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QUARTERLY

Basic properties of the pyruvate dehydrogenase complex isolated from aurochs heart

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The purified aurochs (*Bison bonasus*, European bison) heart pyruvate dehydrogenase complex (PDC) has a set of subunits typical of mammalian PDC. PDC from aurochs heart contains firmly bound tiamine pyrophosphate in the amount providing over 50% of the maximal activity of the complex. The apparent value for activation energy of PDC is 60 kJ/mol.

The Michaelis constant values for aurochs heart PDC are 22.4 ± 1.0, 3.3 ± 0.1 and 24.4 ± 3.6 μM for pyruvate, CoA and NAD, accordingly. Acetyl-CoA is a competitive inhibitor with respect to CoA (K_i = 14.2 ± 0.4 μM), whereas NADH gives the same inhibition with respect to NAD (K_i = 46.9 ± 10.0 μM). The K_m for CoA and NAD of the aurochs heart PDC are lower than that of domestic animals PDC.

Biochemical adaptation and evolution of organisms is greatly based on changes in some of the functional and regulatory properties of key enzymes of metabolism [1, 2]. Such changes were established in the comparative studies of enzymes from tissues of evolutionarily distinct organisms [3, 4]. However, it was not completely elucidated whether the properties of the enzymes from tissues of evolutionarily related wild and domestic animals, which lived in different conditions, were changed. Thus, we have undertaken the investigation of precisely this aspect of the metabolically important pyruvate dehydrogenase complex (PDC)[1] from a new source — the heart of an aurochs. The acquirement of information about the properties of aurochs heart PDC will provide the possibility for comparison with the properties of the sufficiently studied PDC from bovine heart and other organs [5–8].

In this short article we have represented only the basic properties of aurochs heart PDC which have been established in the first stages of our investigation.

As it has been known, the pyruvate dehydrogenase multienzyme complex catalyses the overall reaction:

$$\text{Pyruvate} + \text{CoA} + \text{NAD} \rightarrow \text{Acetyl-CoA} + \text{NADH} + H^+ + CO_2$$

The mammalian PDC consist of multiple copies of three catalytic components, pyruvate dehydrogenase, E_1 (EC 1.2.4.1), lipoamide acetyltransferase, E_2 (EC 2.3.1.12), lipoamide dehydrogenase, E_3 (EC 1.6.4.3) and some copies of regulatory enzymes, E_1-kinase and E_1-phosphatase [6, 9]. The pyruvate dehydrogenase component uses thiamine pyrophosphate (TPP) as coenzyme for catalysis. This first component of the complex respond to a number of regulatory signals by means of a phosphoryla-

[1]Abbreviations: PDC, pyruvate dehydrogenase complex; TPP, thiamine pyrophosphate.

tion-dephosphorylation mechanism [10–11]. The total relative molecular mass of the bovine heart PDC is approximately 8.5×10^6 [6, 11].

MATERIALS AND METHODS

As a source to obtain highly purified PDC preparation we used hearts of 7 aurochs (*Bison bonasus*, European bison) eliminated in the National Park of Białowieża (Poland).

PDC was purified according to the method suggested for the same complex from bovine heart [12]. The initial PDC specific activity in homogenates of aurochs heart was 0.04 U/mg protein. The purified PDC preparation had specific activity of 11.2 U/mg protein. Therefore, the degree of purification was 280 fold. One unit of PDC activity is defined as 1 μmole of NADH formed per minute and was based on the initial rate. SDS-polyacrylamide gel electrophoresis was performed essentially by the method of Laemmli [13], using 3% stacking gels and 7.5% running gels. Gels were stained with Coomassie Brilliant Blue.

