KINETIC EVIDENCE FOR THE PRESENCE OF TWO FORMS OF M_2-TYPE PYRUVATE KINASE IN RAT SMALL INTESTINE

Th.J.C. van Berkel, H.R. de Jonge, J.F. Koster and W.C. Hülsmann

Department of Biochemistry I, Faculty of Medicine, Erasmus University Rotterdam (The Netherlands)

Received July 22,1974

SUMMARY. Some kinetic properties of pyruvate kinase from rat small intestine have been investigated. The relative insensitivity of the enzyme to ATP inhibition and the amino acid inhibition pattern allows the conclusion that intestinal pyruvate kinase belongs to the M_2-type. The pyruvate kinase activity as a function of the phosphoenol pyruvate concentration is characterized by two different n values. The activity correlating with the low n value is stimulated by Fru-1,6-P_2, whereas the activity at higher phosphoenol pyruvate concentrations is not influenced by this glycolytic intermediate. These results, together with the partial relief of the amino acid inhibition by Fru-1,6-P_2, show that two forms of the enzyme are present with different kinetic properties. The metabolic implication of the kinetic properties of pyruvate kinase for rat small intestine is discussed.

Recent work has demonstrated that there are at least three non-interconvertible types of pyruvate kinase[1-3] (ATP: pyruvate phosphotransferase, EC 2.7.1.40) in rat tissues. The L-type is present in liver, erythrocytes and kidney[1], the M_1-type in muscle and brain[2], while the third type of pyruvate kinase, called M_2-type by Imamura et al.[1], is the most widely distributed type. The occurrence of M_2-type pyruvate kinase has now been clearly shown in adipose tissue[4], kidney[2,3], leucocytes[5], liver[1,6] and hepatomas[1,7]. Pogson[4] has shown that this type can occur in two interconvertible forms, called Pyk-A and Pyk-B. Isolation of pyruvate kinase from adipose tissue in the absence of EDTA yields the high-affinity form (Pyk-B), while the low-affinity form (Pyk-A) is obtained by isolation in the presence of EDTA. Although this condition is rather unphysiological, we were able to show that in the liver at 1 mM Mg^{2+}_{free} (a physiological condition for liver) these two forms of the M_2-type are in equilibrium[8]. This indicates that the presence of these two forms may be of physiological importance. From incubation experiments it was also shown that a prolonged storage of

the enzyme in the low-affinity form makes its conversion to the high-affinity form more difficult[8]. This makes it possible to conclude that when pyruvate kinase is isolated from a tissue under conditions which favour the B-form, that the detection of the A-form indicates its presence in vivo. This paper shows that this is indeed the case for pyruvate kinase from rat small intestine.

MATERIALS AND METHODS. Villous- and crypt cell suspensions originating from the duodenal, ileal and jejunal parts of rat small intestine were harvested separately according to the high-frequency vibration technique of Harrison and Webster[9] in a medium containing 0.01 M Tris-HCl buffer (pH 7.5), 0.13 M NaCl and 5 mM EDTA. Cells and cell sheets were collected by centrifugation for 15 sec at 800 g_{max}. The cells were washed two times with the isolation medium except that EDTA was omitted. Homogenates were prepared in a medium containing 0.5 M Tris-HCl (pH 7.5) + 1 mM $MgCl_2$, by Polytran treatment as described earlier by de Jonge[10]. A more heterogeneous cell population originating from the whole length of the small intestine was prepared by gently scraping off the intestinal mucosa with a glass slide. Homogenization medium and technique were similar to those mentioned above.

Pyruvate kinase was assayed as described earlier[6]. The assay mixture contained: 25 mM Tris HCl pH 7.5, 225 mM KCl, 1 mM ADP, 0.12 mM NADH, 23 mM $MgCl_2$, 0.1-0.2 mg lactate dehydrogenase and [PEP] as indicated in the legends to the figures. Duplicates were run with twice the amount of lactate dehydrogenase to exclude possible effects on this enzyme reaction.

