ATP-DEPENDENT INACTIVATION OF HEART MUSCLE PYRUVATE DEHYDROGENASE AND REACTIVATION BY Mg^{++}

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1. Introduction

During *in vitro* studies on the regulatory properties of heart muscle pyruvate dehydrogenase we observed that, in addition to acetyl-CoA [1,2], ATP is a potent inhibitor of the enzyme reaction. In contrast to the action of acetyl-CoA, the effect exerted by ATP was dependent on time of incubation with the enzyme and also on temperature, suggesting some chemical interaction between ATP and the enzyme protein. Very recently, Linn et al. [3] have reported that pyruvate dehydrogenase from beef kidney mitochondria is susceptible to inhibition by ATP, and that this effect is due to enzymatic phosphorylation of the enzyme. Conversely, reactivation of the dehydrogenase was observed, in these studies [3], at increased concentrations of Mg^{++}, and this was ascribed to Mg^{++}-dependent stimulation of enzymatic dephosphorylation of the protein. The results presented in this paper strongly indicate that the pyruvate dehydrogenase complex from pig heart muscle is similarly regulated by ATP-dependent phosphorylation (kinase reaction) and by hydrolytic dephosphorylation (phosphatase reaction) of the enzyme protein.

2. Materials and methods

Pyruvate dehydrogenase complex was isolated from pig heart, and enzyme activity was determined by spectrophotometrical measurement of NAD reduction with pyruvate as substrate as described [2]. Coenzymes and other reagents were the same as used in the earlier studies [2]. The various nucleoside triphosphates and the cyclic-3′, 5′-AMP preparations were either gifts or commercial samples of Boehringer, Mannheim, Germany. Detailed information on the purity of these compunds will be given elsewhere [4].

3. Results

3.1. *Inactivation of pyruvate dehydrogenase by ATP*

If pyruvate dehydrogenase from pig heart muscle is incubated with ATP, the enzyme is successively

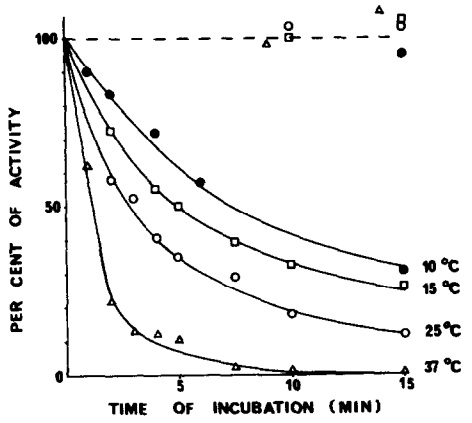

Fig. 1. Time course of inactivation of heart muscle pyruvate dehydrogenase by ATP. 21.4 μg pyruvate dehydrogenase were incubated with 1×10^{-4} ATP in microcuvettes (d = 1 cm) at different temperatures as indicated. At the intervals indicated the reaction mixture was completed for optical assay by adding the components described in table 2, and enzyme activity was measured. pH = 7.51.

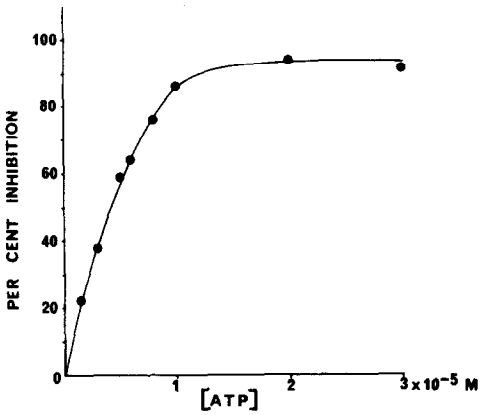

Fig. 2. Inactivation of heart muscle pyruvate dehydrogenase as a function of ATP concentration. 10 μl samples of pyruvate dehydrogenase, corresponding to 32 μg protein, containing 0.5 mM $MgCl_2$ and 5 mM mercaptoethanol were incubated with ATP in the concentrations indicated, to give a final volume of 20 μl, for 15 min at 25°C. Enzyme activity was then determined spectrophotometrically by transferring the preincubated samples into microcuvettes containing, in a final volume of 0.19 ml, Tris-Cl, pH 8, 75 mM; $MgCl_2$ 0.53 mM; mercaptoethanol, 2.6 mM; thiamine pyrophosphate 1.05 mM; NAD 2.1 mM; pyruvate 5.3 mM; CoA-SH, 0.53 mM. The reaction was started by adding CoA-SH, and the increase in optical density was recorded as indicated in table 2.

inactivated. Fig. 1 represents the dependency of this inactivation on time as measured at different temperatures. At incubation of the enzyme with increasing concentrations of ATP, a typical saturation curve was obtained (fig. 2). From these data, half maximal inactivation occurs at about 4×10^{-6} M ATP.

