

Phosphorylation of the skeletal muscle AMP-deaminase by protein kinase C

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Protein kinase C catalyzes phosphorylation of the rat skeletal muscle AMP-deaminase in the presence of calcium ions and phosphatidylserine. At the same time, the catalytic subunit of cAMP-dependent protein kinase fails to phosphorylate AMP-deaminase. Ca^{2+} , phosphatidylserine-dependent phosphorylation decreases three-fold (from 0.6 to 0.2 mM) the K_m value and does not affect V_{max} . Protein kinase C-induced phosphorylation of AMP-deaminase, besides ADP-ribosylation, is suggested to be involved in regulating the AMP-deaminase activity in vivo.

AMP-deaminase; Phosphorylation; Protein kinase C

1. INTRODUCTION

AMP-deaminase (5'-AMP-aminohydrolase, EC 3.5.4.6) catalyzes deamination of AMP that leads to accumulation of equimolar amounts of IMP and NH_3 . The enzyme is widely spread in numerous animal tissues, its maximal activity being revealed in skeletal muscles [1,2]. A number of authors have shown that AMP-deaminase is an allosteric enzyme which is regulated by ATP, ADP, GTP, and monovalent cations [1-4].

Among possible ways of regulation of the AMP-deaminase catalytic activity is covalent modification of the protein molecule. In particular, the allosteric properties of skeletal muscle AMP-deaminase have recently been shown to be regulated through arginine-specific mono-ADP-ribosylation [5].

Since at present there is no doubt that the processes of protein phosphorylation-dephosphorylation are of great importance in the regulation of cell metabolism [6-11], we decided to investigate the possibility of AMP-deaminase phosphorylation by cellular protein kinases.

2. MATERIALS AND METHODS

AMP, ATP, and phosphatidylserine were purchased from Sigma; [γ - ^{32}P]ATP, from Amersham. CaCl_2 , glycerol, SDS, imidazole were from Serva, and dithiothreitol (DTT) from Calbiochem. Rat skeletal muscle AMP-deaminase was purified by the method of Smiley et al. [12] with the following modifications. The frozen leg muscles were homogenized in 89 mM K-phosphate buffer, pH 6.5, containing

180 mM KCl, 1 mM DTT, and centrifuged ($6000 \times g$ for 30 min). The supernatant was adsorbed on phosphocellulose (Whatman). The enzyme was eluted with 1 M of KCl, dialyzed, rechromatographed on a column with phosphocellulose (1.5×10 cm), and concentrated by dialysis against K-phosphate buffer, containing 80% glycerol. The enzyme preparation was subjected to additional purification by gel filtration on Sephadex G-200 (Pharmacia).

Protein kinase C from human brain was partially purified by the method described in [13] using ion-exchange chromatography on DEAE-Toyopearl 650M (Toyo-Soda), hydrophobic chromatography on phenyl-Sepharose (Pharmacia) and HPLC on a TSK G-2000 SW carrier (LKB). Protein kinase C activity was assayed according to [14].

The catalytic subunit of cAMP-dependent protein kinase was kindly provided by I.D. Grozdova of the Research Center of Molecular Diagnostics, USSR Ministry of Health.

Deaminase phosphorylation by protein kinase C was carried out in the reaction mixture (final volume = 100 μl), containing 50 mM Tris-HCl (pH 7.0), 5 mM DTT, 0.5 mM CaCl_2 , 50 μM ATP, [γ - ^{32}P]ATP (16 kBq), phosphatidylserine (50 $\mu\text{g}/\text{ml}$) and 80 μg deaminase. The reaction was initiated by addition of 1 μg of protein kinase C, followed by incubation for 10 min at 37°C. To terminate the reaction, aliquots (40 μl each) were applied to Whatman 3 MM cellulose filters (1.5×1.5 cm). The filters were washed thrice with 10% solution of trichloroacetic acid containing 100 mM $\text{Na}_4\text{P}_2\text{O}_7$, 100 mM Na_2WO_4 and 100 mM K_2HPO_4 , each time for 10 min, and dried. The ^{32}P incorporation in AMP-deaminase was determined using an SL-40 liquid scintillation counter (Intertechnique).

In autoradiography experiments on deaminase phosphorylation, the reaction was terminated by addition of 10% ice-cold trichloroacetic acid solution.

The effect of protein kinase C on AMP-deaminase activity was analyzed in the following procedure. The phosphorylation reaction was performed as described above but without addition of ^{32}P -labeled ATP. Following the incubation, 20 μl of the mixture were collected and the AMP-deaminase activity was assayed as in [15]. The reaction mixture (of a 2 ml final volume) contained 50 mM imidazole-HCl buffer, pH 6.5, 100 μM KCl, and 2.5 mM AMP. The reaction was initiated by addition of the enzyme. The rate of hydrolysis was determined by accumulation of the reaction product (IMP) and an increase in the optical density at 285 nm using a Hitachi 220 A spectrophotometer.

