

## Some regulatory properties of the 2-oxoglutarate dehydrogenase complex from European bison heart

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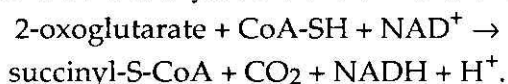
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**Basic regulatory properties of the 2-oxoglutarate dehydrogenase complex (OGDC) isolated and purified from the heart muscle of European bison (*Bison bonasus*) were studied. Kinetic studies have shown that in the absence of phosphate ions OGDC exhibits kinetic attributes of negative cooperativity with respect to 2-oxoglutarate. ADP and phosphate lower  $S_{0.5}$  value of OGDC for 2-oxoglutarate without changing the maximum reaction rate. NADH inhibits OGDC *versus* both 2-oxoglutarate and  $NAD^+$ . Moreover, bison heart OGDC shows negative kinetic cooperativity for  $NAD^+$  and positive kinetic cooperativity for CoA at low CoA concentrations. The latter property has not been observed in earlier studies on OGDC from bovine and pig heart and other tissues of these animals.**

The multienzyme 2-oxoglutarate dehydrogenase complex (OGDC) forms the limiting step in the citric acid cycle [1] and therefore plays a very important role in mitochondrial metabolism. OGDC catalyses the overall reaction:



Animal OGDC consists of multiple copies of three enzymes, designated as  $E_1$ ,  $E_2$  and  $E_3$ .  $E_1$  is 2-oxoglutarate dehydrogenase (EC 1.2.4.2.) which utilises thiamine pyrophosphate as the essential co-factor.  $E_2$  is dihydrolipoamide succinyl transferase (EC 2.3.1.61) with a covalently attached lipoic acid, and  $E_3$  is an FAD-containing dihydrolipoamide dehydrogenase (EC 1.6.4.3) [2, 3]. The total relative molecular mass of mammalian heart OGDC is about  $2.7\text{--}3.3 \times 10^6$ . Electron micrographs of the complex show a polyhedral structure with the diameter of about 26 nm [2, 3].

Detailed studies on kinetic and regulatory properties of OGDC from various sources revealed species-specific and tissue-specific differences between complexes of various origin [4–10]. Therefore, in comparative and evolutionary aspects [11, 12], we have undertaken a study on regulation of OGDC from a new source, the heart of European bison. Such a study may contribute to an interesting comparison of the properties of the same enzyme complex from tissues of evolutionary related domestic animals [7, 9, 13] and lead to a better understanding of OGDC regulation in general.

### METHODS

As a source for the OGDC preparation we used hearts of 7 European bisons (*Bison bonasus*) eliminated in the National Park of Białowieża (Poland). OGDC was purified according

to the method applied for the same complex from bovine heart [14]. The initial OGDC specific activity in homogenates of bison heart was 0.06 IU/mg protein. The purified preparation had a specific activity of 7.6 IU/mg protein. Therefore, the degree of purification was about 126 fold. One unit of OGDC activity is defined as 1  $\mu$ mole of NADH formed per minute at 30°C and was based on the initial rate. The purified OGDC showed 3 clear-cut protein bands (Fig. 1) on sodium dodecylsulfate-polyacrylamide gel electrophoresis [15], which is characteristic for mammalian OGDC [2, 5, 16].

The initial rate of the OGDC-catalysed reaction was recorded by measuring NADH formation at 340 nm with DU-640 spectrophotometer (Beckman) using a thermostated cuvette (30°C). The basic assay mixture contained 50 mM potassium phosphate or Mops-buffer, pH 7.8 (this value was optimal), 1 mM dithiothreitol, 1 mM MgCl<sub>2</sub>, 0.2 mM thiamine pyrophosphate, 0.1 mM CoA, 2 mM NAD<sup>+</sup> and 10 mM 2-oxoglutarate (Na salt). Changes in the concentration of the substrates and the effectors added are shown in the legends to figures. The reaction was started by the addition of 0.5–1.2  $\mu$ g protein of the OGDC preparation.

All experiments were carried out in triplicate. Kinetic parameters were calculated using double reciprocal and Hill plots. The data were analysed by linear least square regression [17].

