ALLOSTERIC INHIBITION OF MUSCLE PYRUVATE KINASE BY PHENYLALANINE

Enrique ROZENGURT, Luis JIMÉNEZ DE ASÚA and Héctor CARMINATTI

Instituto de Investigaciones Bioquimicas "Fundación Campomar" and Facultad de Ciencias Exactas y Naturales, Obligado 2490, Buenos Aires (28), Argentina

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1. Introduction

Pyruvate kinases (EC 2.7.1.40) isolated from different sources exhibit kinetic properties of the allosteric type [1]. On the other hand, there is no kinetic evidence so far, suggesting that the enzyme from muscle, which has been extensively investigated [2], might have allosteric properties. Recently it was reported that type M pyruvate kinase from skeletal muscle [3] and other tissues [3, 4] is inhibited by phenylalanine in a competitive way. It was also found that L-alanine can reverse this inhibition [3].

In this communication we present evidence showing that phenylalanine acts as an allosteric inhibitor of muscle pyruvate kinase. The inhibition is strongly dependent on pH and is reversed by serine, cysteine and alanine.

2. Materials and methods

Crystalline pyruvate kinase (Sigma) was dialyzed overnight against 4000 volumes of 50 mM tris-HCl buffer, pH 7.5–100 mM KCl– 1 mM mercaptoethanol, prior to use. Enzyme activity was measured spectrophotometrically by coupling the system with excess lactate dehydrogenase [5]. All other materials and methods were as described elsewhere [6].

3. Results and discussion

Various amino acids were tested at 5.0 mM for their effect on muscle pyruvate kinase activity. It was found that phenylalanine is a specific inhibitor. Other amino acids, such as alanine, glycine, cysteine, serine, threonine, methionine, aspartate, valine, leucine, isoleucine, glutamate, proline, histidine and tryptophan had no significant effect. Among all the above mentioned amino acids tested only cysteine, serine and alanine reversed the phenylalanine inhibition.

The curves of enzyme activity as a function of phenylalanine concentration are sigmoidal, in the presence or absence of the reactivating amino acids, as it is shown in fig. 1A.

It can be observed that serine, cysteine or alanine greatly increase the concentration of phenylalanine which produces half maximal inhibition. They do not appreciably change the slopes of the Hill plots for phenylalanine (about 1.5 under these conditions). Double reciprocal plots of initial rate as a function of phosphoenol pyruvate (PEP) concentration gave linear relationships both in the absence or in the presence of phenylalanine at 6 or 10 mM. The kinetic of inhibition was of the mixed type. It has also been found that 2-phosphoglyceric acid (2-PGA), an analog of PEP, competitively inhibits the enzyme. Furthermore, the inhibition curve of 2-PGA, in contrast to that of phenylalanine, is hyperbolic. The experiment presented in fig. 1B shows that alanine has a strong reactivating effect when the enzyme is inhibited by phenylalanine but it does not reverse the inhibition of an isosteric inhibitor, such as 2-PGA. These results suggested that phenylalanine behaves as an allosteric inhibitor.

We have shown that the allosteric properties of the hepatic pyruvate kinase (type L) are strongly dependent on pH [7], as is also the case for other regulatory

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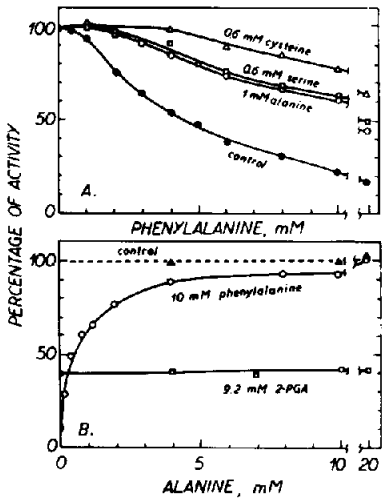

Fig. 1. (A). Variation of the activity of muscle pyruvate kinase with phenylalanine concentration in the presence or absence of 0.6 mM serine or cysteine or 1 mM alanine. Assay conditions were described in Materials and methods. The incubation mixture was as follows: 100 mM TES-TMA (*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid-tetramethylammonium hydroxide) pH 7.5, 100 mM KCl, 8 mM $MgSO_4$, 3 mM ADP, 0.15 mM PEP, 0.2 mM NADH, excess of commercial lactate dehydrogenase, and different concentrations of phenylalanine. The incubation was carried out at 30°. (B) Effect of alanine concentration on the reaction rate of muscle pyruvate kinase in the absence or presence of phenylalanine or 2-PGA. Enzymatic assays were conducted as described in (A), but with different concentrations of alanine. The presence of 10 mM phenylalanine or 9.2 mM 2-PGA was indicated on the curves.

