BBA 66652

THE EFFECT OF PHOSPHOENOLPYRUVATE, FRUCTOSE 1,6-DIPHOSPHATE AND pH ON ALLOSTERIC PYRUVATE KINASE IN MUSCLE TISSUE OF THE BIVALVE *MYTILUS EDULIS* L.

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(Received March 15th, 1972)

SUMMARY

1. Pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) in muscle tissue of the sea mussel *Mytilus edulis* possesses properties which are similar to allosteric L-type pyruvate kinase of the rat liver with respect to stimulation by phosphoenolpyruvate and Fru-1,6-P_2. The action of these modulators is pH dependent. In contrast to L-type there is an increasing stimulation by phosphoenolpyruvate and Fru-1,6-P_2 by lowering the pH within the range from 8 to 6.

2. A possible regulatory role of the pH influence on enzyme activity is discussed.

INTRODUCTION

Tanaka *et al.*[1] reported that there are at least two types of pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) in the rat, types L and M, which predominate in liver and muscle, respectively. Type L, found in liver, kidney cortex[2] and erythrocytes[3] is a regulatory enzyme. Its activity becomes modulated through allosteric stimulation by phosphoenolpyruvate (PEP) and fructose 1,6-diphosphate (Fru-1,6-P_2)[2]. Type M has no allosteric properties and is distributed in various tissues such as brain, heart, skeletal muscle, kidney and testes[1,4]. Fru-1,6-P_2 has no influence on this type[5]. In the course of a study concerning the regulation of the anaerobic carbohydrate metabolism in the sea mussel (*Mytilus edulis* L.), we found that pyruvate kinase from the adductor muscle has allosteric properties and we report here the influence of Fru-1,6-P_2 and of the pH on the enzyme.

MATERIALS AND METHODS

Sea mussels were obtained from the Institute of Mussel Research (Texel, Netherlands) and were collected from beds in the Wadden sea. Posterior adductor muscles were cut out and homogenized with an Ultra-Turrax (Type TP 18/2) for 1 min with 4 vol. of ice-cold buffer, pH 6.9 (10 mM potassium phosphate–1 mM

2-mercaptoethanol–2 mM disodium EDTA). The following procedures were carried out at 0–2 °C. The homogenate was centrifuged at 50 000 × *g* for 30 min. Ammonium-sulphate was added to the supernatant. At least 95% of the pyruvate kinase precipitated within the range 45–65% saturation. After centrifugation the pellet was resuspended in buffer, pH 6.9 (50 mM potassium phosphate–1 mM 2-mercapto-ethanol–5 mM $MgSO_4$) and dialysed against this buffer. After concentration in an Amicon ultrafiltration cell the sample was placed on a DEAE-Sephadex column (1.2 cm × 50 cm) and eluted with 50 mM phosphate buffer (pH 6.9) containing increasing concentrations of KCl. Pyruvate kinase emerged in the effluent at a KCl concentration of 0.1 M. The fractions containing enzyme activity were pooled and dialyzed overnight against 0.1 M Tris–HCl buffer, pH 7.6. The enzyme was stable for a few months when stored at 0 °C. Pyruvate kinase activity was measured according to the method of Bücher and Pfleiderer[6].

ADP, PEP, NADH, lactate dehydrogenase and Fru-1,6-P_2 were obtained from Boehringer (Mannheim, W. Germany). Other chemicals were of analytical grade.

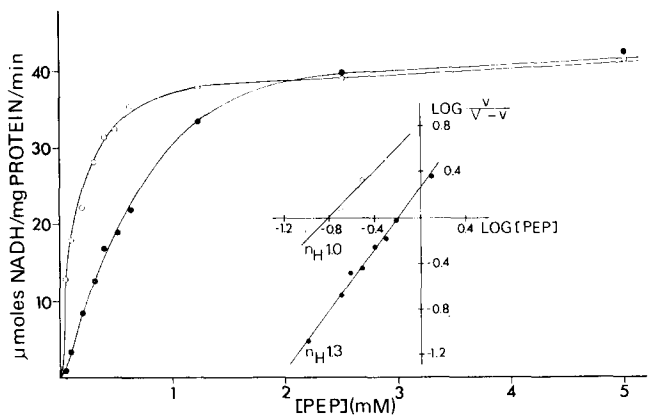

Fig. 1. Relation between pyruvate kinase activity and phosphoenolpyruvate concentration at pH 7.6 in the presence (○) and absence (●) of 0.1 mM Fru-1,6-P_2. Assay conditions: Tris–HCl buffer 0.1 M, [ADP] = 5 mM, [$MgSO_4$] = 8.3 mM, [KCl] = 67 mM, [NADH] = 0.067 mM, excess of lactate dehydrogenase, pyruvate kinase 1.6 μg (spec. act. about 50 units/mg). A Hill plot of the data is inserted.

RESULTS

Fig. 1 shows the substrate concentration–velocity curves in the presence and absence of Fru-1,6-P_2 at pH 7.6. Without Fru-1,6-P_2 the saturation curve is slightly sigmoidal. The Hill plot shows positive cooperativity (n_H = 1.3) for phosphoenol-pyruvate whereby an apparent K_m of 0.63 mM is found. With 0.1 mM Fru-1,6-P_2 the kinetics shift to hyperbolic with a Hill coefficient of 1.0 and an apparent K_m of 0.15 mM (stimulation).

