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Effects of Metal Ions on the Activity of Cytosolic Phosphoenolpyruvate Carboxykinase from Camel Kidney

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ABSTRACT. The presence of a divalent metal ion together with a catalytic amount of inosine-5'-diphosphate (IDP) are essential for the activation of phosphoenolpyruvate carboxykinase purified from kidney of Arabian camels. With decreasing order of effectiveness, the carboxylating activity was supported by the metal ions, Mn^{2+}, Cd^{2+} and Mg^{2+}. Combination of Mg^{2+} with micromolar concentrations of Mn^{2+} or Co^{2+} recovered full activity. However, high concentration of Cd^{2+} proved to be inhibitory, when combined with Mn^{2+} or Co^{2+} plus Mg^{2+}. Gluconeogenesis regulation is assumed to run principally via the control of PEPCK. This plays an important role in maintaining a high blood glucose level in camels, in contrast to ruminants *(in jensu strictu)* which have a low glucose level in blood.

Phosphoenol pyruvate carboxykinase (PEPCK) catalyzes the conversion of oxaloacetate to phosphoenolpyruvate (PEP). The enzyme has been isolated from several animal tissues including guinea pig liver, rate liver and chicken liver (Holten and Nordlie 1965, Ballard and Hanson 1969, Hebda and Nowak 1982). It is also widely distributed in bacteria, yeast and plants (Utter and Kolenbrander 1972). The major biochemical significance of PEPCK is that it is the key enzyme in gluconeogenesis (Rognstad 1979). The enzyme is located in both the cytosol and mitochondria of the liver and the kidney. However, the subcellular distribution of the enzyme varies depending on the species. In the human and in the guinea pig, PEPCK is equally distributed between the mitochondria and the cytosol of hepatocytes (Utter and Kolenbrander 1972). Reduced enzyme activity in human has been associated with hypoglycaemia and failure to develop (Clayton *et al.* 1986).

The species under investigation here was the Arabian Camel *(Camelus dromedarius)* which is exceptional in that it can maintain much higher blood glucose levels than any of the other species mentioned above. This observation may reflect an intense gluconeogenic activity which plays a role in maintaining a high blood glucose level, even during long periods of starvation. In an earlier publication, we described a method for the isolation and purification of PEPCK and confirmed its high activity in camel liver and kidney tissues (Al-Ali *et al.* 1988). Presently we, report a detailed study of the effect of metal ions on the catalytic activity of the enzyme.

Experimental

Reagents were obtained from Sigma and Aldrich Chemical Companies. Purification procedures were described previously (Al-Ali *et al.* 1988) In the assay procedure all reactions were started by the addition of the enzyme (50 µl) to the complete reaction mixture. The activity was determined spectrophotometrically at 25°C by monitoring the decrease of absorbance at 340 nm. The carboxylation of phosphoenlpyruvate (PEP) to oxaloacetate was measured (pH 7.2) in a 2 ml reaction mixture containing 56 mM hepes, 1mM Inosine diphosphate (IDP), 2 mM PEP, 47 mM $NaHCO_3$, 1 mM $MnCl_2$, 0.25 mM NADH and 22 units of malate dehydrogenase (Colombo *et al.* 1981). One unit of activity is defined as the amount of enzyme that catalyzes the formation of one micromole of product per minute under the above conditions. The velocity measured by this assay is linear with time over a 5 minute period and is linear with protein concentration used in the assay procedure. In the determination of the kinetic parameters for the carboxylating activity, 1 mM $MnCl_2$ was always present. Protein concentrations were determined by the Biorad protein assay (Bradford 1976).

Results

The reaction catalyzed by PEPCK followed Michaelis-Menten kinetics. The Lineweaver-Burk plots were linear. The apparent K_m for PEP and IDP were 62 and 260 µM respectively with Mn^{2+} as activity supporting metal ion.

Both Mn^{2+} and Mg^{2+} ions supported the enzyme activity with the former ion having the greatest effect (Fig. 1). However, when Mn^{2+} at very low concentrations of 20 and 5 µM was present in the reaction mixture together with various concentrations of Mg^{2+}, the carboxylation of PEP was strikingly increased (Fig. 2). For purpose of comparison, the activity obtained with various concentrations of Mg^{2+} and 29 µM Co^{2+} is also reported. Cadmium also supported enzymatic activity,

but again to a lower extent than Mn^{2+} (Fig. 3a). However, high concentration of Cd^{2+} proved to be inhibitory. When 2 mM Mg^{2+} was included in combination with a range of Cd^{2+} concentrations synergistic activation of PEPCK was obtained. Maximum activation was similar when Co^{2+} or Mn^{2+} were used under similar experimental conditions, but the activation pattern was different (Fig. 3b). Furthermore, Cd^{2+} decreased the activity obtained in the presence of 2 mM Mg^{2+} plus 40 μM Mn^{2+}, 16 μM Co^{2+} or 0.5 mM Mn^{2+}. Incubation of the enzyme with mercaptoethanol resulted in a complete loss of activity.

