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The author wishes to dedicate this minireview to the memory of his Master, Professor Włodzimierz Mozołowski (1895–1975), the first Editor-in-Chief of Acta Biochimica Polonica

Regulatory effects of the lipid-cytosolic enzyme interaction: AMP deaminase

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Although for many years soluble (cytosolic) intracellular enzymes were usually distinguished from those particle-bound, since the 1960-ies evidence has accumulated that some enzymes regarded so far as typical cytosolic once (e.g., glycolytic enzymes) may be bound to cellular membranes [1], at least in a transient way.

Fifteen years ago we have isolated AMP deaminase (AMP-D)[1] from pig heart [2]. This enzyme, known to be localised in cytoplasm [3], has been shown to be activated by ATP, but the ATP-activated enzyme, when preincubated with phospholipid bilayers (liposomes), displayed an unexpected property: the ATP-activated AMP-D from heart showed an increase in V_{max} almost by 100% after preincubation with egg yolk phosphatidylcholine-containing liposomes (Fig. 1). This activatory effect of model membranes on the soluble enzyme kinetics raised two questions:

- 1. What is the molecular nature of the enzyme-lipid interaction occurring apparently between the heart AMP-D and phosphatidylcholine;
- 2. What is the metabolic significance of this phenomenon.

As a first approach to answer these questions we checked whether the lipids present in the natural membranes of cardiomyocytes display the same effect as the egg yolk phosphatidylcholine used previously. It has been found [4] that the lipids extracted from mitochondria or microsomes influence the pig and rat heart AMP-D kinetics in the same way as in the experiment presented in Fig. 1. The same did the liposomes composed of a mixture of lipids in a proportion corresponding to the average composition of lipids in the natural heart muscle intracellular structures [4].

However, the lipid composition of some intracellular membranes is known to undergo changes under the influence of a variety of factors [5, 6]. For example the proportion of sphingomyelin in lysosomal membranes seems to increase appreciably under some conditions [7]. We investigated the effect of liposomes composed of sphingomyelin only and have found that they display an inhibitory effect on the enzyme both in the presence and in the absence of ATP [8] (Fig. 2). This may be of some metabolic significance. Also some other phospholipid species (e.g. phosphatidate [9] or cardiolipin [9]), which are inhibitory, seem to be potential candidates for being regulators of AMP-D activity in the heart. It may be seen in Fig. 3 that the inhibitory effect of phosphatidate is exerted even on the non-activated enzyme [9], and Fig. 4 shows that this is a noncompetitive inhibition with a K_i value as low as 15 ×

[1] Abbreviations used: AMP-D, AMP deaminase (EC 3.5.4.6); DOPC, dioleoylphosphatidylcholine; DMPC, dimirystoroylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine.

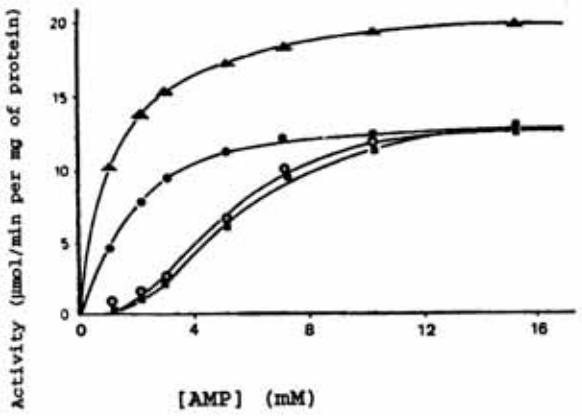

Fig. 1. The effect of phosphatidylcholine-containing liposomes on ATP-activated AMP deaminase from pig heart.
Incubations were carried out without effectors added (○); in the presence of: 1 mM ATP (●); 375 nmol of egg yolk phosphatidylcholine (■) and in the presence of 1mM ATP plus 375 nmol phosphatidylcholine (▲). After [2].