RESULTS

In preliminary experiments we isolated villus- and crypt cells from the duodenal, ileal and jejunal parts of rat small intestine to investigate whether the isoenzyme patterns in the fast-dividing crypt and the non-proliferative villus cells would be similar and also whether differences along the length of the small intestine would occur. In these experiments (not shown) no significant differences in kinetic properties between the various celltypes were observed and the same activity dependence from the PEP concentration was found as shown in Fig. 1 for the intestinal scrapings isolated in the absence of EDTA. Scrapings obtained in the absence of EDTA followed by isolation of the cell fraction in the presence of 1 mM Mg^{2+} results in a kinetic pattern characterized by two different n values (see Hill plot insert Fig. 1). Addition of Fru-1,6-P_2 (0.5 mM) only stimulates the pyruvate kinase activity corresponding with the n value of 1.2, whereas the activity at higher PEP concentration is not influenced. This result indicates

---

Abbreviation: phosphoenol pyruvate PEP

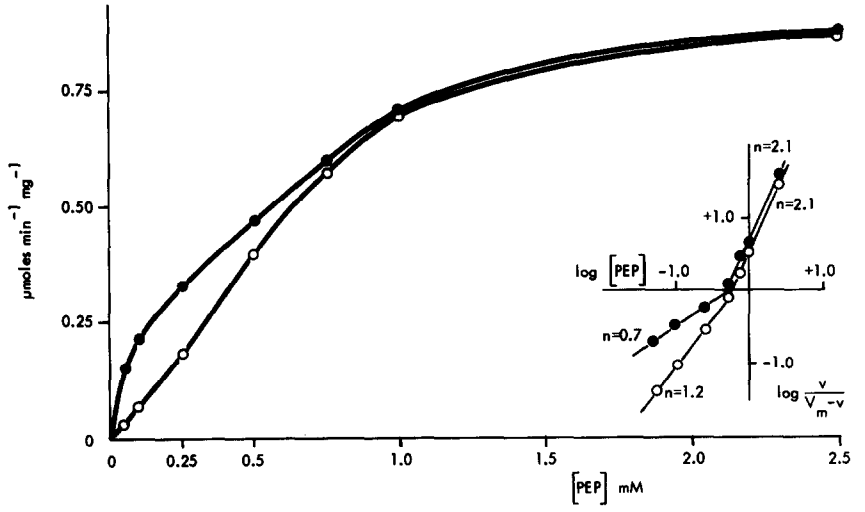

Fig. 1 Rat small intestine pyruvate kinase activity as a function of the PEP concentration.
0-0, activity in the absence of Fru-1,6-P_2; ●-●, 0.5 mM Fru-1,6-P_2 present. The insert is the Hill plot of the values obtained.

that a Fru-1,6-P_2 sensitive and an insensitive form of pyruvate kinase may be detected in small intestine. To clarify the nature of this phenomenon we studied the effect of alanine and ATP on the pyruvate kinase activity. Fig. 2 shows that in the presence of 1 mM alanine a strong inhibition of the pyruvate kinase activity is obtained while addition of Fru-1,6-P_2 to this inhibited enzyme does only relieve the alanine inhibition at low PEP concentrations. In comparison with 1 mM alanine, ATP (2 mM) is a weak inhibitor of intestinal pyruvate kinase (Fig. 3). This relative insensitivity of the intestinal pyruvate kinase activity for ATP indicates that intestinal pyruvate kinase is of the M_2-type. This conclusion is further strenghtened by the amino acid inhibition pattern (TABLE I) which is similar to that of the M_2-type from liver[11] and leucocytes[5] and differs from those of the L- and M_1-types[2]. Fig. 4 shows the influence of increasing alanine concentrations on the enzymatic activity. It can be seen that the presence of 0.5 mM alanine results already in a 70% inhibition of the activity at 1 mM PEP, whereas the influence of increasing ATP concentrations (Fig. 5) illustrates that ATP is far less effective as inhibitor. Fru-1,6-P_2 (0.5 mM) stimulates the alanine-inhibited enzyme at the lower PEP concentration only at higher alanine concentrations.

