EFFECT OF DIVALENT IONS ON PIGEON KIDNEY PYRUVATE CARBOXYLASE

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1. Introduction

In pigeon kidney the carboxylation of pyruvate catalysed by pyruvate carboxylase (pyruvate: CO_2 ligase (ADP), EC 6.4.1.1) in the following way (eq. 1) shows an absolute requirement not only of acetyl-CoA, an allosteric effector [1], but also of extra Mg^{2+} [2].

$$\text{Pyruvate} + HCO_3^- + MgATP^{2-} \xrightarrow{\text{Acetyl-CoA, } Mg^{2+}} \text{Oxaloacetate} + MgADP + P_i \rightarrow \quad (1)$$

The apparent co-operative effect of acetyl-CoA on this enzyme from different sources has been shown by various authors [1, 3–10]. Although this enzyme is known to be found in appreciable quantities only in the liver and the kidney, hardly any attempt has been made to justify the inter-relationship between the positive effectors acetyl-CoA and Mg^{2+} in this enzyme system. Furthermore, it is also known that Mg^{2+} cannot be replaced by any other metal ion in the reaction catalysed by this enzyme and that Ca^{2+} inhibits the enzymic activity of this enzyme [7, 11]. On the other hand Ca^{2+} has been reported to activate other mitochondrial enzymes [12–14]. It is therefore of value to contribute further the results of experiments showing the effect of Ca^{2+} and Mg^{2+} (divalent ions), in the presence of the allosteric effector acetyl-CoA, on pyruvate carboxylase from pigeon kidney. This communication attempts to explain the interrelationship between the allosteric effectors Mg^{2+} and acetyl-CoA, and the effect of Ca^{2+} and Mg^{2+} on this enzyme. The results of this communication differentiate pigeon kidney pyruvate carboxylase from pyruvate carboxylase obtained from other sources.

* The author wishes to dedicate this communication to his teacher, Professor Th. Wieland on his 60th birthday.

2. Materials and methods

Pigeon kidney pyruvate carboxylase was purified and enzymic activity was measured by an optical assay of the rate of NADH-oxidation (eq. 1 and 2). The reaction mixture contained in 2.0 ml: 100 μmoles Tris–HCl buffer, pH 7.7; 80 μmoles $KHCO_3^-$; 16 μmoles $MgCl_2$ (or as shown in figures); 0.4 μmoles NADH; 3 μmoles ATP; 6 μmoles sodium pyruvate; 10.5 U malate dehydrogenase, 0.5 mg serum albumin and 0.15 μmoles acetyl-CoA (or as shown in figures). The concentration of $CaCl_2$ was varied as indicated in fig. 3.

$$\text{Oxaloacetate} + NADH + H^+ \xrightarrow{MDH} \text{malate} + NAD^+ \rightarrow \quad (2)$$

Coenzymes and enzymes were purchased from Boehringer (W. Germany). All the other reagents were of the highest purity commercially obtainable. All solutions were freshly prepared prior to the experiments and all assays were carried out against a control without ATP and acetyl-CoA.

3. Results and discussion

All pyruvate carboxylases purified from mammalian and avian species have been found to be inactive in the absence of acetyl-CoA. In contrast this enzyme from

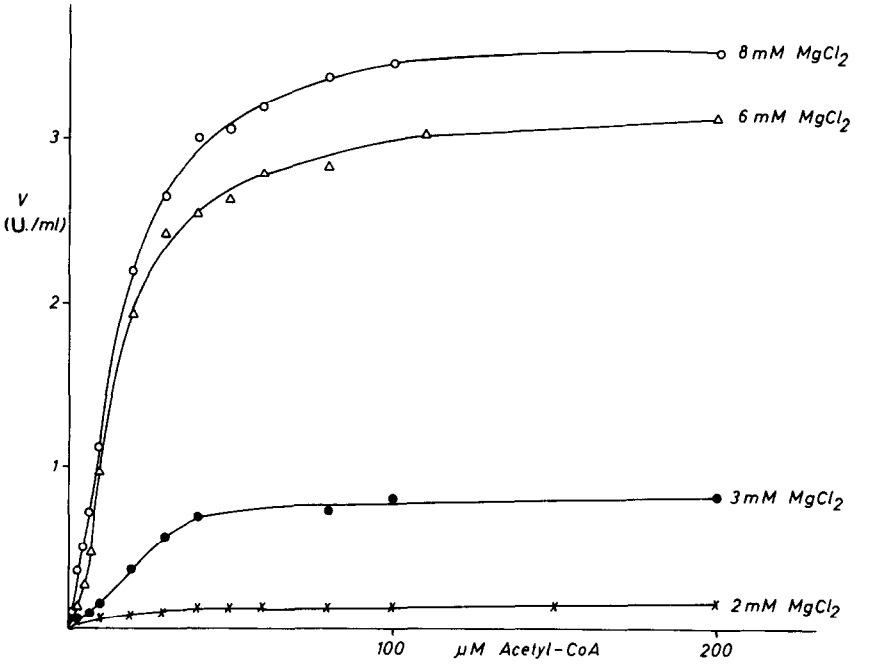

Fig. 1. Allosteric activation of pigeon kidney pyruvate carboxylase by acetyl-CoA at various $MgCl_2$ concentrations, as indicated in the figure. The assay system is as described in Materials and methods.