The initial rate of the PDC-catalyzed reaction was recorded by measuring NADH formation at 340 nm with spectrophotometer Specord UV/VIS (Carl Zeiss, Jena) using a thermostated cuvette (30°C). The basic reaction mixture contained 50 mM potassium phosphate buffer, pH 7.8, 1 mM dithiothreitol, 1 mM $MgCl_2$, 0.2 mM TPP, 0.2 mM pyruvate (K^+ salt), 0.1 mM CoA and 2 mM NAD. To investigate the inhibitory capacities of the reaction products 0.05 mM acetyl-CoA and 0.1 mM NADH were used. The reaction was started by the addition of 0.4 to 1.6 mg of the PDC preparation. Kinetic parameters were calculated using the double reciprocal plot. The results are mean values for 4 determinations. Data were analysed by linear least-squares regression and statistically counted [14].

RESULTS AND DISCUSSION

SDS-polyacrylamide gel electrophoresis of the purified pyruvate dehydrogenase complex from aurochs heart gave 4 clear-cut and 1 indistinct protein bands (Fig. 1) with estimated relative molecular mass of 72000, 55000, 50000, 41000 and 36000. Such a set of subunits is typical for the same complex from other animal sources [6, 15]. Bands 74000, 55000, 41000 and 36000 belong to lipoamide acetyltransferase, lipoamide dehydrogenase, β-subunit and α-subunit of pyruvate dehydrogenase, respectively. The indistinct band 50000 belongs to protein X which contributes to the binding and function of lipoamide dehydrogenase [11]. Subunits of E_1-kinase and E_1-phosphatase we are not able to see because only a few molecules of these enzymes are in composition of the PDC.

The measurements of the PDC activity at various pH value in the presence of added TPP showed that pH optimum of the aurochs heart PDC was 7.8 in potassium phosphate buffer and 8.2 in Tris/HCl buffer (Fig. 2). In the TPP-free reaction medium the PDC also demonstrated sufficient high activity which was obviously provided by tightly bound endogenous coenzyme. In this case the pH optimum was shifted towards the pH value of 7.4 and 7.6 in phosphate and Tris/HCl buffer, respectively. It is possible that ionization of essential groups in active centers is changed at full saturation of PDC by TPP.

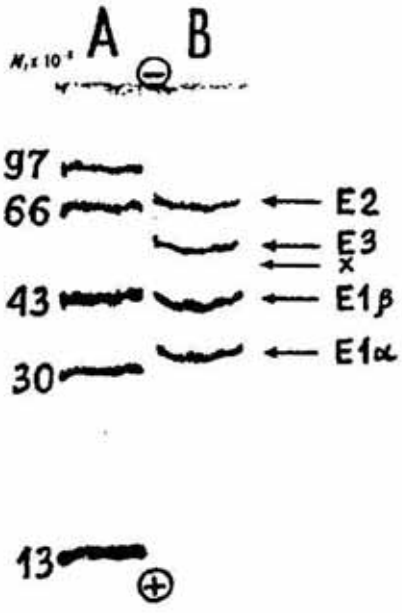

Fig. 1. SDS-polyacrylamide gel pattern of purified pyruvate dehydrogenase complex (10 μg) from aurochs heart (lane B).
Relative molecular mass markers (lane A): phosphorylase *b* from rabbit muscle (97000), bovine serum albumin (66000), ovalbumin (43000), human carbonic anhydrase (30000), cytochrome c (13000).

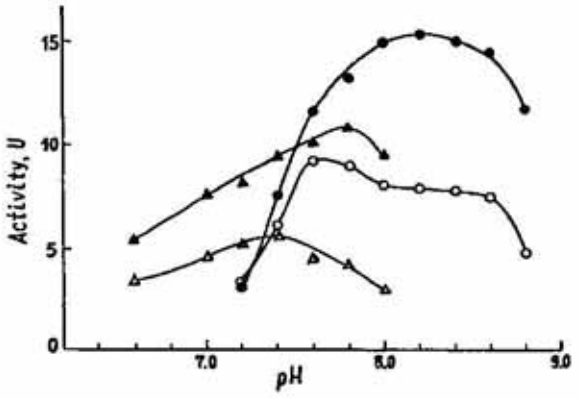

Fig. 2. Dependence of PDC activity on pH of the reaction medium.
●, Tris/HCl buffer, in the presence of 0.2 mM TPP; ○, Tris/HCl buffer, without added TPP; ▲, K-phosphate buffer, in the presence of 0.2 mM TPP; △, K-phosphate buffer, without added TPP.