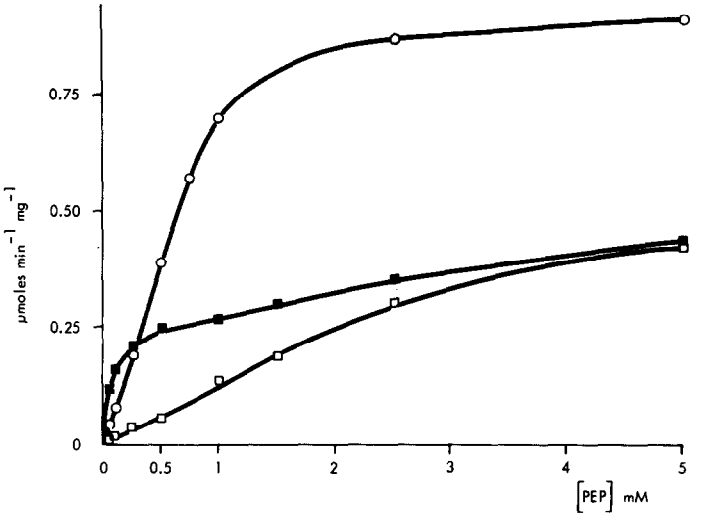

Fig. 2 Rat small intestine pyruvate kinase activity as a function of the PEP concentration in the presence of alanine. 0-0, control; □ -□, 1 mM alanine present; ■- ■, 1 mM alanine + 0.5 mM Fru-1,6-P_2 present.

TABLE I

THE INFLUENCE OF VARIOUS AMINO ACIDS ON INTESTINAL PYRUVATE KINASE

Addition	Concentration (mM)	% of activity - Fru-1,6-P_2	% of activity + Fru-1,6-P_2 (0.5 mM)
L-Phenylalanine	1	18	39
	5	8	33
L-Valine	1	57	68
	5	22	39
L-Proline	1	75	77
	5	32	47
L-Tryptophan	1	50	59
	5	20	32
L-Glutamate	1	98	91
	5	77	81
L-Alanine	1	21	41
	5	12	38
L-Threonine	1	48	59
	5	22	42
L-Cysteïne	1	24	42
	5	16	37
L-Histidine	1	83	88
	5	62	72

The 100% value is the activity at [PEP] = 1.0 mM. Further conditions are indicated in the materials and methods section.

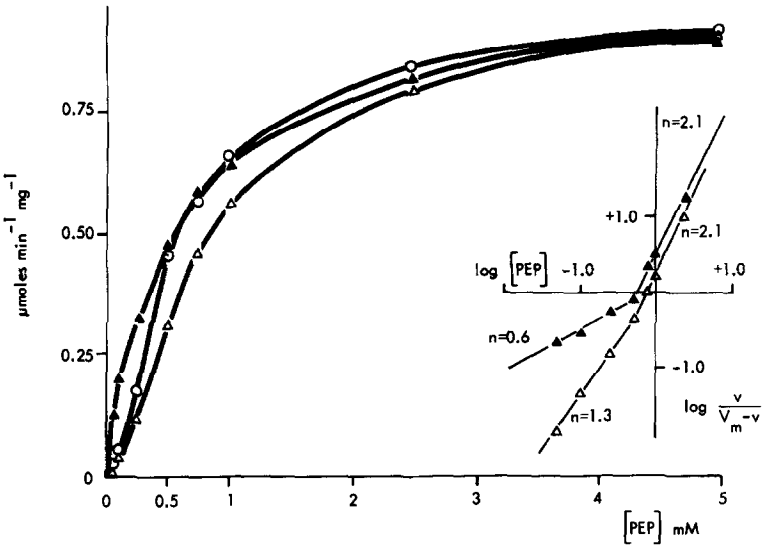

Fig. 3 Rat small intestine pyruvate kinase activity as a function of the PEP concentration in the presence of 2 mM ATP.
O-O, control; Δ-Δ, 2 mM ATP present; ▲-▲, 2 mM ATP + 0.5 mM Fru-1,6-P_2 present. The insert is the Hill plot of the values obtained.

