) is nearly 7 times that for porcine heart PDC [11]. However, other authors obtained much higher $K_m$ value for CoA (21 μM) of animal heart muscle PDC [12].

Studies on the effect of the end products of the reaction on the initial rate of pyruvate oxidative decarboxylation have demonstrated that acetyl-CoA produces a competitive type inhibition of human heart PDC with respect to CoA (Fig. 1b), whereas NADH exhibits the same type inhibition with respect to NAD (the graph is not presented). The $K_i/K_m$ ratio for NADH and NAD is
somewhat lower than 1 (0.72), which enables us to consider the inhibition by NADH as important for control of this multienzyme complex from human heart. The PDC inhibition induced by acetyl-CoA may be less significant for the regulation since $K_i$ amounts to 35 μM, which is nearly 3-fold the $K_m$ value for CoA.

The dependence of the initial rate of the human heart OGDC-catalyzed reaction on the 2-oxoglutarate concentration is of a more complex character. The Lineweaver-Burk plot is represented by two lines with different slopes (Fig. 2). This may be interpreted in terms of OGDC having two types of active sites with different affinities for the substrate. The $[S]_{0.5}$ values for these sites vary by 4-fold (30 and 120 μM). This property has not been described for OGDC from animal heart. Only in measuring the rate of bovine kidney
2-oxoglutarate-catalyzed reaction by the use of a system containing artificial electron acceptors kinetic results were obtained which point to the presence of three types of active sites with different affinities for 2-oxoglutarate [13, 14]. Our data on the rate of the complete 2-oxoglutarate oxidative decarboxylation reaction indicate that human heart OGDC also shows heterogeneity of its active sites.

When ADP was used at a physiological concentration (1 mM), it proved to be a positive effector for OGDC from human heart and lowered the \([S]_{0.5}\) value for 2-oxoglutarate by a half but did not change the maximum reaction half rate on saturating the complex with the substrate. The pattern of the ADP effect was similar to that observed by ourselves [15] and other authors [13, 16] for OGDC from other animal sources.

Studies on the inhibition of the oxoglutarate dehydrogenase reaction by the reaction products have shown that succinyl-CoA produces a competitive type inhibition of OGDC with respect to CoA (Fig. 3a), whereas NADH gives a mixed type inhibition with respect to NAD (Fig. 3b). Considering the values

![Graph](image)

Fig. 3. Double reciprocal plot of the dependence of the initial rate of OGDC-catalyzed reaction on the concentration of CoA (A) and NAD (B). A: ○, without succinyl-CoA; ●, in the presence of 100 μM succinyl-CoA; B: ○, without NADH; ●, in the presence of 50 μM NADH.

for the \(K_i/K_m\) ratio and the concentrations of these compounds in heart tissue [17], one can draw the conclusion that the NADH-induced inhibition may be most significant for control of OGDC activity in situ. The mixed type of OGDC inhibition by NADH with respect to NAD indicates that it is manifested not only at the active site of the lipoamide dehydrogenase component. More detailed studies are needed to solve this problem. Nevertheless, our data on kinetic properties of OGDC and PDC from human heart seem to be useful for elucidation of pathways and mechanisms of metabolic control in cardiomyocyte mitochondria.
REFERENCES