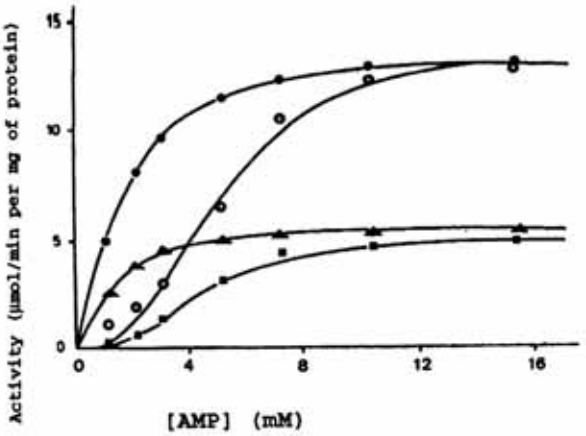

Fig. 2. The effect of sphingomyelin-containing liposomes on AMP deaminase.
Incubations were carried out without effectors added (○); in the presence of: 1 mM ATP (●); 375 nmol sphingomyelin (■) and in the presence of 1 mM ATP plus 375 nmol sphingomyelin (▲). After [8].

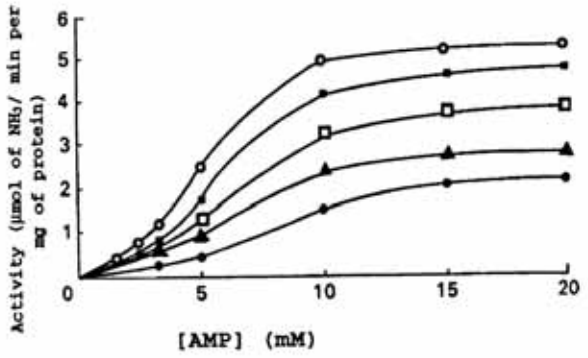

Fig. 3. The effect of dioleoylphosphatidate-containing liposomes on the non-activated AMP deaminase from pig heart.
The incubations were carried out without liposomes (○) or with liposomes containing phosphatidate at concentrations of: 4 μM (■); 8 μM (□); 16 μM (▲) and 20 μM (●). After [9].

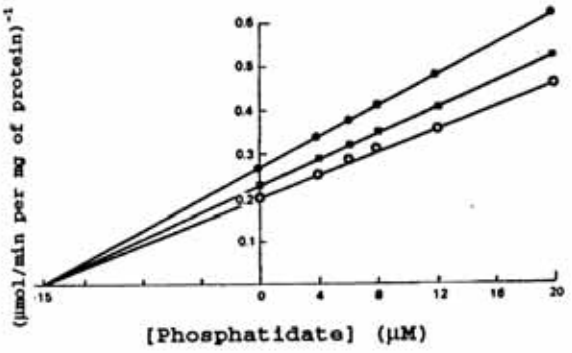

Fig. 4. Dixon plot presenting the effect of dioleoylphosphatidate-containing liposomes on ATP-activated pig heart AMP deaminase.
The incubations were carried out in the presence of 1 mM ATP and AMP at concentrations of: 3 mM (●); 5 mM (■) and 10 mM (○). After [9].

10^{-6} M. This inhibition constant is three orders of magnitude lower than for orthophosphate, regarded before as a "physiological" inhibitor of AMP-D [10]. The inhibitory effect is displayed by those phosphatidate species only which contain unsaturated fatty acids in their molecule; however dilauryphosphatidate (DLPA), a saturated species, if incubated at pH 7.9, becomes also inhibitory. This happens probably because, at the alkaline pH, the transition temperature of this lipid becomes lowered from 33°C to 15°C [9].

The diversity of the effects of unsaturated and saturated phosphatidate species on AMP-D activity indicates that the enzyme-lipid interaction occurs probably within the hydrophobic regions of both enzyme and phospholipid. This is true for the inhibitory effect of phosphatidic acid but also for the activatory effect of phosphatidylcholine. In Fig. 5 an experiment is presented in which dioleoylphosphatidylcholine (DOPC), but not dipalmitoylphosphatidylcholine (DPPC) has been demonstrated to activate the ATP-activated AMP-D from the heart [11]. This difference could also be caused by the fact that only those phospholipid species which contain unsaturated fatty acids are able to interact with AMP-D.

This has been confirmed by Fourier-transform infrared spectroscopy (Table 1). It may be seen that only the kinetically effective phospholipid species caused a change in the beta/alpha conformation ratio of the enzyme protein.

Thus, one may try to answer the first of the questions mentioned before, by saying that the phospholipid species which modify (regulate) enzyme kinetics are interacting with the enzyme protein in a way changing its secondary structure.

