Acta Biochimica Polonica

Vol. 40 No. 3/1993

QUARTERLY

Comparison of the kinetic properties of the pyruvate dehydrogenase complex from pig kidney cortex and medulla*

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Received 7 April, 1993

The activity of the pyruvate dehydrogenase complex (PDC) purified from pig kidney medulla was affected by K^+, Na^+, Cl^-, HCO_3^-, HPO_4^{2-} and changes in ionic strength. Increased ionic strength influenced the activity of PDC from medulla by decreasing the V_{max} and $S_{0.5}$ for pyruvate and increasing the Hill coefficient. The magnitude of these changes was smaller than the corresponding changes for PDC purified from the cortex.

In the presence of K^+ (80 mM), Na^+ (20 mM), Cl^- (20 mM), HCO_3^- (20 mM), HPO_4^{2-} (10 mM) and at ionic strength of 0.15 M the $S_{0.5}$ for pyruvate of PDC from medulla was 117 μM and the enzyme complex was saturated by 1.1 mM pyruvate. Under these conditions the $S_{0.5}$ for pyruvate of PDC derived from cortex was 159 μM and the enzyme was saturated at 4.5 mM pyruvate.

Based on the results presented in this report it is suggested that PDC in kidney medulla may be regulated not only by a phosphorylation/dephosphorylation system and end-product inhibition but also *via* changes in ionic strength.

Kidney is a tissue with a high degree of specialization. Individual regions of kidney show considerable morphological and functional differences [1 - 3]. In the cortex fatty acids, ketone bodies, lipids, amino acids, lactate and glucose are all metabolic fuels for respiration [4 - 6]. Kidney cortex is the only tissue except liver where glucose is synthesized [4, 5, 7]. Kidney contributes 20 - 30% of blood glucose under normal conditions and as much as 50% during starvation or diabetes [8]. It has been shown that glucose metabolism is involved in bicarbonate transport [9] and potassium secretion [10]. The free-water clearance and maintenance of the glomerular filtration rate also are dependent on glucose metabolism [11, 12]. Glucose can be metabolized by glycolysis (to lactate or pyruvate) and the pentose phosphate pathway. The rate of entry of glucose carbon into the tricarboxylic acid cycle is controlled by the pyruvate dehydrogenase complex (PDC)[1]. This enzyme links glycolysis with other metabolic pathways such as ketogenesis, lipogenesis and the tricarboxylic acid cycle. Renal carbohydrate metabolism is regulated by free fatty acids, which alter PDC activity [8, 13]. The metabo-

*Supported by a grant from the NIH, HL-24654 (MSO)

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[1]Abbreviatons used: PDC, pyruvate dehydrogenase complex; PDH, pyruvate dehydrogenase (E1); PDH_a, active form of PDH; E2, dihydrolipoamide acetyltransferase; TPP, thiamine pyrophosphate; TEA, triethanolamine

lism of outer medulla is characterized by rapid rates of aerobic and anaerobic glycolysis [14 - 16]. In contrast, inner medulla, especially the papillary tip, is unable to oxidize glucose significantly even at high oxygen tension [13]. Such different metabolic activities are closely related to the electrolyte transport functions of the kidney [17 - 19]. The cells of the kidney medulla are the only mammalian cells, which are normally exposed to high concentrations of such solutes as urea and NaCl. It has been found that extracellular concentrations of urea and NaCl in renal papilla can reach 1.5 M and 0.45 M, respectively [20]. Intracellular concentration of potassium plus sodium salts is not as high as the extracellular concentration [21]. Indeed, an intracellular osmolality equal to the extracellular osmolality in medulla cells is maintain by high concentrations of betaine [22], lactate [23], glycerolphosphocholine, inositol, and sorbitol [24], amino acids and their derivatives [25, 26]. Some of these compounds contribute to ionic strength of the cell. Thus, the intracellular ionic strength of renal medulla cells can be much higher than in other cells, including renal cortex cells.

In addition, it has been reported that the intracellular concentration of these solutes changes in response to alterations in the diuretic state [27 - 29]. Large quantities of amino acids are present in outer and inner medulla, and their accumulation is osmoregulated by the state of hydration [26]. In the light of these facts it was of interest to compare the regulatory properties of the pyruvate dehydrogenase complex from kidney cortex and medulla.