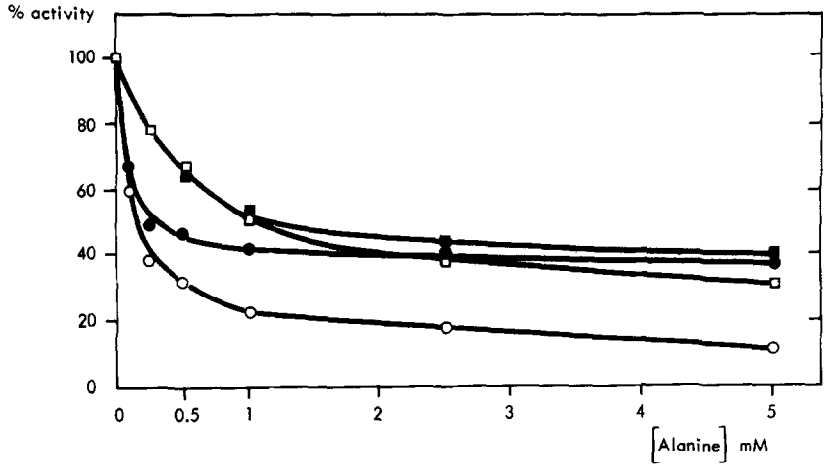

Fig. 4 Influence of alanine on rat small intestine pyruvate kinase activity at two PEP concentrations in the absence and presence of Fru-1,6-P_2 (0.5 mM).
O-O, 1 mM PEP present; ●-●, 1 mM PEP + 0.5 mM Fru-1,6-P_2 present; □-□ , 5 mM PEP present; ■-■ , 5 mM PEP + 0.5 mM Fru-1,6-P_2 present. The 100% value is the activity in the absence of alanine.

DISCUSSION

From the sensitivity of the intestinal pyruvate kinase activity towards ATP and alanine and from the amino acid inhibition pattern we can conclude that intestinal pyruvate kinase has to be

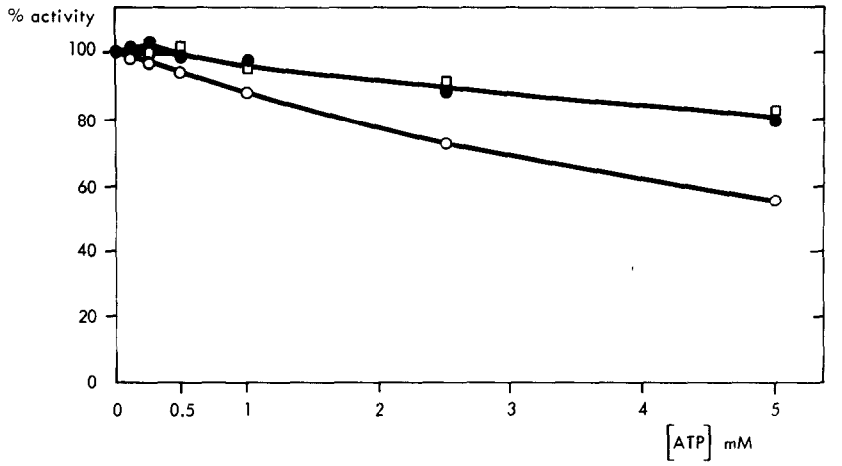

Fig. 5 Influence of ATP on rat small intestine pyruvate kinase activity at two PEP concentrations in the absence and presence of Fru-1,6-P_2 (0.5 mM).
0-0, 1 mM PEP present; ●-●, 1 mM PEP + 0.5 mM Fru-1,6-P_2 present; □-□, 5 mM PEP present and 5 mM PEP + 0.5 mM Fru-1,6-P_2 present. The 100% value is the activity in the absence of ATP.

