Effects of Mn^{2+} and Mg^{2+} on the Pigeon Liver Pyruvate Kinase

Pigeon liver pyruvate kinase[1], like the enzyme of other different cells[2–4], has an obligatory requirement of divalent cations. We present data showing that Mn^{2+} and Mg^{2+} activate the pigeon liver pyruvate kinase in a distinct was, and that Mn^{2+} is able to influence the enzyme already activated by saturating concentrations of Mg^{2+}. Some of the data were given in a previous communication[5].

Material and method. Fed male pigeons were used as liver donors. The whole liver was homogenized with two parts of 0.25 *M* sucrose and centrifuged for 10 min at $10{,}000 \times g$. The collected supernatant was centrifuged again for 1 h at $105{,}000 \times g$. In order to restore the allosteric properties of the enzyme, the $105{,}000 \times g$ supernatant was incubated at 25 °C for 2 h before being used for the enzyme assay[1]. Pyruvate kinase activity was assayed by BÜCHER and PFLEIDERER[6] method. The volume of the reaction mixture was always 1.0 ml and the final concentrations were as follows: *Tris*-HCl buffer pH 7.0 50 m*M*; KCl 0.1 *M*; NADH 0.15 m*M*; ADP 1.0 m*M*; lactate dehydrogenase 0.5 U. The concentration of phosphoenolpyruvate, $MgCl_2$ and $MnCl_2$, were as indicated in the legend of the figures. The enzyme activity was expressed as μMol of NADH oxidized $\times \min^{-1} \times g^{-1}$ of liver and calculated from initial velocity.

Results and discussion. Mg^{2+} produces the strongest activatory effect when it is present in concentration of 3.0 m*M* or higher and when phosphoenolpyruvate is 1.0 m*M* (Figure 1). In our assay conditions, a concentration of phosphoenolpyruvate above 0.5 m*M* saturates the enzyme[1]. Mn^{2+} is a more powerful activator when the enzyme is in the presence of low concentrations of phosphoenolpyruvate and when the assay is performed with a content of divalent cations lower than 0.5 m*M*.

Figure 2 shows the effect of Mn^{2+} on the pyruvate kinase activated by a constant concentration of Mg^{2+}. Mn^{2+} is a powerful activator of the enzyme assayed in the presence of low concentrations of phosphoenolpyruvate. In this assay condition the maximal activatory effect is about 7 times.

The effect of Mn^{2+} in the presence of 0.3 m*M* phosphoenolpyruvate is complex. At low concentrations Mn^{2+} acts as an activator, and by increasing the Mn^{2+} concentration the original stimulatory effect of Mn^{2+} is partially reduced; further increases of Mn^{2+} content are ineffective. At 1.0 m*M* phosphoenolpyruvate, the effect of Mn^{2+} on the enzyme activity is similar to that observed at 0.3 m*M* phosphoenolpyruvate, with the difference that the activatory effect is very small and that, on increasing the Mn^{2+} concentration, this effect is completely lost and an inhibitory effect is observed. When Mn^{2+} is present in concentration higher than 3.0 m*M*, the enzyme specific activity is fixed at a constant value that is independent from the phosphoenolpyruvate concentration.

The allosteric nature of the pigeon liver pyruvate kinase, in particular the cooperative effect of the phosphoenolpyruvate[1], suggests that the different sensitivity of the enzyme towards divalent cations could be related to different conformations of the enzyme protein. The data on Figure 2 clearly indicate that the phosphoenolpyruvate concentration influences the sensitivity of the pyruvate kinase towards Mn^{2+}.

It has been suggested that divalent cations[7–9] can play an important role in the control of carbohydrate metabolism by affecting the catalytic activity of the related enzymes.

According to this view, the possible physiological effect of Mn^{2+} on the pigeon liver pyruvate kinase has to be studied in a range of concentration close to Mn^{2+} content in the liver, that is, 0.1–0.15 m*M*[9]. The hepatic content of Mg^{2+} is 5.0–10 m*M*[10], phosphoenolpyruvate 0.03–0.15 m*M*[11,12]. In the range of the concentrations given

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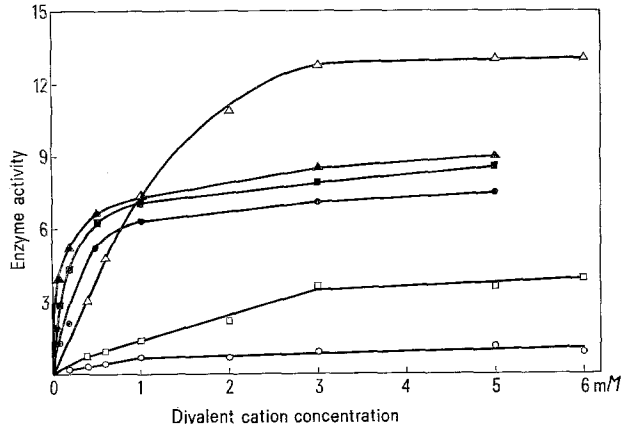

Fig. 1. Effect of Mn^{2+} and Mg^{2+} on pigeon pyruvate kinase activity. The assay system was as described in the text. ○, ●, 0.1 m*M* phosphoenolpyruvate; □, ■, 0.3 m*M* phosphoenolpyruvate; △, ▲, 1.0 m*M* phosphoenolpyruvate. Open symbols $MgCl_2$; colsed symbols $MnCl_2$.