The activity of the pyruvate dehydrogenase complex in mammalian cells is regulated by end-product inhibition [30, 31] and by interconversion between the active dephosphorylated form and the inactive phosphorylated form by a specific phosphatase/kinase regulatory system [32]. In extensive studies on this system it has been shown that cofactors, univalent and bivalent cations, and reaction products regulate both the kinase and phosphatase activity (for review, see [33, 34]). The pyruvate dehydrogenase interconversion occurs *in vivo* in response to metabolic conditions. In kidney, marked changes in the amount of PDH_a were observed in response to starvation and diabetes [35 - 37].

In previous reports [38, 39] we have shown that the activity of pyruvate dehydrogenase complex from pig kidney cortex is affected by a number of mono- and divalent ions, such as K^+, Na^+, Cl^-, HCO_3^-, HPO_4^{2-}, and changes in ionic strength. In the present study it is shown that the pyruvate dehydrogenase complex from pig kidney medulla is less sensitive to changes in ionic strength than the enzyme purified from the cortex. In the presence of the mentioned above ions the regulatory properties of PDC from medulla are different from those of the cortex-derived enzyme complex.

EXPERIMENTAL PROCEDURES

Materials. Pyruvic acid, imidazole, NAD^+, cysteine hydrochloride, bovine serum albumin, Tris (Tris[hydroxymethyl] aminomethane), Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid), Mops (3-[*N*-morpholino]propanesulfonic acid) were obtained from Sigma Chemical Co. (St. Louis, MO). Thiamine pyrophosphate (TPP) was from Calbiochem-Behring (La Jolla, CA). Coenzyme A was from P-L Biochemicals (Milwaukee, WI). Triethanolamine (TEA) was purchased from Fischer Scientific (Fair Lawn, NJ). All other chemicals were of the highest grade commercially available. Highly purified preparations of the pyruvate dehydrogenase complex from pig kidney cortex and outer medulla (14 and 13 μmol/min per mg protein, respectively) were prepared as described previously [38]. Both enzyme complexes were 100% in the dephosphorylated active form. The activation was performed by incubation of PDC preparations prior to the second polyethylene glycol precipitation with added partially purified phosphatase in the presence of 10 mM $MgCl_2$ [38].

Methods. The activity of pyruvate dehydrogenase complex was determined by monitoring NADH formation at 340 nm and 30°C with an Aminco DW2 spectrophotometer. The reaction mixture contained 2.5 mM NAD^+, 0.5 mM TPP, 2.6 mM cysteine hydrochloride, 1.0 mM $MgCl_2$, Tris/Hepes buffer, pH 7.8, and 1.0 μg of PDC. Pyruvate (K^+ or Tris salt) was added at varying concentrations (see Figure legends). A stock solution containing the pyruvate dehydrogenase complex (0.2 mg/ml) in Tris/Hepes buffer, pH 7.8, 1.0 mM $MgCl_2$, 2.6 mM cysteine

hydrochloride, 0.5 mM TPP was incubated for 45 min at 0°C before use. The ionic strength and pH were adjusted as required by varying the buffer concentration. The reaction was initiated by addition of coenzyme A following a preincubation period of 45 s at 30°C. Protein was determined by the biuret procedure [40] using bovine serum albumin as a standard.

SDS-polyacrylamide gel electrophoresis was performed essentially by the procedure of Laemmli [41]. Proteins were separated using 3% stacking gels and 7.5% running gels. The stacking gels contained 57 mM Tris / phosphate buffer, pH 6.7, and the running gels contained 258 mM Tris/HCl buffer, pH 8.9. Gels were stained with Coomassie Brillant Blue G-250.

Estimates of the concentrations of conjugate base and conjugate acid for all buffers used in the experiments were calculated from the Henderson-Hasselbalch equation with corrections for total ionic strength as is shown in ref. 42. The ionic strength of assay mixtures were calculated on the basis of the concentration of the ionic form of all additions.