classified as M_2-type. This finding is consistent with the absence of gluconeogenesis in this tissue[12]. Recently we have shown for the liver that this type of pyruvate kinase at physiological Mg^{2+}_{free} concentrations can occur in two forms[8]. The so-called Pyk-B form[4] has a high affinity for the substrate PEP and is inhibited by amino acids, an inhibition which is completely relieved by the addition of Fru-1,6-P_2[5-6]. At lower Mg^{2+}_{free} concentrations the Pyk-A form of the enzyme is formed which has less affinity for PEP. This form is more sensitive to amino acid inhibition and this inhibition is not abolished by the addition of Fru-1,6-P_2[8]. The kinetic behaviour of pyruvate kinase from rat small intestine can be explained completely by the presence of both forms. Fig. 1 shows that the form with high affinity for PEP is sensitive to Fru-1,6-P_2, whereas the low affinity form is not. Fru-1,6-P_2 relieves only the alanine inhibition of the high affinity form (Fig. 2). Fig. 4 shows that especially at higher alanine concentrations Fru-1,6-P_2 relieves the inhibition, indicating that the form which is less sensitive to alanine (Pyk-B) can be influenced by Fru-1,6-P_2. For reason that intestinal pyruvate kinase was isolated and assayed under conditions which favour formation of the Pyk-B form it can be concluded that "in vivo" in the small

intestine M_2-type pyruvate kinase is at least partially present in the Pyk-A form.

We have discussed earlier (ref. 8) that the B⇌A equilibrium may provide an additionalproperty for the most widely distributed type of pyruvate kinase to accommodate its properties to the specific requirements of the several tissues. As rat small intestine is the first tissue for which it is shown that a considerable amount of Pyk-A type is present "in vivo" one might speculate about its physiological function. As the major difference between B and A type is the amino acid sensitivity, the presence of A type might indicate that glycolysis will be inhibited when a protein-rich diet is ingested. Preliminary experiments with isolated intestinal cells indeed indicate that glycolysis is inhibited in the presence of alanine (H.R. de Jonge, unpublished results). A similar inhibitory effect of amino acids from the diet on the glycolytic rate "in vivo" may be important because it will induce a glucose sparing effect, which can be of importance during limited carbohydrate uptake. Experiments are in progress to investigate if the relative amounts of Pyk-B and Pyk-A in intestine can be regulated by the diet.

ACKNOWLEDGEMENTS

Messrs. J.K. Kruyt and L.M. Scheek are thanked for their expert technical assistance. Miss A.C. Hanson is thanked for typing the manuscript. The Netherlands Foundation for Fundamental Medical Research (FUNGO) is acknowledged for partial financial support (grant 13-39-05).

REFERENCES

1. Imamura, K. and Tanaka, T. (1972) J. Biochem. Tokyo 72, 1001-1015.
2. Carbonell, J., Feliu, J.E., Marco, R. and Sols, A. (1973) Eur. J. Biochem. 37, 148-156.
3. Ibsen, K.H. and Trippel, P. (1973) Arch. Biochem. Biophys. 156, 730-744.
4. Pogson, C.I. (1968) Biochem. J. 110, 67-77.
5. Van Berkel, Th.J.C. and Koster, J.F. (1973) Biochim. Biophys. Acta 293, 134-139.

6. Van Berkel, Th.J.C., Koster, J.F. and Hülsmann, W.C. (1973) Biochim. Biophys. Acta 321, 171-180.
7. Walker, P.R. and Potter, V.R. (1973) J. Biol. Chem. 248, 4610-4616.
8. Van Berkel, Th.J.C., submitted for publication.
9. Harrison, D.D. and Webster, H.L. (1969) Exp. Cell. Res. 55, 257-260.
10. De Jonge, H.R. (1973) Biochem. Pharm. 22, 2659-2677.
11. Jiménez de Asúa, L., Rozengurt, E., Devalle, J.J. and Carminatti, H. (1971) Biochim. Biophys. Acta 235, 326-334.
12. Lamers, J.M.J., Kurpershoek-Davidov, R. and Hülsmann, W.C. (1974) Biochim. Biophys. Acta 343, 427-430.