From the ratio of the PDC-reaction rate in presence of saturating concentration of exogenous TPP and in its absence we may suggest that the PDC from aurochs heart did not completely lose TPP during purification. This was also shown for the PDC from pig heart [16], human heart [17] and bovine adrenal glands [8]. With the allowance for different pH optima the degree of saturation of aurochs heart PDC by endogenous tightly bound TPP was above 50%. This parameter of the aurochs heart PDC was higher then that of the PDC from other sources [8, 16, 17]. The occurrence of great amount of tightly bound TPP in highly purified preparation of the aurochs heart PDC is interesting. It may become the object of a very thorough study in future.

Studies on the dependence of the initial rate of PDC reaction to temperature gave the next results. The Arrenius plot of this dependence was linear up to 39°C (Fig. 3). The apparent value for activation energy of the PDC was 60 kJ/mol. At 39°C the Arrenius plot had a curvature which may indicate that above this temperature the PDC were capable of undergoing conformational changes (the possibility of denaturation) accompanied by an alteration in the catalytic activity.

Using the sufficiently broad range of substrates concentrations we found that the dependence of the initial rate of the aurochs heart PDC-catalyzed reaction to the concentration of each of the three substrates (pyruvate, CoA and NAD) obeyed Michaelis-Menten equation. The values for the Michaelis constants, as calculated using the double reciprocal plots (Figs. 4–6) are 22.4 ± 1.0 μM, 3.3 ± 0.1 μM and 24.4 ± 3.6 μM for pyruvate, CoA and NAD, respectively.

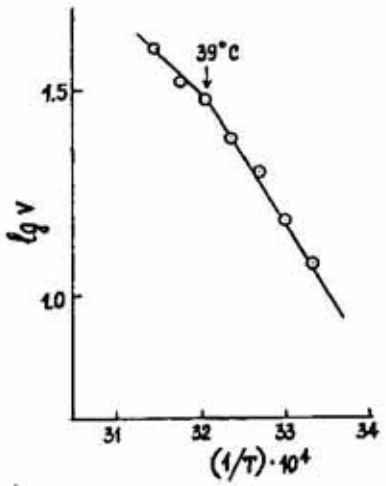

Fig. 3. Dependence of PDC reaction rate on temperature (Arrenius plot).

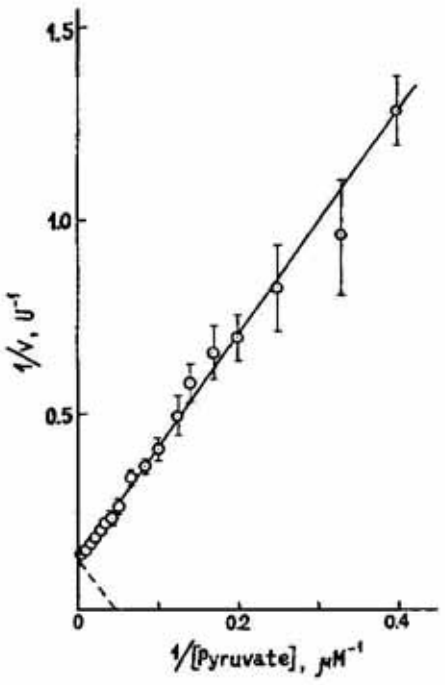

Fig. 4. Double reciprocal plot of the dependence of the initial rate of PDC-catalyzed reaction on pyruvate concentration.
Each point indicates mean value ± S.E.