RESULTS

SDS-polyacrylamide gel electrophoresis analysis of the purified pyruvate dehydrogenase complexes from pig kidney cortex and medulla yield nearly identical subunit characteristics (Fig. 1). The gel pattern shows five bands corresponding to dihydrolipoamide acetyltransferase (E2), dihydrolipoamide dehydrogenase (E3), the α- and β- subunits of pyruvate dehydrogenase (E1), and protein X migrating near dihydrolipoamide dehydrogenase. Evidence of proteolysis of the subunits of PDC was not observed (Fig. 1) [43 - 45]. Thus, it can be assumed that the enzyme complexes isolated from both kidney medulla and cortex were structurally intact.

The data presented in Fig. 2 indicate that the activity of PDC from pig kidney medulla was dependent upon ionic strength. Experiments in which different molarities (up to 0.3 M) of mannitol, inositol or sucrose were used showed that the activity of both PDC isolated from medulla

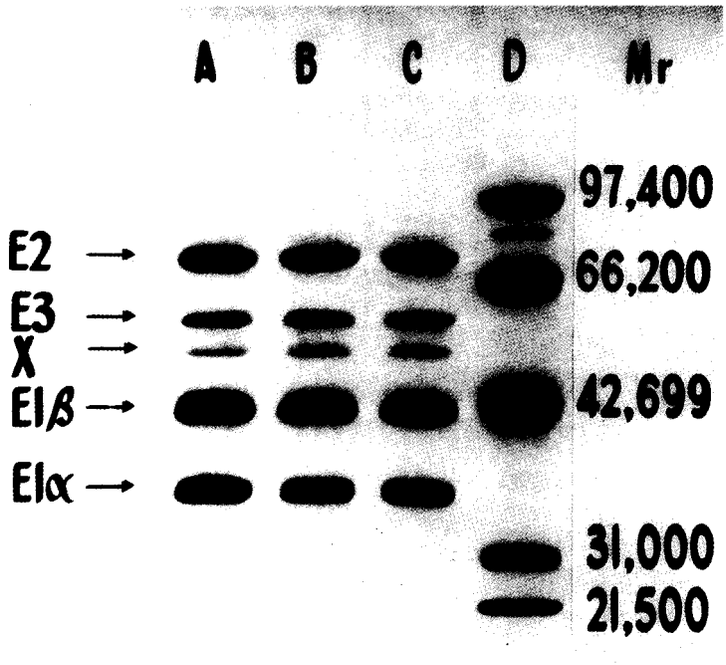

Fig. 1. SDS-polyacrylamide gel pattern of purified pyruvate dehydrogenase complexes.
The pyruvate dehydrogenase complex (25 μg) from pig kidney cortex and medulla are shown in lanes A and C, respectively. Mixed pyruvate dehydrogenase complex from pig kidney cortex (12 μg) and medulla (12 μg) is shown in lane B. The proteins used as molecular markers are: phosphorylase B (rabbit muscle) (97,400), bovine serum albumin (66,200), ovalbumin (42,699), carbonic anhydrase (bovine) (31,000), soybean trypsin inhibitor (21,500).

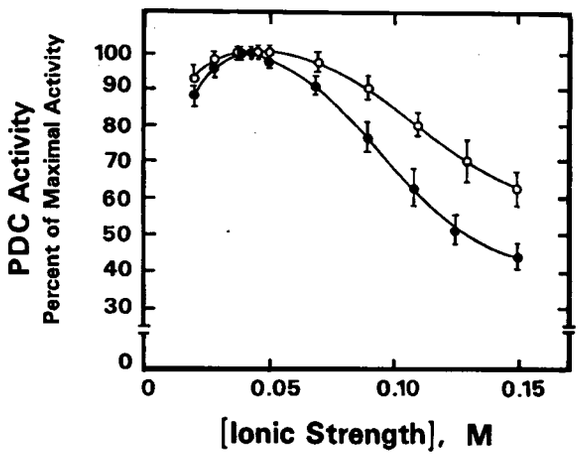

Fig. 2. Effect of ionic strength on the activity of pyruvate dehydrogenase complexes from pig kidney. The activity of PDC from medulla (○) and cortex (●) was determined at pH 7.8 and in the presence of 3.0 mM pyruvate. The results are mean values ± S.D. for 6 parallel determinations on 2 different preparations.