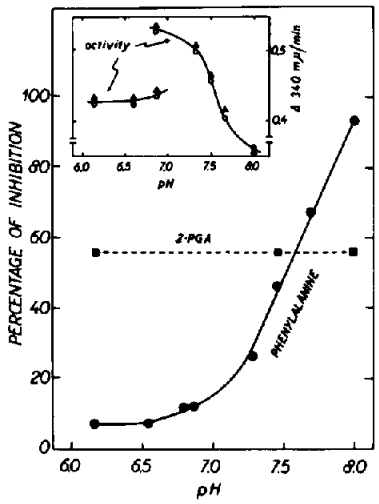

Fig. 2. Percentage of inhibition by phenylalanine or 2-PGA at different pH values. The conditions were as in fig. 1A except that the PEP concentration was 0.1 mM. MES-TMA (2-(*N*-morpholino)ethanesulfonic acid-tetramethylammonium hydroxide) and TES-TMA were used as buffers: the former for determinations in the acid range and the other for pH values above 7. Closed symbols represent the percentage of inhibition by 5 mM of phenylalanine (–●–) or 9.2 mM 2-PGA (–■–). *Inset:* pyruvate kinase activity at different pH values. –○–, enzyme activity; –△–, activity in the presence of both, phenylalanine (5 mM) and serine (5 mM).

enzymes (for references see [6]). In order to test if there is any effect of pH on the allosteric behavior of muscle pyruvate kinase, its inhibition by phenylalanine was further studied at different hydrogen ion concentration. It can be observed in fig. 2 that at a pH lower than 7.0 the inhibition of enzyme activity by phenylalanine is negligible and is maximal at pH 8.0. On the other hand, pyruvate kinase activity in the absence of the inhibitor is higher at pH 7.0 than at pH 8.0. It is important to indicate that the inhibition by 2-PGA, the isosteric inhibitor, does not vary in the whole range of pH values tested, thus indicating an essentially intact active site. Furthermore, serine at 5 mM reversed completely the phenylalanine inhibition at all the pH values tested.

The homotropic cooperative effect of phenylalanine is also affected by changes in the pH of the incubation medium. The data summarized in table 1 show that the enzyme has a sigmoidal response to the inhibitor, which became more pronounced as the pH was raised. The Hill coefficient increases from 1.3 at pH 7.25 to 2.1 at pH 8.0 and this increase is associated with a decrease in the $I_{0.5}$ values. In connection to these results it is pertinent to mention that the enzyme undergoes conformational changes when the pH is shifted from 7.8 to 7.0 [8].

The results presented in this communication indicate that the inhibition of the skeletal muscle pyruvate kinase by penylalanine is of allosteric nature. This conclusion is supported by the reactivating effect of cysteine, serine and alanine, not found with the isosteric inhibitor, and by the fact that at pH lower than 7.0 the inhibition by phenylalanine disappears whereas the effect of 2-PGA is independent of the pH of the assay medium. Finally, at pH 7.5 or higher the homotropic interaction of phenylalanine is

Table 1
Variation of the $I_{0.5}$ and the Hill coefficient for phenylalanine as a function of pH.

pH	$I_{0.5}$ (mM)	n_H
7.25	8.0	1.3
7.50	4.1	1.5
7.75	3.0	1.8
8.0	1.7	2.1

Assay conditions were similar to those described in fig. 1 A expect for the pH values and the PEP concentrations which was 0.1 mM.

clearly observed, in contrast to the hyperbolic inhibition curve displayed by 2-PGA.

At present the physiological significance of these results is not clear. Further studies will be carried out on the M isoenzymes of pyruvate kinase from other tissues, in order to find out if similar effects are also present.

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