ALLOSTERIC PROPERTIES OF ADIPOSE TISSUE PYRUVATE KINASE

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Received February 18, 1971

SUMMARY. The pyruvate kinase of rat adipose tissue is strongly inhibited by physiological concentrations of alanine. This inhibition is counteracted by FDP+ in the μmolar range of concentrations. These effects can be observed independently of the homogenization medium employed and in partially purified preparations of the enzyme. Aging of the preparations tends to produce the loss of the inhibitory and activatory effects, pointing to their allosteric nature.

Pyruvate kinase, which catalyses the third irreversible step in glycolysis presents important regulatory properties in gluconeogenic tissues (1). In adipose tissue the possible existence of two interconvertible enzyme forms with regulatory implications was suggested (2,3) mainly on the basis of some different responses in the kinetics respect to phosphoenolpyruvate when adipose tissue was homogenized in a medium with and without 5 mM EDTA, although the physiological significance of this effect has been criticised (1). Further studies on the regulatory properties of pyruvate kinase in different rat tissues (4) have led to the characterization of some definite allosteric properties of potential physiological significance in the pyruvate kinase from both white and brown adipose tissue.

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+ Abbreviations: FDP, fructose-1,6-diphosphate; PEP, phosphoenolpyruvate.

Materials and Methods. Epididymal adipose tissue of white Wistar rats of about 100 g of weight and brown adipose tissue from the interscapular burse of newborn rats were used in these experiments. The rats were decapitated and the tissue was rapidly removed and homogenized in the cold (2-4°) in 0.25 M sucrose. The extracts were centrifuged at 30,000 g x 20 min and the supernatants were used in the enzyme assays, except in the case of the pyruvate carboxylase where a suspension of the pellet of this centrifugation was used after freeze drying.

Pyruvate kinase, enolase, FDPase and PEP carboxykinase, were measured as previously described (1). Hexokinase, phosphofructokinase, glucosephosphate isomerase, glucose 6-phosphate dehydrogenase, malate dehydrogenase and glutamic oxaloacetic transaminase were measured essentially as described by Vallejo et al. (5), 6-phosphogluconate dehydrogenase as Vallejo et al. (6), pyruvate carboxylase as Marco et al. (7) and aldolase as Sillero et al. (8).

Glutamic pyruvic transaminase was measured using a similar assay mixture as that for glutamic oxaloacetic transaminase, with alanine instead of aspartate and lactate dehydrogenase instead of malate dehydrogenase. Lactate dehydrogenase was assayed similarly as malate dehydrogenase, with pyruvate instead of malate.

L-α-glycerophosphate dehydrogenase was measured following the decrease in absorbancy at 340 nm with 50 mM imidazole HCl pH 7, 0.1 mM NADH and 0.2 mM dihydroxyacetone-P. Phosphoglycerate kinase was measured in 50 mM imidazole HCl pH 7, 0.1 M KCl, 5 mM $MgCl_2$, 2 mM ATP, 0.1 mM NADH, 2 mM 3-P-glycerate and 2 units of glyceraldehyde-3-P dehydrogenase. Phosphoglycerate mutase was measured in 50 mM imidazole HCl pH 7, 0.1 M KCl, 5 mM $MgCl_2$, 2 mM ATP, 0.1 mM NADH, 0.15 mM 2,3-diphosphoglycerate, 2 mM 2-P-glycerate, 2 units of phosphoglycerate kinase, and 2 units of glyceraldehydephosphate dehydrogenase.

Enzyme activities are expressed in μmoles/min. Protein was measured as Lowry et al. (9).

Results and Discussion. Fig. 1 shows the kinetics of white adipose tissue pyruvate kinase with respect to its substrate PEP. In the presence of 2 mM alanine, a typical sigmoid curve appears with a $S_{0.5}$ of about 0.5 mM, while in its absence it is of about 0.1 mM. FDP at 10 μM concentration completely removes the inhibition and transforms the kinetics from sigmoid to hyperbolic. These results have been obtained with imidazole buffer and with extracts stored in the cold (2-4°). Preincubation of the extracts did not change the response of the enzyme and similar results could be obtained using phosphate buffer. Sometimes when the extracts were stored in the refrigerator for a long period of time (about one week) the described effects were lost, without significant decrease in the activity of the enzyme, pointing to the allosteric nature of these effects.