and cortex were not affected by changes in osmolarity (data not shown). The pyruvate dehydrogenase complex from kidney medulla was less sensitive to changes in ionic strength than the enzyme purified from cortex (Fig. 2). Upon increasing the ionic strength from 0.05 M to 0.15 M, there was a 58% decrease in the activity of the cortex enzyme, but only a 37% decrease in the activity of PDC from medulla. The data in Fig. 3 shows that increasing ionic strength caused a decrease in $S_{0.5}$ for pyruvate, and an increase in Hill coefficient. The observed change in Hill coefficient for PDC purified from medulla was the same as for the enzyme complex derived from cortex. Increasing ionic strength from 0.05 to 0.2 M decreased the $S_{0.5}$ for pyruvate from 145 μM to 85 μM for PDC purified from medulla. The same change in ionic strength causes a decrease of this parameter from 125 μM to 58 μM for enzyme derived from cortex (Table 1). As with PDC from cortex [39], ionic strength markedly influenced the pH dependence of the activity of PDC from medulla. At an ionic strength of 0.05 M the activity of PDC from medulla was 95--100% of optimum activity over the pH range from 7.4 to 8.3. At higher ionic strength (0.15 M) the pH optimum for PDC activity from medulla was in range from pH 7.9 to 8.1 (Fig. 4).

Like the pyruvate dehydrogenase complex purified from kidney cortex [38], the activity of the enzyme from medulla was affected by K^+ and Na^+. The relationship between the activity of PDC from medulla and the ion concentration

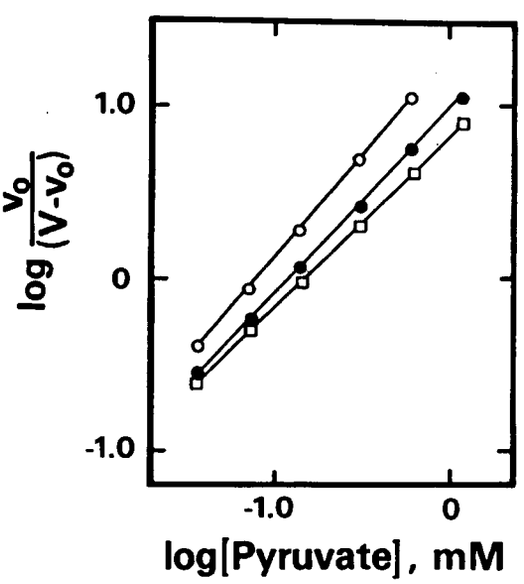

Fig. 3. Effect of ionic strength on the activity of pyruvate dehydrogenase complex from kidney medulla. Hill plot.
The activity of PDC was determined at pH 7.8 and ionic strength of: 0.05 M (□); 0.10 M (●); 0.15 M (○). The results are mean values for 3 determinations.

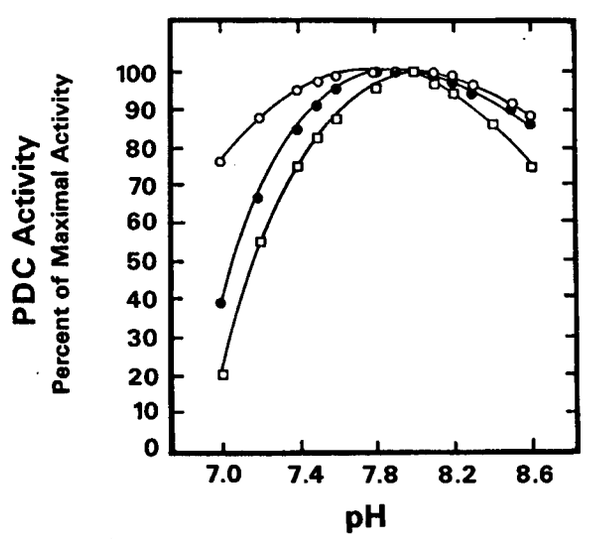

Fig. 4. Effect of pH and ionic strength on the pyruvate dehydrogenase activity from kidney medulla.
The activity of PDC was determined in the presence of 3.0 mM pyruvate and ionic strength of 0.05 M (○), 0.10 M (●) and 0.15 M (□). The results are mean values from 3 determinations.