2-Oxoglutarate, thiamine pyrophosphate, ADP and Mops were obtained from Sigma (U.S.A.), CoA from Fluka (Switzerland), NAD<sup>+</sup> and NADH from Merck (Germany), and dithiothreitol from Loba (Austria).

## RESULTS

SDS-polyacrylamide gel electrophoresis of purified OGDC from bison heart gave 3 clear-cut protein bands (Fig. 1) with estimated relative molecular mass of about 100 000, 55 000 and 43 000. Such a set of subunits is typical for the same complex from mammalian tissues (pig heart, bovine kidney) [5, 14, 16]. Bands 100 000, 55 000 and 43 000 belong to 2-oxoglutarate dehydrogenase, dihydrolipoamide dehydrogenase and dihydrolipoamide succinyl transferase, respectively.

Using a sufficiently wide range of 2-oxoglutarate concentration (0.02–1.00 mM) we found

that the dependence of the initial rate of bison heart OGDC-catalysed reaction on the concentration of this substrate in the presence of phosphate obeyed the Michaelis-Menten equation. The  $K_m$  value, calculated from the Lineweaver-Burk plot, was 0.10 mM (Fig. 2).

However, omission of phosphate ions from the reaction mixture resulted in a non-linear double reciprocal plot with respect to 2-oxoglutarate (Fig. 3). The presence of effectors, ADP and NADH, did not change the shape of the kinetic curves. From Hill plots (Fig. 3b) the Hill coefficients ( $h$ ) were determined as  $0.71 \pm 0.02$  and  $0.69 \pm 0.01$  in the absence and the presence of NADH, respectively. Under these two conditions the plots appeared linear throughout the concentration range tested. Yet, in the presence of ADP the Hill plot seemed to be composed of two lines with different slopes corresponding to  $h$  values of 0.43 and 0.72 for low and high 2-oxoglutarate concentrations, respectively. The values of  $h$  below 1 may be regarded as a kinetic attribute of negative cooperativity [17]. In the case of OGDC the negative cooperativity applies to the 2-oxoglutarate binding sites.

From Fig. 3b the  $S_{0.5}$  values of 1.28, 0.55 and 3.20 mM 2-oxoglutarate in the absence and the

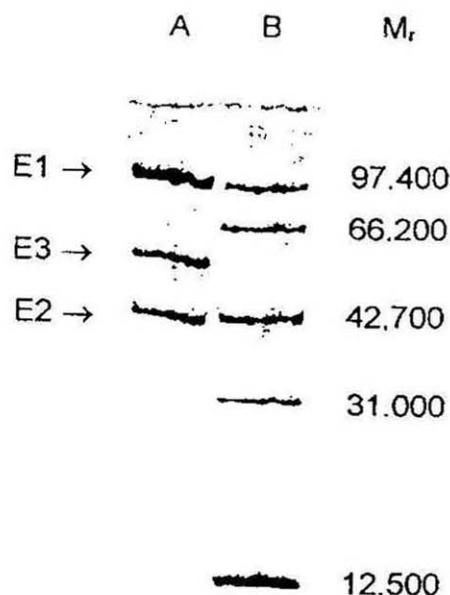


Fig. 1. SDS-polyacrylamide gel pattern of the purified 2-oxoglutarate dehydrogenase complex from European bison heart (lane A).

Relative molecular mass of markers (lane B): phosphorylase *b* from rabbit muscle (97 400), bovine serum albumin (66 200), ovalbumin (42 700), human carbonic anhydrase (31 000), cytochrom *c* (12 500).