3.2. *Nucleotide specificity of inactivation of pyruvate dehydrogenase*

As shown in table 1, among the various nucleotides investigated only ATP, and – to a marked lesser extent – ADP is effective in inactivating pyruvate dehydrogenase. The ADP effect is probably due to the action of myokinase still present in the enzyme preparation yielding sufficient inhibitory amounts of ATP from ADP. Potassium pyrophosphate, in a concentration of 2.5×10^{-4} M, was without effect.

3.3. *Effect of Mg^{++} on the ATP-dependent inactivation of pyruvate dehydrogenase*

Among the various components of the pyruvate dehydrogenase system, Mg^{++}, when added at high concentrations to the preincubation medium, completely abolished the inactivation of the enzyme by ATP (table 2). The other cofactors such as thiamine pyrophosphate, NAD, and CoA-SH remained ineffective in this respect. Protection of the enzyme against ATP inactivation – though to a lesser degree – was also seen in the presence of pyruvate.

Table 1

Nucleotide specificity of inactivation of heart muscle pyruvate dehydrogenase. Experimental conditions of enzyme preincubation and optical assay were as indicated in table 2.

Nucleotide	Concentration (M)	PDH-activity, ΔE/min/334 mμ
No addition	–	0.140
ATP	6.25×10^{-5}	0.010
GTP	6.25×10^{-5}	0.150
ITP	6.25×10^{-5}	0.130
UTP	6.25×10^{-5}	0.135
3′,5′-cyclo AMP	6.25×10^{-5}	0.140
Dibutyryl-3′,5′-cyclo AMP	6.25×10^{-5}	0.105
No addition	–	0.115
ATP	5×10^{-4}	0.005
CTP	3×10^{-4}	0.093
GTP	5×10^{-4}	0.120
ITP	5×10^{-4}	0.120
UTP	5×10^{-4}	0.115
3′,5′-cyclo AMP	5×10^{-4}	0.145
Dibutyryl-cyclo-AMP	5×10^{-4}	0.110
No addition	–	0.090
ADP	6.25×10^{-5}	0.060
ADP	5×10^{-4}	0.022
AMP	6×10^{-4}	0.087

3.4. *Reversibility of ATP-dependent inactivation of pyruvate dehydrogenase*

Inactivated pyruvate dehydrogenase can be completely reactivated by high concentrations of Mg^{++}.

Table 2
Inactivation of pig heart pyruvate dehydrogenase by ATP.

Pyruvate dehydrogenase preincubated with		Enzyme activity after preincubation (ΔE/min/334 mμ)
ATP (M)	Cofactors (M)	
–	–	0.100
6.25×10^{-5}	–	0.005
6.25×10^{-5}	Thiamine pyrophosphate 8×10^{-3}	0.010
6.25×10^{-5}	NAD, 1.6×10^{-2}	0.005
6.25×10^{-5}	CoA-SH, 8×10^{-4}	0.005
6.25×10^{-5}	$MgCl_2$, 6.25×10^{-5}	0.005
6.25×10^{-5}	$MgCl_2$, 8×10^{-3}	0.160
–	–	0.132
2×10^{-4}	–	0.007
2×10^{-4}	Pyruvate, 3.3×10^{-3}	0.073

107 μg pyruvate dehydrogenase was incubated with ATP, final concentration 6.25×10^{-5} M, and the cofactors indicated, in a final volume of 25 μl for 10 min at 25°C. Thereafter, 5 μl aliquots were removed and assayed for enzyme activity. The reaction mixture contained, in a final volume of 0.2 ml: Tris-HCl, pH 8, 10 μmoles, mercaptoethanol, 0.35 μmoles, $MgCl_2$ 1.4 μmoles, thiamine pyrophosphate 0.14 μmoles, NAD 0.07 μmoles, EDTA 0.07 μmoles, sodium pyruvate 0.2 μmoles, pyruvate dehydrogenase 21.4 μg. The reaction was started by adding 0.02 μmoles CoA-SH. The increase in optical density was recorded at 334 mμ, Eppendorf-Photometer with recorder attachment, amplification twofold. Light path 1 cm, temperature 25°C.