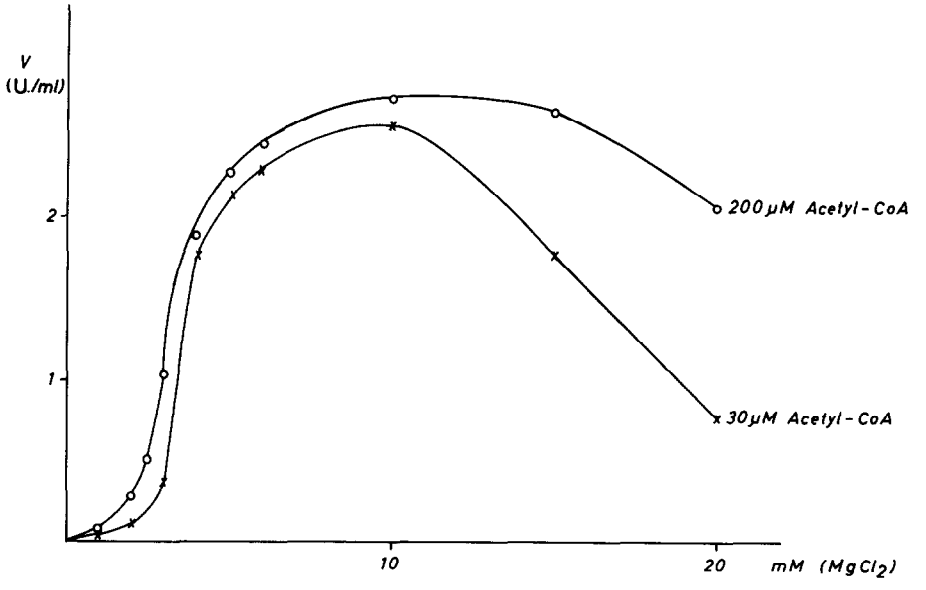

Fig. 2. Allosteric activation of pigeon kidney pyruvate carboxylase by Mg^{2+} at different concentrations of acetyl-CoA, as indicated in the figure. The other experimental conditions were the same as described in Materials and methods.

Table 1
Influence of Mg^{2+} concentration on the binding of acetyl-CoA to pyruvate carboxylase from pigeon kidney.

n (Hill coefficient)	R_S	$S_{0.5}$ (μM)	$MgCl_2$ (mM)
1.5	11	22.0	3
1.3	25	15.5	6
1.1	40	14.0	8

Pseudomonas citronellolis and *Aspergillus niger* [15] shows maximal activity in the absence of an acetyl-CoA. Pyruvate carboxylase from *Saccharomyces cerevisiae* [16–18] is also active in the absence of acetyl-CoA but either CoA-SH or its acetyl derivative is capable of inducing a stimulation of enzymic activity. The pyruvate carboxylases which are essentially inactive in the absence of added acetyl-CoA are interesting since the situation is rarely observed for activators other than metal ions.

As shown in fig. 1, Mg^{2+} greatly influences the affinity of pigeon kidney pyruvate carboxylase for acetyl-CoA. The n-(Hill coefficients), R_S- and $S_{0.5}$ values calculated from the data of fig. 1 are represented in table 1. These data demonstrate an increase in n- and $S_{0.5}$ values, with increasing concentration of Mg^{2+}. These R_S- and n values < 81 and > 1 respectively suggest the positive co-operative effect [20, 21]. This means that the homotropic co-operative effect of acetyl-CoA is decreased by the increasing concentration of the heterotropic effector Mg^{2+}. This can be understood by applying the theory of Monod et al. [19], which predicts that the heterotropic effector, Mg^{2+}, should reduce the homotropic interactions of acetyl-CoA. The values at 2 mM Mg^{2+} are not given further attention, as hardly any appreciable enzymic activity is found (fig. 1). This further supports the suggestion that free Mg^{2+} is essential for the reaction catalysed by pyruvate carboxylase from pigeon kidney [2].

Fig. 2 shows that when the initial reaction velocity is plotted as a function of $MgCl_2$ concentration at two different acetyl-CoA concentrations, a sigmoid kinetic is obtained, suggesting that free Mg^{2+} may exhibit a homotropic positive co-operative effect, and that more than one Mg^{2+} may be involved, in addition to the allosteric effector acetyl-CoA in the reaction catalysed by this enzyme [2]. Contrary to the results illustrated in fig. 1 acetyl-CoA shows hardly any effect on the binding of Mg^{2+} to this enzyme. The $S_{0.5}$ values were found to be 3.6 mM and 3.1 mM at 30 μM and 200 μM acetyl-CoA concentrations respectively. The R_S values were found to be approx. 3 in both the cases.