SDS-PAGE was run by the method of Laemmli [16] in 10% poly-

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acrylamide slabs ($9 \times 12 \times 0.04$ cm). The protein concentration was determined using the method proposed by Bradford [17].

3. RESULTS AND DISCUSSION

AMP-deaminase was earlier shown to consist of four identical subunits with a molecular mass around 69–76 kDa each [18]. However, our previous SDS-PAGE experiments allowed us to reveal two additional minor polypeptides with molecular masses of 42 and 33 kDa [15]. These polypeptides did not possess the catalytic activity and were apparently the products of proteolytic degradation of the native enzyme (fig.1, lane 1). The autoradiogram of electrophoretic separation of the enzyme phosphorylated by human brain protein kinase C demonstrated incorporation of the ^{32}P -labeled phosphate of ATP into the protein bands corresponding to the main enzyme subunit and the minor 39 kDa polypeptide (fig.1, lane 2).

The AMP-deaminase preparation exhibited no endogenous phosphotransferase activity; moreover, under our experimental conditions, we failed to detect autophosphorylation of protein kinase C (data not shown). Protein kinase C-induced phosphorylation of AMP-deaminase was highly specific (according to our data, the process was characterized by a sufficiently low K_m value of $2.3 \pm 0.3 \mu\text{M}$, which is comparable with the values of the same constant found for some other physiological substrates of protein kinase C [6]) and observed only in the presence of both cofactors (Ca^{2+} ions and phosphatidylserine). The absence of at least one of

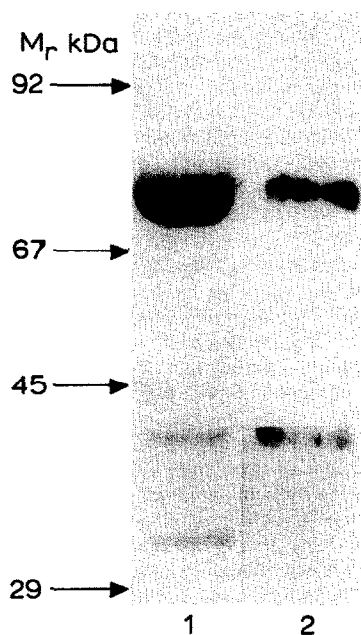


Fig.1. Electrophoregram of rat skeletal muscle AMP-deaminase phosphorylated by protein kinase C. Lane 1, gel stained with Coomassie blue R-250; lane 2, autoradiogram of the same gel. The molecular masses of the marker proteins are shown by arrows.

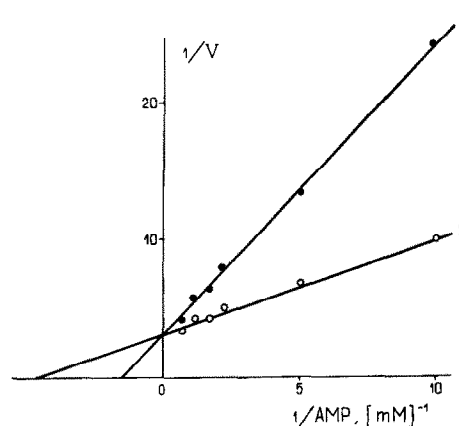


Fig.2. Effect of phosphorylation by protein kinase C on AMP-deaminase activity. Deaminase was preincubated with 0.5 mM CaCl_2 , phosphatidylserine (50 $\mu\text{g}/\text{ml}$), and 50 μM ATP in the absence (1) and the presence of 1 μg of protein kinase C (2).

the activators eliminated incorporation of the labeled phosphate into the enzyme.

We have also studied the effect of the capacity of cAMP-dependent protein kinase upon AMP-deaminase. Incubation of deaminase with the catalytic subunit of cAMP-dependent protein kinase and subsequent electrophoretic separation did not reveal a ^{32}P incorporation into the protein bands corresponding to deaminase. Thus, the phosphotransferase was shown to be incapable of modifying the deaminase molecule.

In the last series of experiments, the effect of the AMP-deaminase phosphorylation on its enzymatic activity has been investigated. As can be seen in fig.2, protein kinase C-dependent phosphorylation did not affect the maximal reaction rate but caused a 3-fold decrease in the K_m value from 0.6 to 0.2 mM. Thus, the Ca^{2+} , phospholipid-dependent phosphorylation of AMP-deaminase results in activation of this enzyme, an effect similar to that described for some other cellular enzymes (guanylate cyclase [8], tyrosine hydroxylase [9], phosphofructokinase [6,7,10,11]). In conclusion, a novel substrate of protein kinase C, namely AMP-deaminase, has been identified. The physiological significance of this phenomenon is yet to be established.

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