Table 1

Effect of ionic strength on the kinetic constants of the pyruvate dehydrogenase complex from kidney medulla and cortex

The activity of PDC was measured at pH 7.8. The V_{max} values have been normalized to 100%, which represents activities of 11.1 - 14.2 and 11.8 - 15.2 μmol/min per mg protein for PDC preparations from medulla and cortex, respectively. The results are mean values ± S.D. for 5 parallel determinations on 3 different preparations.

Ionic strength	V_{max}		$S_{0.5}$ for pyruvate	
	cortex PDC	medulla PDC	cortex PDC	medulla PDC
(M)	(%)	(%)	(μM)	(μM)
0.05	100	100	125 ± 11	145 ± 11
0.10	68 ± 6	85 ± 8	91 ± 14	120 ± 12
0.20	38 ± 9	60 ± 7	58 ± 9	85 ± 9
0.30	32 ± 7	52 ± 6	42 ± 9	72 ± 10

was sigmoidal for Na^+ and K^+ (not shown). Both K^+ and Na^+ at concentrations of 20 and 80 mM stimulated the activity of PDC from medulla by about 5 and 20%, respectively (Table 2). The stimulation of PDC purified from the cortex by Na^+ was quite different from that observed for K^+ at concentrations ranging up to 40 mM [38]. Both Na^+ and K^+ affected the activity of PDC purified from medulla by changing the V_{max} and the Hill coefficient (Table 2).

The activity of the pyruvate dehydrogenase complex purified from pig kidney medulla

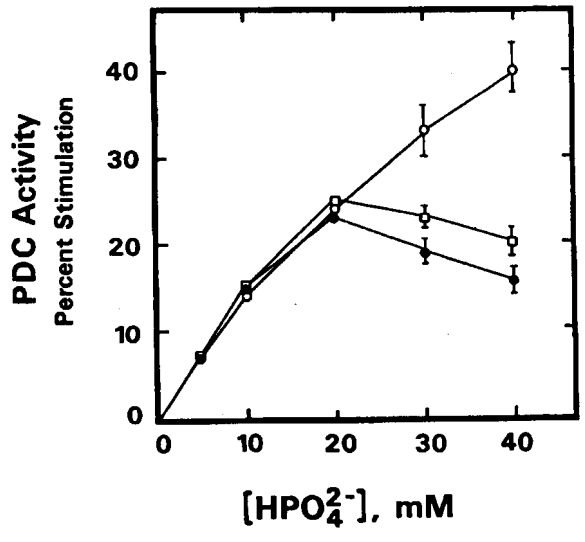

Fig. 5. Effect of HPO_4^{2-} on the activity of pyruvate dehydrogenase complex from kidney medulla.
The activity of PDC was measured at 3.0 mM pyruvate, pH 7.8 and ionic strength of 0.13 M. Reaction mixture contained: HPO_4^{2-} as Tris salt (○); HPO_4^{2-} as Na^+ salt (□); HPO_4^{2-} as K^+ salt (●). The results are mean values ± S.D. for 4 determinations on 2 different preparations.

was affected by HPO_4^{2-}. In the presence of 40 mM HPO_4^{2-} a 40% increase in the activity of PDC from medulla was observed (Fig. 5). Our previous data [38] showed that the effect of HPO_4^{2-} on the activity of PDC purified from kidney cortex was half that on the activity of enzyme purified from kidney medulla. The presence of Na^+ or K^+ at concentrations lower than 40 mM had no effect on the HPO_4^{2-}-mediated stimulation of the activity of PDC from medulla. However, the effect of HPO_4^{2-} on the activity of this enzyme was markedly diminished when Na^+ or K^+ were present in concentrations higher than 40 mM (Fig. 5). In the presence of 80 mM Na^+ or K^+ the activity of PDC was stimulated by 40 mM HPO_4^{2-} by 20% and 15%, respectively (Fig. 5). In contrast, in the presence of K^+ or Na^+ at a concentration of 80 mM the activity of PDC purified from cortex was stimulated by HPO_4^{2-} (40 mM) by only 2 and 10%, respectively [38].

Cl^- and HCO_3^- at concentrations of 80 mM decreased the activity of PDC purified from medulla by 30% and 19%, respectively (Fig. 6). Inhibition of the activity of PDC from medulla caused by Cl^- and HCO_3^- was additive (not shown).