Fig. 2 shows the activity at pH 6.0. The sigmoidicity of the saturation curve undergoes a sharp in increase with n_H = 1.8 and the apparent K_m = 3.5 mM. Again Fru-1,6-P_2 changes the saturation curve to a hyperbole with n_H = 1.0 and the apparent K_m = 0.25 mM (stimulation). So the enzyme behaves opposite to L-type

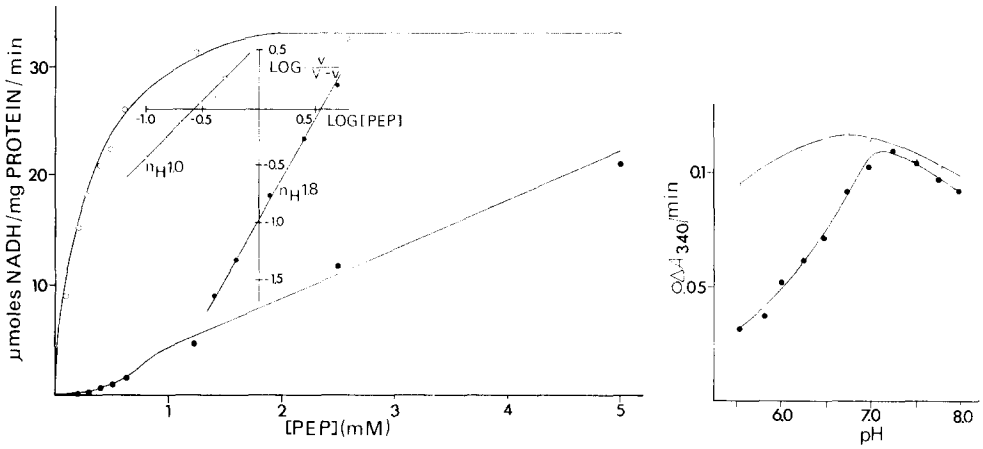

Fig. 2. Relation between pyruvate kinase activity and phosphoenolpyruvate concentration at pH 6.0 in the presence (○) and absence (●) of 0.1 mM Fru-1,6-P_2. Buffer: 0.1 M Tris–maleic acid, other assay conditions as in Fig. 1. A Hill plot of the data is inserted.

Fig. 3. Effect of Fru-1,6-P_2 on the pH optimum (●, no Fru-1,6-P_2; ○, with 0.1 mM Fru-1,6-P_2). Buffer: 0.1 M Tris–maleic acid, [phosphoenolpyruvate] = 5 mM, [ADP] = 5 mM, pyruvate kinase 2.4 μg (spec. act. about 50 units/mg). Other assay conditions as in Fig. 1.

pyruvate kinase from rat liver and erythrocytes which both show normal hyperbolic kinetics at pH < 7, with no Fru-1,6-P_2 stimulation[3,7].

Fig. 3 shows a pH optimum for the pyruvate kinase at about 7. At pH > 7 the activity decreases slowly but at pH < 7 there is rapid drop in activity. In the pre-presence of Fru-1,6-P_2 the pH optimum is displaced to pH 6.7 but the activity changes only marginally between pH 5.5 and 8.0.

DISCUSSION

Poikilothermic organisms show marked differences from mammalian muscle tissues with respect to pyruvate kinase. Fru-1,6-P_2 stimulation of trout pyruvate kinase from white muscle[8] and of oyster pyruvate kinase from the adductor muscle[9] have been reported. But in both cases the enzyme shows non-allosteric kinetics. On the contrary Somero and Hochachka [10] described interconversion between pyruvate kinase types with hyperbolic and sigmoidal kinetics in leg muscle of the Alaskan king-crab. This interconversion was temperature dependent but was not sensitive to Fru-1,6-P_2. The finding of a pyruvate kinase with hyperbolic and sigmoidal kinetics in muscle tissue of the sea mussel with the shift of the kinetics dependant on Fru-1,6-P_2 is therefore remarkable.

It is striking that the influence of pH on the enzyme kinetics is opposite to that observed with the L-type pyruvate kinase of mammalian tissues. In our opinion this might be of physiological significance. Intertidal bivalves such as *Mytilus edulis* have to be able to withstand anaerobic conditions for many days due to their habitat. It was shown by one of us[11,12] that alanine and dicarboxylic acids accumulate, among the latter succinate being the major end product. As the formation of the dicarboxylic

acids takes place by carboxylation of phosphoenolpyruvate rather than of pyruvate[11,12], there is a need for suppressing pyruvate kinase activity, thus leaving phosphoenolpyruvate to be carboxylated to oxaloacetate. Gradual decrease of pH, caused by formation of dicarboxylic acids, mostly succinate, would modify pyruvate kinase activity .Conversion of phosphoenolpyruvate to the initial end product alanine would be prevented in favour of the further formation of succinate.

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