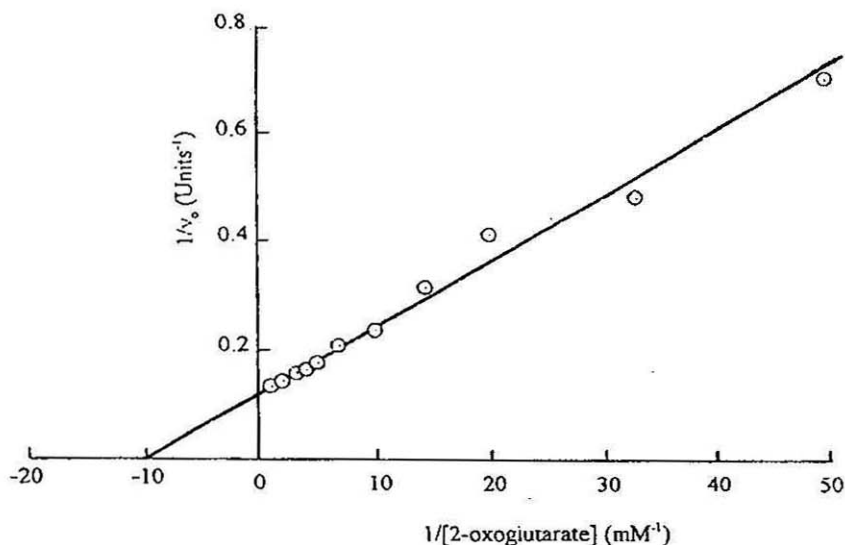


Fig. 2. Lineweaver-Burk plot of the dependence of the 2-oxoglutarate dehydrogenase complex activity on the concentration of 2-oxoglutarate in phosphate buffer, pH 7.8.

presence of ADP and NADH, respectively, were calculated. ADP acted by decreasing the  $S_{0.5}$  value for 2-oxoglutarate without considerably changing the maximum reaction rate. So, this positive effector caused an increase of the apparent affinity of OGDC to its substrate. In contrast, the negative effector NADH (0.05 mM) acted by increasing the  $S_{0.5}$  value for 2-ox-

oglutarate 2.5 times and slightly decreasing the  $V_{max}$ . Comparison of the data from Fig. 2 and Fig. 3 indicated a significant regulatory effect of phosphate ions. In the presence of phosphate the  $K_m$  value for 2-oxoglutarate was a 12.8 times lower than the  $S_{0.5}$  value observed in the absence of phosphate. Changes in the maximum reaction rate were negligible. Moreover,

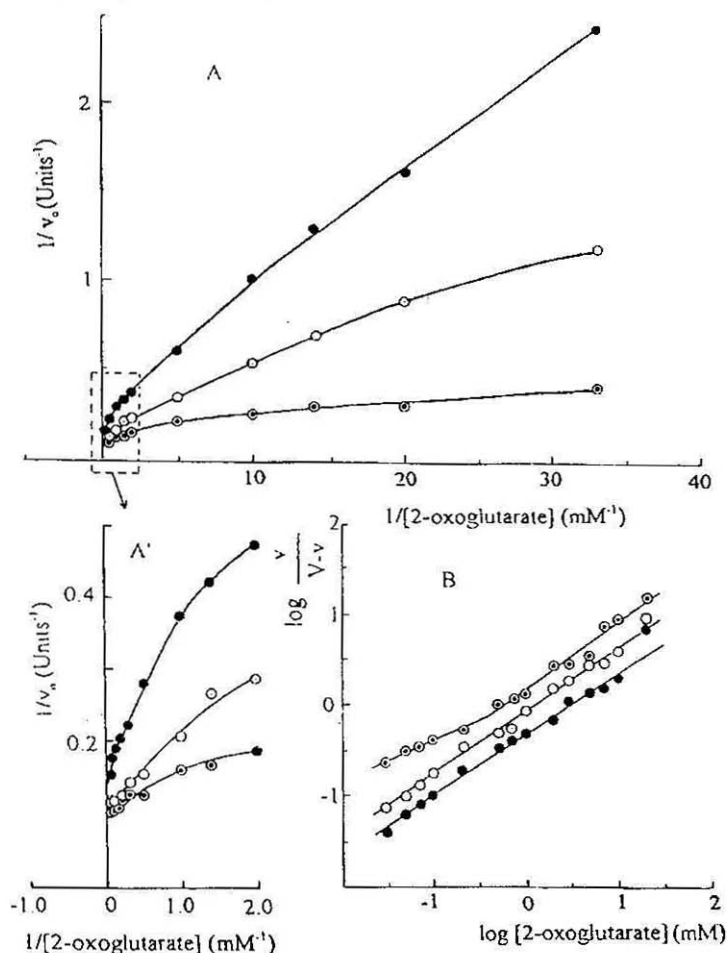


Fig. 3. Activity of the 2-oxoglutarate dehydrogenase complex at various concentrations of 2-oxoglutarate: A, double reciprocal plots; B, Hill plots:  $\circ$ , in the absence of ADP and NADH;  $\bullet$ , in the presence of 0.1 mM NADH;  $\ominus$ , in the presence of 0.5 mM ADP.