In the presence of K^+, Na^+, Cl^-, HCO_3^- and HPO_4^{2-} (at the concentrations indicated in Table 2) the activity of PDC purified from kidney medulla was increased by 32%. Results shown in Table 2 indicate, that K^+, Na^+, Cl^-, HCO_3^- and HPO_4^{2-} affected the activity of PDC from kidney medulla by increasing V_{max} and $S_{0.5}$ for pyruvate. In the presence of these ions a 32% increase in $S_{0.5}$ for pyruvate for PDC

Table 2

Effect of K^+, Na^+, Cl^-, HCO_3^- and HPO_4^{2-} on the kinetic constants of the pyruvate dehydrogenase complex from kidney medulla

The activity of PDC was measured at pH 7.8 and ionic strength of 0.15 M. Values for PDC from the cortex are also shown for comparison (*). At 0.05 M ionic strength the V_{max} of PDC preparation from medulla and cortex was 12.78 and 13.87 μmol/min per mg protein, respectively. The results are mean values ± S.D. for 4 parallel determinations on 2 different preparations.

Additions	Concentrations (mM)	V_{max} (μmol/min per mg)	$S_{0.5}$ for pyruvate (μM)	Hill coefficient
None		8.80 ± 0.32	90 ± 9	1.2
		7.24 ± 0.29*	75 ± 12*	1.3*
Na^+	20	9.22 ± 0.26	91 ± 11	1.1; 1.3
K^+	20	9.16 ± 0.27	92 ± 10	1.1; 1.3
Na^+	80	10.55 ± 0.31	91 ± 9	1.1; 1.7
K^+	80	10.85 ± 0.37	88 ± 10	1.1; 1.7
K^+ Na^+ Cl^- HCO_3^- HPO_4^{2-}	80 20 20 20 10	11.44 ± 0.35 9.55 ± 0.32*	117 ± 9 152 ± 11*	1.1; 1.7 1.1*

from medulla was observed. By comparison, under the same conditions, a 2.1-fold increase in $S_{0.5}$ for pyruvate was observed for PDC from kidney cortex (Table 2). In the presence of K^+, Na^+, Cl^-, HCO_3^- and HPO_4^{2-} (at the concentrations indicated in Table 2) the activity of PDC from kidney medulla was saturated by 1.1 mM pyruvate (not shown). Under these conditions the activity of PDC from kidney cortex was saturated by 4.5 mM substrate [38].

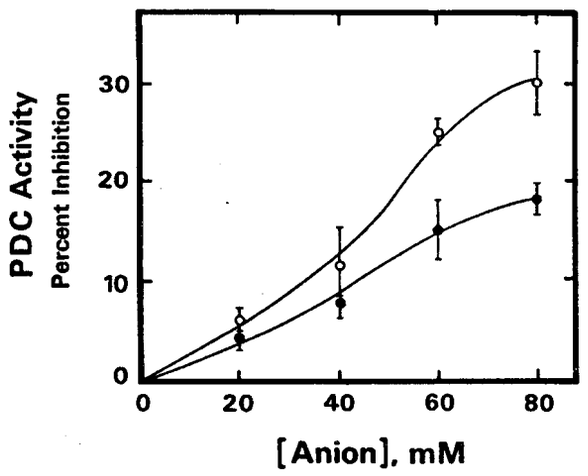

Fig. 6. Effect of Cl^- and HCO_3^- on the activity of pyruvate dehydrogenase complex from kidney medulla.

The activity of PDC was determined at 3.0 mM pyruvate, pH 7.8 and ionic strength of 0.13 M. Reaction mixture contained : Cl^- as Tris salt (○); HCO_3^- as K^+ salt (●). The inhibition by $KHCO_3$ was calculated compared to PDC activity in the presence of adequate concentrations of K^+.