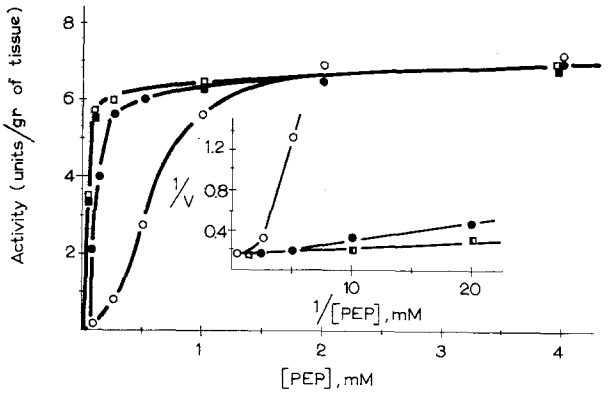

Fig. 1. Adipose tissue pyruvate kinase. Kinetics for the PEP in the presence or absence of the allosteric effectors. The assay mixture contained in 1 ml, 50 mM imidazole-HCl pH 7, 0.1 M KCl, 5 mM $MgCl_2$, 1 mM ADP, PEP at the concentration indicated, 0.15 mM NADH, 0.5 units of lactate dehydrogenase and 20 µl of adipose tissue homogenized in 2 volumes of 0.25 M sucrose; (●), no effectors added; (○), + 2 mM alanine; (■), + 10 µM FDP; (□), + 2 mM alanine and 10 µM FDP. Blanks without ADP or PEP, carried out in parallel, were substracted. A double reciprocal plot of the data appears in the insert.

In Fig. 2A the effect of the concentration of the allosteric inhibitor alanine is shown. At 0.25 mM PEP a concentration of less than 1 mM alanine is enough to produce 50% inhibition. There is no appreciable inhibition in the presence of 10 µM FDP. ATP has no marked inhibitory effect on the kinetics of adipose tissue pyruvate kinase, except the well known inhibition as product at high concentrations (3). Moreover, when the enzyme is assayed with imidazole buffer, the presence of 2 mM MgATP removes the effect of alanine, acting similarly to FDP. Nevertheless, with phosphate buffer, MgATP has no effect either on the kinetics respect to PEP or on the inhibition by alanine and the counterinhibition by FDP. When the imidazole buffer is used, FDP concentrations below 0.1 µM are enough for fully counteracting the alanine inhibition. With 40 mM K^+ phosphate as buffer the concentration of FDP for 50% counterinhibition is of about 1 µM (Fig. 2B). The sigmoidicity of the concentration curves of both effectors (Figs. 2A and 2B) also indicates the allosteric nature of their action. On the other hand, the tendency to desensitization

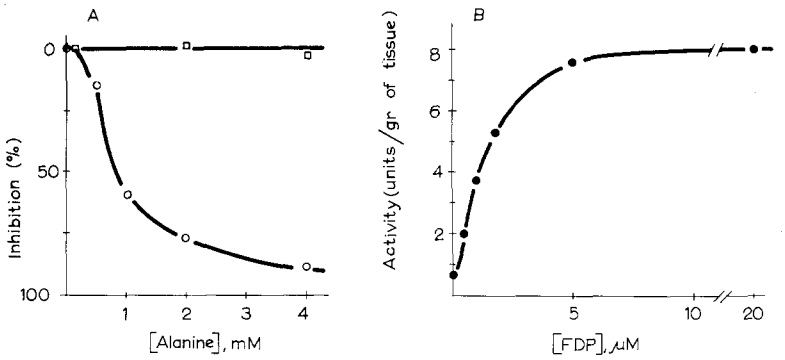

Fig. 2. Effects of the concentration of effectors on the adipose tissue pyruvate kinase activity. A. Inhibition by alanine. The enzyme was assayed as in Fig. 1 at 0.25 mM PEP; (O), no FDP; (□), + 10 μM FDP. B. Counterinhibition by FDP. Assay as in Fig. 1, but with 40 mM potassium phosphate pH 7 as buffer, 0.1 mM PEP and 2 mM alanine.

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with the imidazole buffer, and the definite absence of allosteric inhibition by ATP of the adipose tissue enzyme, explain our previous failure to detect these allosteric properties (1).

Among the analogues of alanine investigated only phenylalanine inhibited adipose tissue pyruvate kinase in a similar degree. Aspartate (2 mM), malate (4 mM), β-alanine (2 mM), glycine (2 mM), or lactate (4 mM) did not inhibit significantly. No counterinhibition was found with 0.5 mM fructose-1-P, 0.5 mM glucose-1-P, 1 mM glucose-6-P or 2.5 mM fructose-6-P. The enzyme could be partially purified by precipitation with $(NH_4)_2SO_4$ without loss of the allosteric properties. No qualitative difference in the allosteric behavior of the adipose tissue pyruvate kinase could be observed when the adipose tissue was homogenized with the buffers described by Pogson (2, 3).