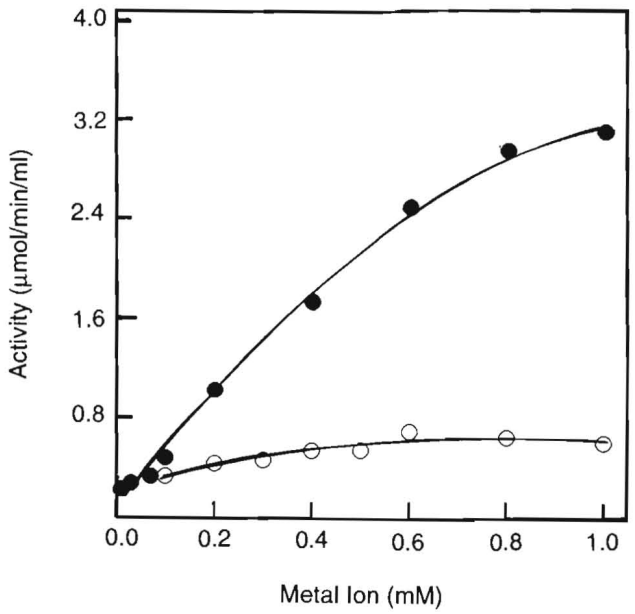

Fig. 1. PEP carboxylating activity of purified PEPCK as a function of concentration of added Mn^{2+} (●) or Mg^{2+} (○).

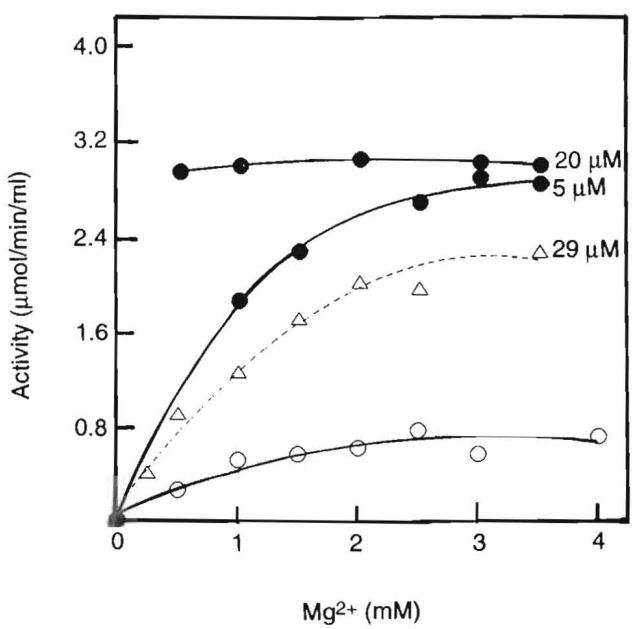

Fig. 2. Effects of Mn^{2+} or Co^{2+} on the activity of PEPCK as a function of the concentration of Mg^{2+}. Variable Mg^{2+} concentration plus fixed level of Mn^{2+} (●),Co^{2+} (Δ) or absence of these ions (○).