A typical experiment is shown in fig. 3. After 15 min, the enzyme was completely inactivated by ATP. 25 min following addition of Mg^{++} in a twentyfold concentration, enzyme activity had been fully restored. From the control values it can further be seen that the enzyme, in the absence of ATP, gains some activity during prolonged incubation in the presence of Mg^{++}.

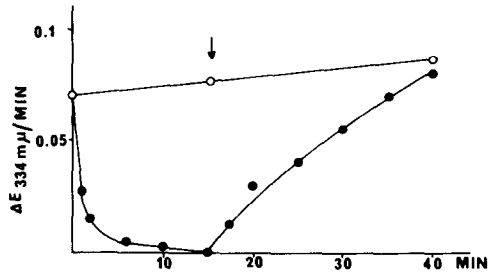

Fig. 3. Inactivation of heart muscle pyruvate dehydrogenase by ATP and reactivation by Mg^{++}. 642 μg pyruvate dehydrogenase, 150 μmoles $MgCl_2$, 1.5 μmoles mercaptoethanol, 9.25 μmoles potassium phosphate buffer, pH 7.0, in a final volume of 0.3 ml, were preincubated for 20 min at 25°C. After determining initial enzyme activity in a 5 μl-aliquot, 10 μl of 3.4 mM ATP was added and incubation was continued for further 15 min at 25°C. At this point (arrow) 5 μl of $MgCl_2$ was added to make a final concentration of 10 mM Mg^{++}. For enzyme determinations, 5 μl aliquots were removed from the incubation mixture at the time intervals indicated, and assayed as described in table 2 with the following alterations: NAD and CoA-SH were increased to 0.36 μmoles and 0.09 μmoles, respectively, and EDTA was omitted. The open circles represent the activity of pyruvate dehydrogenase incubated under identical conditions but without the addition of ATP.

4 Discussion

In contrast to inhibition of pyruvate dehydrogenase by acetyl-CoA [1, 2], the inhibition by ATP as reported here bears some characteristics suggesting chemical interaction between ATP and the enzyme. This is indicated by the dependency of the inactivation process upon time and temperature (fig. 1).

There is also remarkable specificity of the process. From the various nucleotides investigated only ATP is effective. The smaller inactivation produced by ADP (table 1) can be ascribed to the fact that our enzyme preparation still contains some activity of myokinase sufficient to produce inhibitory concentrations of ATP from ADP during the incubation period. ATP-induced inactivation of pyruvate dehydrogenase from beef kidney mitochondria and its reversal by Mg^{++} has recently been reported by Linn et al. [3]. From their studies these authors concluded that the enzyme, during inactivation, undergoes enzymatic phosphorylation by an ATP-dependent kinase, and that it is reactivated due to hydrolytic cleavage of the protein-bound phosphate group by a phosphatase, the latter being activated at high concentrations of Mg^{++}. Although definite proof of phosphate transfer from ATP to the protein is lacking in the present studies, the close similarity of the kinetic properties strongly suggests that the heart muscle enzyme is also subject to ATP-dependent interconvertibility from an active, dephospho-form to an inactive, phosphorylated form. As in the case of the kidney enzyme,

both the kinase and the Mg^{++}-stimulated phosphatase seem to represent subunits which are incorporated into the multienzyme complex of heart muscle pyruvate dehydrogenase. From the data in fig. 2, K_m = 4×10^{-6}M ATP for the heart muscle pyruvate dehydrogenase-kinase can be derived.

The inactivation of pyruvate dehydrogenase by ATP and its reversion by Mg^{++} opens new aspects on the control of pyruvate metabolism in animal tissues. This has been discussed in relation to gluconeogenesis in the kidney [3]. In heart muscle where new formation of glucose does not occur, ATP-dependent control of pyruvate dehydrogenation may serve as a mechanism for sparing pyruvate breakdown when other fuel, such as fatty acid, is supplied to meet the energy requirement of the cell. Increased ATP concentration as well as a decrease in free Mg^{++} concentration at the site of pyruvate dehydrogenase in heart muscle mitochondria would be effective in controlling the enzyme reaction. It remains established, whether these changes do occur in the living cell corresponding to the special metabolic state.

Acknowledgement

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