A qualitatively similar behavior of the pyruvate kinase of brown adipose tissue of the interscapular bursa of rat fetuses and newborn has been observed.

In principle, these allosteric properties of adipose tissue pyruvate kinase may have some specific physiological function in this tissue, unless it were an isoenzyme primarily selected for its value in some other tissue related to adipose tissue in the process of differentiation. In this

Table I. *Enzyme activities in adipose tissue*

	U/g of tissue		U/g of tissue
Hexokinase	0.5	Glucose 6-phosphate dehydrogenase	3 (0.3)
Glucosephosphate isomerase	7 (0.3)	6-Phosphogluconate dehydrogenase	0.5
Phosphofructokinase	0.5 (2)	Fructose 1,6-diphosphatase	none detected
Aldolase	0.3 (0.3)	L-α-Glycerophosphate dehydrogenase	10
Phosphoglycerate kinase	3	Pyruvate carboxylase { Fed	0.08*
Phosphoglyceromutase	3	Pyruvate carboxylase { Starved (48 hr)	0.07*
Enolase	5	Phosphoenolpyruvate carboxykinase { Fed	0.05*
Pyruvate kinase	7	Phosphoenolpyruvate carboxykinase { Starved (48 hr)	0.16*
Lactate dehydrogenase	26	Malate dehydrogenase	31
Glutamic pyruvic transaminase	0.2	Glutamic oxaloacetic transaminase	0.9 (2)

All values were obtained with adipose tissue of normal fed animals except when indicated otherwise. The tissue was homogenized with 0.25 M sucrose as indicated in Materials and Methods except in the cases when the values are followed by a figure in brackets. In these cases the homogenization medium of Reshef and Heller including 10 mM phosphate buffer (17) was used. The ratios between the values obtained with this medium and those obtained with 0.25 M sucrose alone are given in the brackets. Extracted protein was ca. 10 mg/g tissue in all cases.

*These values are the mean of three animals.

case the corresponding gene may be expressed in the latter even if there is in it no physiological benefit from these potentially regulatory allosteric properties. The information on the concentrations of metabolites in adipose tissue is scanty (10-15), based mainly on measurements in incubated fat pads (10-14), and not easy to interpret in terms of actual intracellular concentrations of metabolites (16). No data for alanine are available to our knowledge and the concentration of FDP calculated from available data (13) seems rather high (0.1 mM).

Table I shows the activities in white adipose tissue of some glycolytic and other related enzymes. As observed by Reshef and Heller (17) there are some differences in results depending on the homogenization medium used. There is a considerable difference in the activities of the key enzymes of the higher part and the lower part of the glycolytic pathway. While hexokinase and phosphofructokinase have an activity of about 0.5 units/g of tissue, pyruvate kinase has an activity of about 7 units/g. Therefore, a possible physiological implication of the allosteric properties above described could be the coordination of the overall functioning of the glycolytic pathway. The FDP activation of pyruvate kinase in adipose tissue could have two important consequences: 1), to maintain a steady state concentration of FDP and dihydroxyacetone-P high enough to produce L-glycerol-1-P in amounts sufficient for lipogenesis, and 2), to prevent the accumulation of pyruvate and lactate (and consequently of alanine) in quantities greater than those which can be transformed in acetyl-CoA in adipose tissue, preventing the loss of these intermediates (pyruvate and lactate) to the extracellular space.

On the other hand the possible physiological significance of the existence of a phosphoenolpyruvate carboxykinase activity in adipose tissue has been emphasized by Reshef et al. (18), and Goring et al. (19) using it for explaining the isotopic labeling of glycerol from pyruvate. As can be seen in Table I, pyruvate kinase activities exceed by about two orders of magnitude the phosphoenolpyruvate carboxykinase even in fasted young rats ((19) and Table I). Therefore only with a strict regulation of pyruvate kinase could the PEP formed from oxaloacetate be transformed into glycerol to a significant extent. Nevertheless, the

possible physiological significance of this potential glycerogenic pathway is not very clear, as it should become operative only when no glucose is available, i.e. in conditions when lipogenesis is decreased and lipolysis is increased in adipose tissue.

Acknowledgements. J.C. had a fellowship of the Spanish Ministry of Education and Science. The advice and help of Dr. A. Sols and the technical assistance of Miss Ciotilde Estévez is gratefully acknowledged.

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