DISCUSSION

The data presented in this contribution indicate that the pyruvate dehydrogenase complex derived from pig kidney medulla has kinetic properties similar but not identical to those of the enzyme from pig kidney cortex. The inhibitory effect of increasing ionic strength was markedly weaker on PDC from medulla than for the enzyme complex from cortex. However, varying ionic strength in the range from 0.05 M to 0.15 M caused the same percentage change in Hill coeficient and $S_{0.5}$ for pyruvate for the enzyme from these two regions of the kidney. The observed increases in the Hill coefficient concomitant with increased ionic strength suggest that changes in the conformation of the complex occur such that a better positive site--site interaction can be achieved. The observed

decrease in V_{max} of PDC as a consequence of increased ionic strength could be the result of a change in protein conformation causing a decrease in the number of catalytic sites or binding sites available for substrate. This finding suggests that varying of the ionic strength may produce a similar effect on the affinity for pyruvate of the two PDCs, but it may have a greater effect on the conformation of PDC purified from pig kidney cortex than enzyme from medulla.

It should be emphasized, that data presented in this contribution come from the experiments on 100% dephosphorylated enzyme complex. Therefore, it could be speculated that partially phosphorylated PDC may exhibit different sensitivity to variation in ionic strength. In the previous report [46] we have shown that the activities of PDC components are differentially sensitive to changes in ionic strength. Component E1 and E2 being the most sensitive. In addition, the activity of pyruvate dehydrogenase kinase is also affected by changes in ionic strength [47]. Within the pyruvate dehydrogenase component there are three seryl residues that are substrates for pyruvate dehydrogenase kinase. The pyruvate dehydrogenase catalyzes the first two steps of the overall reaction, i.e., TPP-dependent decarboxylation of pyruvate to 2-(1-hydroxyethylidene)-TPP, and the reductive acetylation yielding an acetyl group covalently attached to the lipoyl moietes of dihydrolipoamide acetyltransferase [48]. Reductive acetylation is the rate limiting step in the overall reaction [49]. If the phosphorylation of E1 component alters its sensitivity to variation in ionic strength, then the rate of the overall reaction should undergo the same change.

It has been reported that cells in the inner and, to the lesser extent, in outer medulla are surrounded by a highly hypertonic environment [20, 26]. To maintain an equal intracellular to extracellular osmolality, medullary cells accumulate a number of organic solutes at relatively high concentrations [24, 26, 29]. Some of these substances, such as lactate or amino acids, contribute to the maintenance of a high ionic strength. Based on available data it is difficult to draw a conclusion concerning possible reasons for the observed differences in sensitivity to changes in ionic strength of the two enzyme complexes studied. Since there is no evidence of genetic differences in mammalian PDC, it can be speculated that the lower sensitivity to high ionic strength of PDC from medulla is a reflection of fine adaptional changes in the structure of this multienzyme complex.

The results presented in this report indicate that K^+, Na^+, Cl^-, HCO_3^- and HPO_4^{2-} exert different effects on the activity of PDC purified from cortex and medulla, but interpretation of these differences is difficult due to the unavailability of data on the concentration (activity) of these ions in mitochondria from kidney cortex and medulla. In addition, as mentioned above, the data presented here concern PDC in fully dephosphorylated state. In the presence of phosphatase and active kinase and their cofactors the effect of these ions is more complex. We have reported [46] that HPO_4^{2-} stimulates the activity of PDC by activating the E2 component. It was also shown that HPO_4^{2-} decreased the activity of pyruvate dehydrogenase kinase [47]. Cl^- and HCO_3^- inhibit the activity of PDC by acting on E1 component [46] and also decreased the activity of pyruvate dehydrogenase kinase [47]. In the presence of K^+, Na^+, Cl^-, HCO_3^- and HPO_4^{2-} and at ionic strength of 0.15 M the $S_{0.5}$ for pyruvate of PDC from medulla was 117 μM, and the enzyme complex was saturated by 1.1 mM pyruvate. As can be seen in Table 1, an increase in ionic strength by 0.05 M caused a decrease in $S_{0.5}$ for pyruvate by ≈25 μM.

If assumed that ionic strength in medullary cells is much higher and is susceptible to fluctuation, it is likely that under such conditions PDC may be regulated not only by a phosphorylation/dephosphorylation system and end-product inhibition but also *via* changes in ionic strength. In contrast, the kinetic properties of PDC from kidney cortex [38, 39] allow us to speculate that the activity of this enzyme *in situ* may be regulated additionally *via* relatively small changes in the concentration of substrate.

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