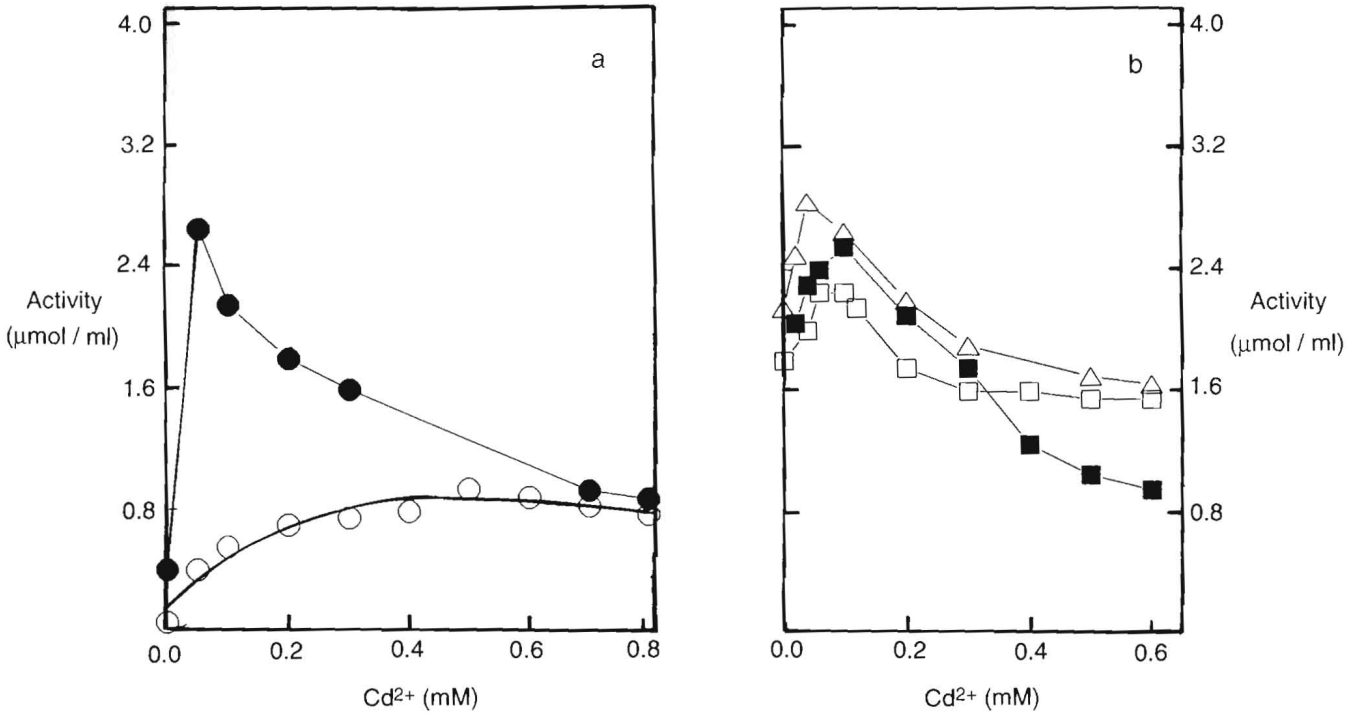

Fig. 3. Activity of PEPCK as a function of the concentration of $CdCl_2$.

(a) Oxaloacetate formed with varied concentrations of Cd^{2+} in the absence (○) or the presence of (●) of 2 mM Mg^{2+}.

(b) Inhibition of oxaloacetate formation in the presence of 2 mM Mg^{2+} plus 40 µM Mn2+ (❑), 2mM Mg^{2+} plus 16 mM Co^{2+} (Δ), Or 0.5 mM Mn^{2+} (■), as function of varying concentration of Cd^{2+} indicated on the abscicca.

Discussion

The Km values of 62 and 260 μM for PEP and IDP found in the camel are similar to those reported for rat liver cytosolic PEPCK of 48.6 and 71.5 μM for PEP and IDP respectively (Colombo *et al.* 1978). Moreover, similar values were reported for other species such as chicken liver PEPCK (Hebda and Nowak 1982).

The enzyme required divalent metal ions for the support of its activity in the direction of oxaloacetate formation. At the concentrations indicated Mn^{2+} was more effective in stimulating enzyme activity than Mg^{2+}. Both metal ions have been reported to stimulate PEPCK from other species (Goto *et al.* 1980). Micromolar concentration of Mn^{2+} or Co^{2+} in combination with millimolar concentration of Mg^{2+} stimulated the enzymatic activity of PEPCK. Cadmium on the other hand was not as effective as Mn^{2+} or Co^{2+} in supporting PEP carboxylation. Nevertheless, in the presence of 2 mM Mg^{2+}, the maximal activation decreased. This may be due to the formation of a less active Cd^{2+}/IDP complex. The results suggest a dual-cation requirement in the carboxylation of PEP catalyzed by camel kidney cytosolic PEPCK. This indicates that an activating cation in addition to that chelated by the nucleotide are required to obtain significant carboxylation of PEP. These effects have also been reported for rat liver cytosolic PEPCK (Colombo *et al.* 1981). With respect to the above cited observations, it is assumed that divalent metal ions have a certain regulatory role on PEPCK in camel kidney tissue and thus on gluconeogenesis in this organ. Camel PEPCK is apparently a sulfhydryl sensitive enzyme as shown by the loss of activity when incubated in mercaptoethanol (Carlson *et al.* 1978). Present data indicates that camel kidney PEPCK is similar to the related enzymes present in other species.