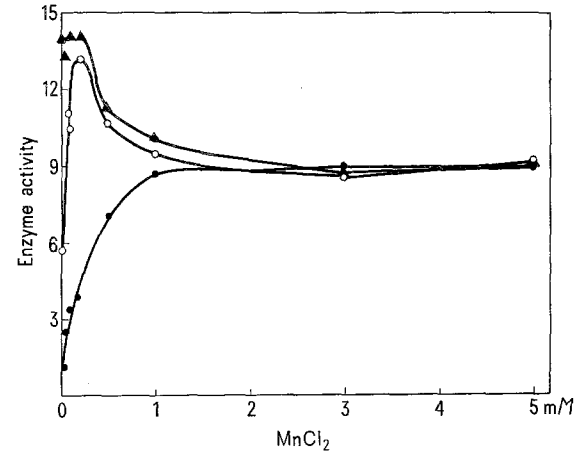

Fig. 2. Effect of Mn^{2+} on the pigeon liver pyruvate kinase activityi n the presence of 5 m*M* Mg^{2+}. The assay system was as described in the text with 5 m*M* $MgCl_2$. ●, 0.1 m*M* phosphoenolpyruvate; ■, 0.3 m*M* phosphoenolpyruvate; ▲, 1.0 m*M* phosphoenolpyruvate.

above, it appears that Mn^{2+} acts on the pigeon liver pyruvate kinase only as a positive effector.

The pigeon liver pyruvate kinase activity can be varied by a small change of Mn^{2+} concentration even when the Mg^{2+} content in the cell is maintained at a constant level. Changes of the Mn^{2+} content in the liver cytoplasm can be postulated, because rat liver mitochondria are able to accumulate Mn^{2+} from a surrounding solution[13]. However, the lack of knowledge of the actual ion environment of the enzyme in the cell precludes the exact valuation of the phenomena in vivo.

Riassunto. Mn^{2+} and Mg^{2+} attivano la piruvato cinasi di fegato di piccione in maniera distinta. In presenza di basse concentrationi di fosfoenolpiruvato Mn^{+} é piú efficace di Mg^{2+} ed é attivatore dell'enzima saturato da Mg^{2+}. Piruvato cinasi (EC 2.7.1.40).

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Conformational Analysis of Proteins from Circular Dichroism Spectra with Reference to Human Erythrocuprein

The interpretation of protein conformation from circular dichroism (CD) spectra has attracted much attention[1-5]. It is assumed that the far ultraviolet CD-spectrum has a basis consisting of the spectra of the α-helical, β-structural and unordered conformations, and is given by a linear combination of these spectra. Although poly-α-amino acid CD-spectra give good approximations in certain cases[1,3], it is doubtful whether these spectra can form a true basis for protein spectra[4-6]. The use of basis spectra calculated from the CD-spectra of reference proteins whose structural composition has been determined by X-ray analysis has therefore been proposed[4-6]. The reservations in this approach have been pointed out[4-6]. We here draw attention to the need to assume that the set of reference and analyzed protein spectra have the same basis. This assumption is amenable to confirmation by rank analysis of the matrix of reference and analyzed protein spectra. For a valid 3-component fit of the analyzed spectra this matrix should have a rank of three. We propose this matrix rank analysis prior to curve fitting of protein CD-spectra with calculated basis spectra as in the computations reported here on CD-spectra of human erythrocuprein.

The profiles of the analyzed human erythocuprein CD-spectra have been reported previously[7]. The spectra were digitized at intervals of 1 or 2.5 nm and the data were smoothed by a quadratic 5-point least squares approximation[8]. Basis spectra were calculated from the CD-spectra of myoglobin, lysozyme and ribonu-

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Table I. CD-spectra of human erythrocuprein preparations

Wavelength (nm)	Mean residue ellipticity (deg. cm²/d mol)		
	Holoprotein	Apoprotein	Partial apoprotein[a]
205	−3410	−2700	−3030
207.5	−3740	−3280	−3310
210	−3600	−3280	−3200
212.5	−3150	−2980	−2810
215	−2560	−2630	−2400
217.5	−1920	−2160	−1920
220	−1230	−1600	−1480
222.5	−530	−1070	−1050
225	130	− 630	− 640
227.5	500	− 420	− 360
230	620	− 250	− 140
232.5	550	− 230	− 70
235	450	− 240	− 50
237.5	370	− 240	10
240	360	− 160	70

[a] Contained 60% of original copper and no zinc.

Table II. Results of rank analysis of a matrix of the CD-sprectra of Table I and the spectra of myoglobin, lysozyme and ribonuclease[6]

Reduced data matrix[a]

−24850	−9580	−7810	− 530	−1070	−1050
0	−7003	−4260	−3111	−2097	−2438
0	0	2960	1471	783	936
0	0	0	− 634	− 621	− 379
0	0	0	0	− 394	− 12
0	0	0	0	0	− 95

Reduced error matrix

1243	479	391	27	54	53
0	776	577	172	145	160
0	0	616	298	208	239
0	0	0	796	504	581
0	0	0	0	855	688
0	0	0	0	0	491

[a] First 6 rows.