phosphate ions abolished the kinetic attributes of the negative cooperativity of the 2-oxoglutarate binding sites. The stimulation of OGDC activity, at low 2-oxoglutarate concentrations, upon addition of potassium phosphate was not due to the presence of additional  $K^+$  as revealed by the observation that addition of 50 mM KCl to Mops buffer had no effect on  $S_{0.5}$  for 2-oxoglutarate.

The next step of our work was to study the dependence of the initial rate of the OGDC-catalysed reaction on the concentration of positive effectors (ADP and phosphate), at a fixed non-saturating (0.05 mM) 2-oxoglutarate concentration, in Mops buffer-containing mixture.

In these conditions the effects of ADP and phosphate were most expressed. Typical hyperbolic curves were obtained in both cases (Fig. 4 and Fig. 5). When  $v_0$  values observed in the absence of ADP and  $P_i$  were subtracted from  $v_0$  values observed in their presence, double reciprocal plots of these data were linear (Fig. 4 b and Fig. 5b). From these plots  $A_{50}$  values of 0.15 mM and 1.33 mM for ADP and phosphate, respectively, could be obtained. One can suggest that these activating effects may have a regulatory significance since they are realised at ADP and phosphate concentrations which can occur *in situ* in various animal tissues [19]. The dependence of the OGDC reaction rate on CoA concentration

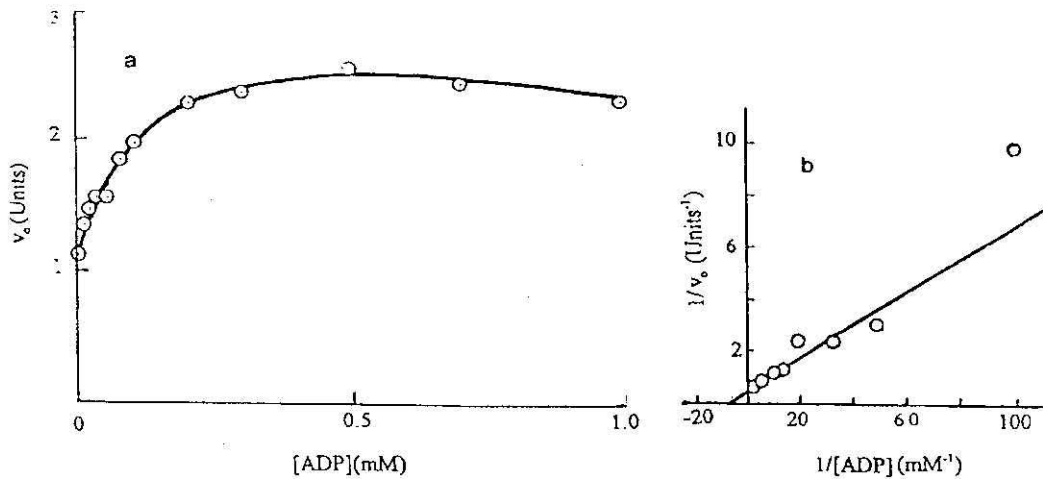


Fig. 4. Dependence of OGDC-catalysed reaction rate on ADP concentration at 0.05 mM 2-oxoglutarate (a); the same data as double reciprocal plot (b).