The ability of the camel to maintain an active gluconeogenesis in the kidney to support the gluconeogenesis in the liver is reflected by its ability to maintain a high glucose level in the blood even after long periods of starvation. In addition the kidney gluconeogenesis is involved in maintaining acid base balance. During starvation when fat metabolism is active the increased acid production is linked to ammonia production through the deamination of glutamine. Hence carbon skeleton of glutamine will act as a substrate for gluconeogenesis in the kidney. This is in contrast to ruminants which maintain a low glucose in the blood which is reflected by a low concentration of PEPCK.

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References

Al-Ali, A.K., Al-Husayni, H. and **Power, D.M.** (1988) Comprehensive biochemical analysis of the blood of the camel. *Comp. Biochem. Physiol.* **89**(B): 35-37.

Ballard, F.J. and **Hanson, R.W.** (1969) Purification of PEPCK from the cytosol fraction of rat liver and the immunochemical demonstration of differences between this enzyme and the mitochondrial PEPCK. *J. Biol. Chem.* **244:** 5625-5630.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Analyt. Biochem.* **72:** 248-254.

Carlson, G.M., Colombo, G. and **Lardy, H.A.** (1978) A vicinal dithiol containing an essential cysteine in PEPCK (GTP) from cytosol of rat liver. *Biochemistry* **17:** 5329-5338.

Clayton, P.T., Hyland, K., Brand, M. and **Leonard, J.V.** (1986) Mitochondrial PEPCK deficiency. *Eur. J. Paediatr.* **145**: 46-50.

Colombo, G., Carlson, G.M. and **Lardy, H.A.** (1978) Phosphoenolpyruvate from rat liver cytosol separation of homogeneous forms of the enzyme with high and low activity by chromatography on agarose-hexane-GTP. *Biochemistry* **17:** 5321-5329.

Colombo, G., Carlson, G.M. and **Lardy, H.A.** (1981) Phosphoenolpyruvate carboxykinase (GTP) from rat liver cytosol. *Biochemistry* **20:** 2749-2757.

Goto, Y., Shimizu, J. and **Shukuya, R.** (1980) Purification and molecular characteristics of mitochondrial PEPCK from bullfrog liver. *J. Biochem.* **88:** 1239-1249.

Hebda, C.A. and **Nowak, T.** (1982) Phosphoenolpyruvate carboxykinase Mn^{2+} and Mn^{2+} substrate complexes. *J. Biol. Chem.* **257:** 5515-5522.

Holten, D.D. and **Nordlie, R.C.** (1965) Comparative studies of catalytic properties of guinea pig liver intra- and extramitochondrial PEPCK. *Biochemistry,* **4**(4): 723-731.

Rognstad, R. (1979) Rate limiting steps in metabolic pathways. *Bio. Chem.* **254:** 1875-1879.

Utter, M.F. and **Kolenbrander, H.M.** (1972) PEPCK, *in the enzymes,* (**Boyer, O.** (ed.) Academic Press, New York, **6:** 117-168.

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تأثير الفلزات ذات الشحنة الثنائية الموجبة في عملية تحفيز أنزيم PEPCK المستخلص من كلية الجمل

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لقد تم استخلاص وتنقية أنزيم Phosphoenolpyruvate Carboxykinase PEPCK من أنسجة كلية الجمل باستخدام تقنية التنقية الكروموتغرافية المائية تحت الضغط العالي . أوضحت الدراسات بأن وجود الفلزات ذات الشحنة الثنائية الموجبة مع كمية معينة من أنوسين ثنائي الفوسفات IDP ضروري لتنشيط الأنزيم وتختلف هذه الفلزات بمدى قدرتها على تحفيز الأنزيم باتجاه تفاعل معين . فبينما يختص المنغنيز بأعلى قدرة من التحفيز فان المغنيسيوم له أقل قدرة على تحفيز الأنزيم وفي حالة وجود فلزين مع بعضهما فان عملية التحفيز تختلف باختلاف الفلزين الموجودين .

إن تنظيم عملية بناء الجلوكوز في الكبد والكلية تتم بصورة رئيسية عن طريق تنظيم فعالية أنزيم PEPCK الذي يعتبر العامل الرئيسي في البناء . وبما أن مستوى سكر الدم في حالة الصيام تعتمد بصورة مهمة على هذه العملية فان دور الأنزيم في ابقاء مستوى سكر الدم يرتبط مباشرة بمدى فعاليته أو تركيزه . يمتاز الجمل عن بقية المجترات بمحافظته على مستوى سكر دم عالي وتنسجم هذه الدراسة مع نتائجنا السابقة حول كون الظاهرة ترجع إلى ارتفاع تركيز الأنزيم في انسجة كلية الجمل عند مقارنته ببقية الحيوانات .