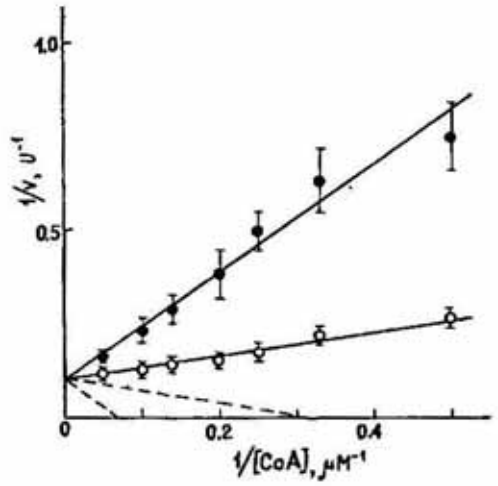

Fig. 5. Double reciprocal plots of the dependence of the initial rate of PDC-catalyzed reaction on CoA concentration.
○, In the absence of acetyl-CoA; ●, in the presence of 50 μM acetyl-CoA. Each point indicates mean value ± S.E.

As expected, the end products of the reaction inhibited the pyruvate oxidative decarboxylation rate. In particular, acetyl-CoA produced a competitive inhibition ($K_i = 14.2 \pm 0.4$ μM) with respect to CoA (Fig. 5), whereas NADH gave the same type inhibition ($K_i = 46.9 \pm 10.0$ μM) with respect to NAD (Fig. 6). The value for the K_i/K_m ratio for NADH and NAD amounted to 1.9. Therefore NADH inhibition seemed to be important in terms of regulation of PDC from the aurochs heart. In this aspect the inhibitory effect of acetyl-CoA was less efficient since the K_i value exceeded the K_m for CoA by more than 4-fold.

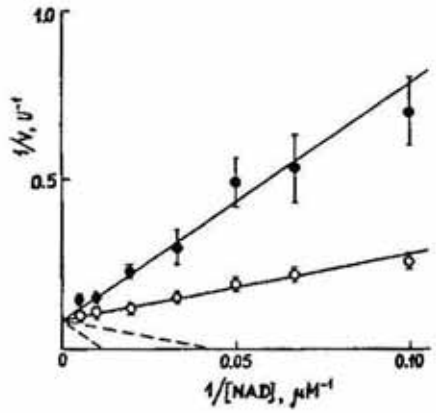

Fig. 6. Double reciprocal plot of the dependence of the initial rate of PDC-catalyzed reaction on NAD concentration.
○, In the absence of NADH; ●, in the presence of 100 μM NADH. Each point indicates mean values ± S.E.

Interestingly, the Michaelis constants for CoA and NAD of the aurochs heart PDC are considerably lower than that of the PDC from bovine tissues [5, 18] (the most suitable material for comparison), other domestic animal tissues [15, 19] and human heart [20, 21]. This may indicate a higher apparent affinity of aurochs heart PDC for these substrates-coenzymes. Possibly the aurochs heart PDC is more catalytically active than PDC of other origins at low substrate-coenzyme concentrations in the medium.

It may well be that there are other peculiarities of aurochs heart PDC regarding for example the mechanism of phosphorylation-dephosphorylation, but more detailed studies are needed to solve this problem.

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REFERENCES

1. Hochachka, P.W. & Somero, G.N. (1973) *Strategies of biochemical adaptation.* W.B. Saunders Company, Philadelphia, London, Toronto.
2. Somero, G.N. & Hochachka, P.W. (1971) Biochemical adaptation to the environment. *Amer. Zoologist* **11**, 159–167.
3. Fortin, D., Coulon, J.F. & Roberge, A.G. (1993) Biochemical parameters and kinetic properties of dopamine-β-hydroxylase activity from cat and rat adrenals. *Comp. Biochem. Physiol.* **104B**, 567–576.
4. Ogawa, H., Gomi, T. & Fujioka, M. (1993) Mammalian glycine *N*-methyltransferases — comparative kinetic and structural properties of the enzymes from human, rat, rabbit and pig liver. *Comp. Biochem. Physiol.* **106B**, 601–612.
5. Linn, T.C., Pelley, J.W., Pettit, F.H., Hucho, F., Randall, D.D. & Reed, L.J. (1972) α-Keto acid dehydrogenase complexes. XV. Purification and properties of the component enzymes of the pyruvate dehydrogenase complexes from bovine kidney and heart. *Arch. Biochem. Biophys.* **148**, 327–342.
6. Barrera, C.R., Namihira, G., Hamilton, L., Munk, P., Eley, M.H., Linn, T.C. & Reed, L.J. (1972) α-Keto acid dehydrogenase complexes. XVI. Studies on the subunit structure of the pyruvate dehydrogenase complexes from