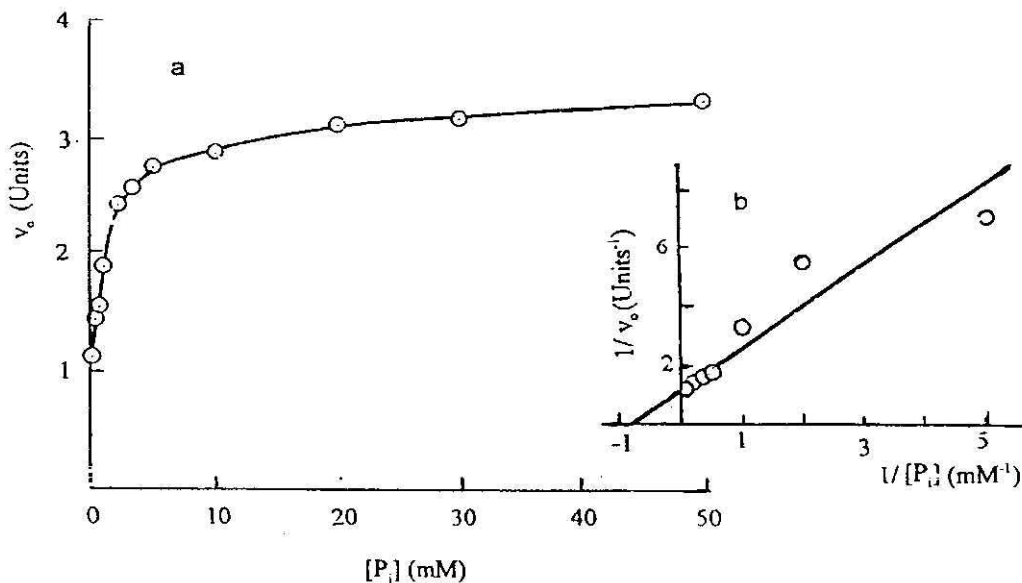


Fig. 5. Dependence of the OGDC-catalysed reaction rate on phosphate concentration at 0.05 mM 2-oxoglutarate (a); the same data as double reciprocal plot (b).

showed a non-linear character in the Lineweaver-Burk plot (Fig. 6a). The Hill plot consisted of two lines with different slopes (Fig. 6b), corresponding to the  $h$  values of 2.57 and 1.00. Therefore, at low CoA concentrations OGDC showed kinetic characteristic of a positive cooperativity with respect to CoA. The  $S_{0.5}$  value for CoA calculated from the Hill plot was 0.004 mM.

Finally, the investigation of the  $\text{NAD}^+$  saturation kinetics for bison heart OGDC showed some peculiarities (Fig. 7). The double reciprocal plots were non-linear. NADH inhibited OGDC in a mixed manner with respect to  $\text{NAD}^+$ . Hill plots revealed  $h$  values of  $0.87 \pm 0.02$  and  $0.80 \pm 0.02$  in the absence and the presence of NADH, respectively. Consequently, in this case there was a kinetic attribute of negative