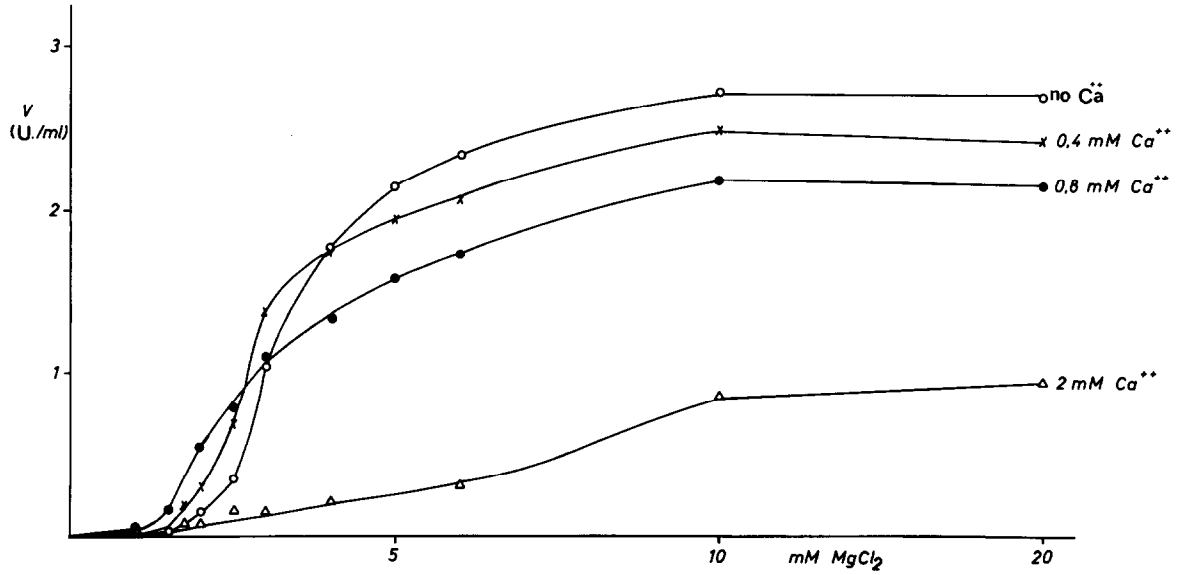

Fig. 3. Effect of Ca^{2+} on the allosteric activation of pigeon kidney pyruvate carboxylase by Mg^{2+}. The Ca^{2+} concentrations were varied as indicated at the curves. The other experimental conditions were similar to those described in Materials and methods.

These values show a pronounced deviation from the values suggested for the classical Michaelis–Menten kinetic [20, 21]. It appears from fig. 2 that above 10 mM concentration, only the inhibitory effect of Mg^{2+} is lower at 200 μM acetyl-CoA concentration.

It is known that pyruvate carboxylase from different sources also requires divalent metal ion for its activation [2, 7, 11, 16, 22, 23], though some divalent metal ions like Ca^{2+} and Zn^{2+} have been reported to show inhibitory effect on this enzyme [7, 11]. Contrary to these results we found that, like pigeon liver pyruvate kinase [12], pyruvate carboxylase from pigeon kidney is activated by Ca^{2+} at lower concentrations of Mg^{2+} (fig. 3). The Ca^{2+} activation effect is completely dependent on the Mg^{2+} concentration and in the absence of Mg^{2+}, no enzymic activity is found. This means that, Mg^{2+} cannot be replaced by Ca^{2+}. The latter shows double and opposite effect on the enzymic activity (fig. 3). Whilst at lower concentration Ca^{2+} activates, at higher Mg^{2+} concentration it shows an inhibitory effect on this enzyme. In otherwords this paradoxial effect of Ca^{2+} is dependent on the concentration of Mg^{2+} as shown in fig. 3. At 2 mM concentration of Ca^{2+} this effect was not very pronounced. Approximately the same results were found for pyruvate carboxylase from pigeon liver [24], even at different concentrations of substrates. Ca^{2+} has also been reported to show an activating effect on other enzymes [12–14]. The mitochondrial concentration of Ca^{2+} is about 3 mM and mitochondria can vary their Ca^{2+} concentration. It could therefore be possible that, at low concentrations of Ca^{2+} and/or Mg^{2+} the enzymic activity is influenced as has been reported also for other enzymes. Even now it is not easy to suggest the *exact* role of Ca^{2+} in the regulation of gluconeogenesis at the level of pyruvate carboxylase, since so many other effectors are also involved in this complex enzyme system. At this stage it can probably be suggested that either at low concentration of Mg^{2+}, the reported high activity of pyruvate carboxylase from other sources could be due to the presence of Ca^{2+} or that the behaviour of this enzyme towards divalent ions varies with different species. Studies with the enzyme from pigeon kidney indicate that the actual kinetics observed with Mg^{2+}, acetyl-CoA or Ca^{2+} are considerably more complex than has been suggested previously. This enzyme appears to be one of the most complex allosteric enzymes that has been studied so far. It becomes particularly difficult to interpret these and other results [2] in the light of current allosteric theoeries [19–21], if attempting to explain these results purely under the heading of a single theory.

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