Although the effect of phospholipids on AMP deaminase from many tissues, except skeletal muscle, has been demonstrated [12], the metabolic significance of this interaction seems to be especially important in the heart. Hypoxia- and ischemia-induced heart injury is connected with dramatic changes in adenine nucleotide metabolism both in rat and human heart [13 - 15]. Preservation of appropriate adenine nucleotide pool in the myocardium is the main task of the metabolism for cell to survive the ischemic shock [16]. ATP concentration decreases during ischemia and AMP concentration may increase even fivefold. Two enzymes are able to catalyse further degradation of AMP: 5'-nucleotidase and AMP-D [10, 17]. Adenosine arises as a product of 5'-nucleotidase activity. This compound is postulated to be a "retaliatory catabolite", playing an essential role in improving blood supply to the heart by influencing coronary vessels [18]. AMP-D catalyses hydrolytic deamination of AMP to IMP, initiating complete degradation of adenylates; also it plays a role in maintaining a constant adenylate energy charge [19]. Low activity of AMP-D causes an increase in AMP concentration, which provides the substrate for adenosine production by 5'-nucleotidase and, thus, is supporting improvement of blood supply to the heart in the case of higher demand.

One can suppose that, at a high concentration of ATP, the cytosolic AMP-D interacts with phosphatidylcholine of the membrane and its activity becomes high. As a consequence of hypoxia or ischemia, phosphatidylcholine may be decomposed to phosphatidate which is in-

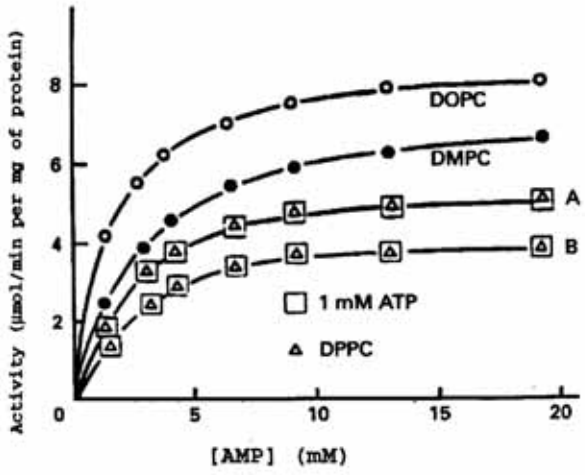

Fig. 5. The effect of dioleoylphosphatidylcholine (DOPC) (○), dimirystoroylphosphatidylcholine (DMPC) (●), and dipalmitoylphosphatidylcholine (DPPC) (Δ) - containing liposomes on ATP-activated AMP deaminase from pig heart; (❑), control experiments in the presence of 1 mM ATP without phospholipid added.

All incubations were carried out at 30°C, except in the case of DPPC which were carried out both at 30°C (curve A) and at 45°C (curve B). After [11].

Table 1

*Estimation of AMP deaminase secondary structure**

The shape of the deconvoluted amide I band of AMP deaminase was simulated by Gaussian/Lorentzian functions. The best fit was obtained with a Gaussian weighting factor of 0.8

	Content (%)					
	30°C, no ATP, no lipids	30°C, ATP, no lipids	30°C, ATP, 3 mg of DOPC	30°C, ATP, 6 mg of DOPC	30°C, ATP, 6 mg of DPPC	45°C, ATP, 6 mg of DPPC
β-Structures	45	46	39	29	46	44
α-Helices	28	31	37	43	34	30
Random	11	9	12	8	9	14
Turns	16	14	12	20	11	12
β/α ratio	1.60	1.48	1.05	0.67	1.35	1.46

*After [11].

hibitory to AMP-D [9]. The interaction of phospholipids with AMP-D can also be changed by a transverse movement of phospholipid in the membrane [20]. This may help to maintain a high AMP concentration required to produce the "retaliatory" adenosine. In this way, the enzyme-lipid interaction may be a part of a regulatory mechanism by which adaptation to the changing heart oxygen supply occurs during increased physical exercise, coronary insufficiency or in the case of other physiological and pathological incidents.

One is tempted to postulate that the described transient interaction of membrane lipids with AMP deaminase in the heart is only an example of a more general phenomenon of subtle regulation of the activity of so-called "cytosolic" (soluble) enzymes by their temporary binding to the cellular membranes.

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