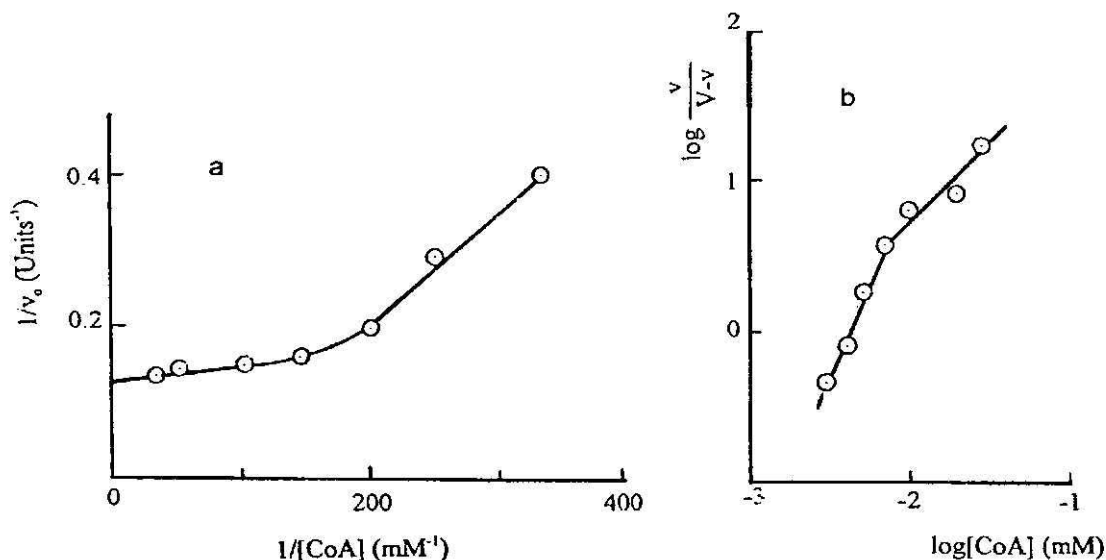


Fig. 6. Double reciprocal plot of the dependence of the OGDC reaction rate on CoA concentration (a); the same data as Hill plot (b).

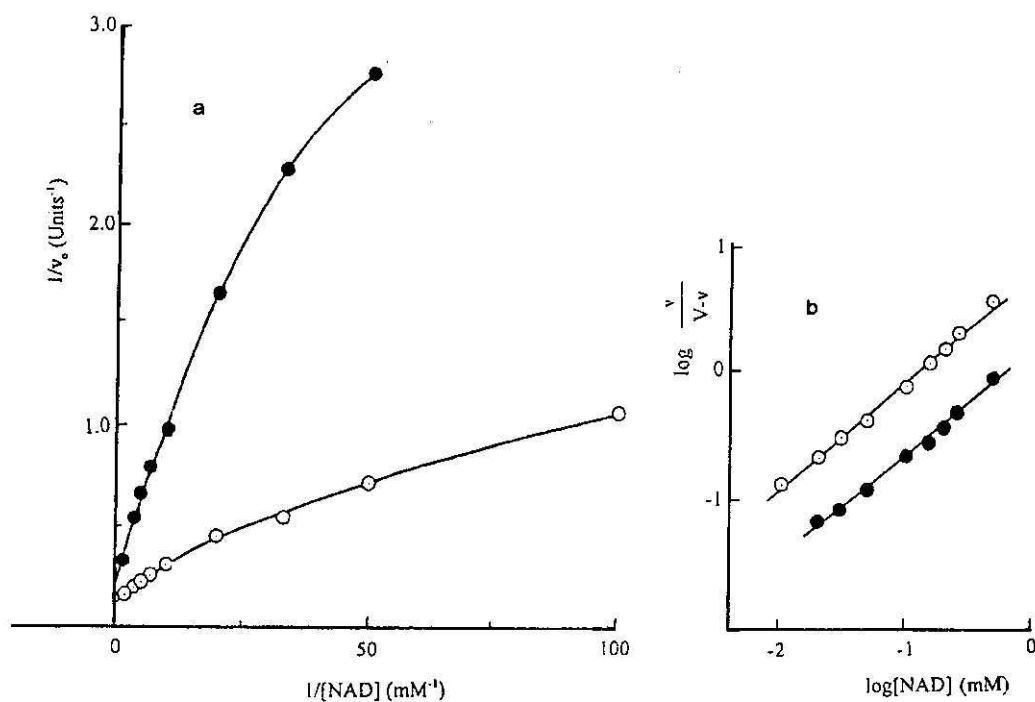


Fig. 7. Double reciprocal plot of the dependence of OGDC on  $\text{NAD}^+$  concentration (a); the same data as Hill plot (b): ○, in the presence of 0.1 mM NADH; ●, in the absence of NADH.



cooperativity with respect to  $\text{NAD}^+$ . In the absence of NADH, the  $S_{0.5}$  value for  $\text{NAD}^+$  was 0.08 mM.

## DISCUSSION

Comparison of the relative molecular masses of subunits of OGDC from various sources showed that the subunit of dihydrolipoamide succinyl-transferase of bison OGDC has lower  $M_r$  value (approx. 43 000) than that of OGDC from bovine kidney (approx. 52 000) [10, 18], adrenal cortex (approx. 52 000) [16] and pig heart (approx. 48 000) [2]. The lower relative molecular mass of the  $E_2$  subunit may not be the result of a proteolytic cleavage because these subunits compose the inner OGDC core which is surrounded by  $E_1$  and  $E_2$  molecules and is inaccessible to proteases. Moreover, the electrophoregram of OGDC does not contain any additional bands (Fig. 1). This interesting difference of  $M_r$  of  $E_2$  subunits requires a more profound investigation in the structural aspect.