bovine kidney and heart. *Arch. Biochem. Biophys.* **148**, 343–358.

7. Hucho, F., Randall, D.D., Roche, T.E., Burgett, M.W., Pelley, J.M. & Reed, L.J. (1972) α-Keto acid dehydrogenase complexes. XVII. Kinetic and regulatory properties of pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase from bovine kidney and heart. *Arch. Biochem. Biophys.* **151**, 328–340.
8. Strumilo, S.A. (1988) Hysteresis behaviour of the pyruvate dehydrogenase complex containing endogenous thiamin pyrophosphate; in *Tiamin pyrophosphate biochemistry* (Schellenberger, A. & Schowen, R.L., eds.) pp. 83–91, CRC Press, Boca Raton, Florida.
9. Wieland, O.H. (1983) The mammalian pyruvate dehydrogenase complex: structure and regulation. *Rev. Physiol. Biochem. Pharmacol.* **96**, 123–170.
10. Yeaman, S.J. (1989) The 2-oxo acid dehydrogenase complex: recent advances. *Biochem. J.* **257**, 625–632.
11. Patel, M.S. & Roche, T.E. (1990) Molecular biology and biochemistry of pyruvate dehydrogenase complexes. *FASEB J.* **4**, 3224–3233.
12. Stanley, C.J. & Perham, R.N. (1980) Purification of 2-oxo acid dehydrogenase multienzyme complexes from ox heart — a new method. *Biochem. J.* **191**, 147–154.
13. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (London)* **227**, 680–685.
14. Keleti, T. (1986) *Basic enzyme kinetics*. Akademiai Kiado, Budapest.
15. Roche, T.E. & Cate, R.L. (1977) Purification of porcine liver pyruvate dehydrogenase complex and characterization of its catalytic and regulatory properties. *Arch. Biochem. Biophys.* **183**, 664–677.
16. Walsh, D.A., Cooper, R.H., Denton, R.M., Bridges, B.J. & Randle, P.J. (1976) The elementary reactions of the pig heart pyruvate dehydrogenase complex. A study of the inhibition by phosphorylation. *Biochem. J.* **157**, 41–67.
17. Kiselevsky, Y.V., Ostrovtsova, S.A. & Strumilo, S.A. (1990) Interaction of the pyruvate dehydrogenase complex from human heart with tiamine pyrophosphate. *Biomed. Biochim. Acta* **49**, 285–287.
18. Tsai, C.S., Burgett, M.W. & Reed, L.J. (1973) α-Keto acid dehydrogenase complexes. XX. A kinetic study of the pyruvate dehydrogenase complex from bovine kidney. *J. Biol. Chem.* **248**, 8348–8352.
19. Siess, E.A., Wittmann, I. & Wieland, O.H. (1971) Interconversion and kinetic properties of pyruvate dehydrogenase from brain. *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 447–452.
20. Kiselevsky, Y.V., Ostrovtsova, S.A. & Strumilo, S.A. (1990) Kinetic characterization of the pyruvate and oxoglutarate dehydrogenase complexes from human heart. *Acta Biochim. Polon.* **37**, 135–139.
21. Strumilo, S., Kiselevsky, Y. & Ostrovtsova, S. (1991) Properties of the 2-oxo acid dehydrogenase complexes from human heart; in *Biochemistry and Physiology of Thiamin Diphosphate Enzymes* (Bisswanger, H. & Ullrich, J., eds.) pp. 200–208, Verlag-Chemie, Weinheim.