The data presented here indicate that the 2-oxoglutarate dehydrogenase complex isolated from European bison heart has kinetic and regulatory properties similar but not identical to those of OGDC from tissues of other animals [5, 7–10, 13, 16, 21]. In particular, bison heart OGDC exerted positive kinetic cooperativity of the CoA-binding sites and negative kinetic cooperativity of the  $\text{NAD}^+$ -binding sites.

On studying the dependence of the OGDC activity on 2-oxoglutarate concentration we confirmed the effects of ADP, phosphate and NADH on the  $S_{0.5}$  value with no considerable change in the maximum rate as reported by other workers [5–7, 13]. All these data showed that ADP and phosphate lowered the  $S_{0.5}$  for 2-oxoglutarate and that NADH increased the  $S_{0.5}$  for 2-oxoglutarate. Similarly to the OGDC from bovine heart and kidney, bison heart OGDC showed kinetic characteristics (based on Hill coefficients below 1.0) of negative cooperativity involving site-site interactions that influenced the binding of 2-oxoglutarate [7, 13]. However, European bison heart OGDC showed the Hill coefficient that was somewhat lower (0.43–0.72) than observed with OGDC from bovine heart (0.84–0.90) [7].

Moreover, OGDC from mammalian tissues (pig heart, bovine kidney) was regulated by the

levels of  $\text{Ca}^{2+}$  and ATP [5, 7, 10]. Micromolar concentrations of  $\text{Ca}^{2+}$  lowered substantially the  $S_{0.5}$  for 2-oxoglutarate and ATP increased the  $S_{0.5}$  for this substrate. Preliminary experiments showed that OGDC from bison heart was sensitive to  $\text{Ca}^{2+}$  and ATP (not shown), but we focused on the effects of ADP and phosphate. Analysis of changes in initial rates as a function of phosphate and ADP concentrations at low level of 2-oxoglutarate yielded typical hyperbolic curves for both activators. The value of  $A_{50}$  for phosphate is almost 10 times higher than that for ADP. These results were in agreement with different levels of phosphate and ADP in animal heart muscle [19]. Earlier we established that OGDC from bovine adrenal glands [9] and human heart [22] showed S-shaped curve of saturation with the allosteric activator ADP. A detailed kinetic study on bison heart OGDC did not reveal cooperative interaction of ADP binding sites.

In the present communication we have evaluated NADH inhibition with 2-oxoglutarate and  $\text{NAD}^+$  as the varying substrates. The apparent non-competitive inhibition of NADH *versus*  $\text{NAD}^+$  can be explained by the fact that NADH inhibits both dihydrolipoamide dehydrogenase and the 2-oxoglutarate dehydrogenase component of OGDC. These two NADH inhibitions together confirm the present knowledge that there is an allosteric site in the 2-oxoglutarate dehydrogenase component of OGDC for inhibition by NADH [9, 13, 23]. It is worth noting that OGDC from bison heart shows higher  $S_{0.5}$  value for  $\text{NAD}^+$  than those observed for OGDC from bovine adrenals [8, 16] and pig heart [20].

Thus, our results indicate that bison heart OGDC, like OGDC from other mammalian tissues (bovine heart, kidney and adrenals, and pig heart), is regulated by the reaction product (NADH) and by energy-linked compounds (phosphate, ADP) with a decreased  $S_{0.5}$  value for 2-oxoglutarate at the lower energy state. Furthermore, there are some kinetic peculiarities (negative cooperativity of  $\text{NAD}^+$ -binding sites, positive cooperativity of CoA-binding sites) which may be of importance in the regulation of the 2-oxoglutarate dehydrogenase complex activity in mitochondria. Possibly, the observed functional peculiarities of bison heart OGDC are connected with possible difference in the  $E_2$ -subunit structure (lower relative

molecular mass, see Fig. 1) in comparison with that of OGDC from other mammalian tissues. Probably, all these peculiarities of the kinetic properties of OGDC may have regulatory significance. These data may be interesting from the comparative aspect.

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