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**DIVERSITY OF THE EFFECT OF PHOSPHATIDYLCHOLINE  
AND SPHINGOMYELIN ON ADENYLATE DEAMINASE FROM PIG  
BRAIN\***

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**Liposomes made of sphingomyelin were found to inhibit both ATP-activated and non-activated AMP deaminase from pig brain, in contrast to liposomes made of egg yolk phosphatidylcholine which exhibited an activating effect on the ATP-activated enzyme, being without effect on AMP deaminase in the absence of ATP. Dioleoylphosphatidylcholine exerted a similar effect as egg yolk phosphatidylcholine but dipalmitoylphosphatidylcholine was without effect.**

Liposomes composed of egg yolk phosphatidylcholine and of sphingomyelin are model systems that reflect the extreme limits of normal lysosomes [1]. The proportion of these two phospholipids in biological membranes may vary considerably [2]. Under some pathological conditions and aging the amount of sphingomyelin was found to increase at the expense of phosphatidylcholine [3]. The changes of the membrane lipid composition may influence not only the permeability of the membrane, but also the properties of some intracellular enzymes susceptible to phospholipids. AMP deaminase (EC 3.5.4.6) is one of the ubiquitous cytoplasmic enzymes the regulatory properties of which are influenced by intracellular phospholipids [4]. Phosphatidylcholine was found to change the kinetics of AMP deaminase isolated from the heart [5, 6] and from some other vertebrate tissues [7, 8, 9]. The influence of sphingomyelin (which may replace phosphatidylcholine in natural membranes) on the kinetic properties of AMP deaminase has not been investigated so far.

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The aim of the present work is to compare the effects of sphingomyelin and a few species of phosphatidylcholine on the kinetics of AMP deaminase isolated from pig brain.

#### MATERIALS AND METHODS

*Reagents.* Dioleoylphosphatidylcholine and dipalmitoylphosphatidylcholine were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Egg yolk phosphatidylcholine was obtained from Serva (Heidelberg, F.R.G.), and sphingomyelin from Koch-Light (Colnbrook, Bucks., England). Cellulose phosphate P-11 was from Whatman (Springfield Mill, England). All other reagents were of highest analytical purity available from POCh (Gliwice, Poland).

*Liposomes.* Liposomes were prepared as described previously [5] with slight modifications: a stream of argon instead of nitrogen was used throughout the procedure. While preparing liposomes from sphingomyelin a mixture of 40% methanol and 60% chloroform had to be used instead of pure chloroform in order to solubilize the phospholipid prior to its deposition on the test tube wall by evaporation and subsequent sonication.

*Enzyme isolation and assay.* Pig brain was brought into the laboratory from the local slaughterhouse in ice within 90 min after the death of the animal. AMP deaminase from the brain hemispheres was purified by cellulose phosphate chromatography as described by Smiley *et al.* [10]. The enzyme preparation displayed specific activity of 11.8  $\mu\text{mol NH}_3/\text{min}$  per mg protein as estimated at 10 mM concentration of substrate and 30°C.

To investigate the effect of liposomes 25 - 50  $\mu\text{l}$  of enzyme solution containing 5 - 10  $\mu\text{g}$  protein was preincubated for 30 min at 4°C with 250  $\mu\text{l}$  of the liposome suspension containing 375 nmol phospholipid, in 100 mM succinate buffer containing 150 mM KCl, pH 6.4. The reaction was initiated by the addition of an appropriate amount of AMP solution to make up the final volume of the mixture to 0.5 ml. Incubation was carried out for 10 min at 30°C and terminated by the addition of phenol reagent used to estimate ammonia liberated.

*Analytical procedures.* Ammonia was estimated by the phenolhypochlorite method as described by Chaney & Marbach [11]. Protein was estimated either by the method of Lowry *et al.* [12] or by the method of Warburg and Christian as described by Layne [13].

#### RESULTS AND DISCUSSION

As shown in Fig. 1, AMP deaminase from pig brain displayed a sigmoidal kinetics in the absence of ATP. The same was observed in the presence of egg yolk phosphatidylcholine. The addition of ATP to the reaction mixture

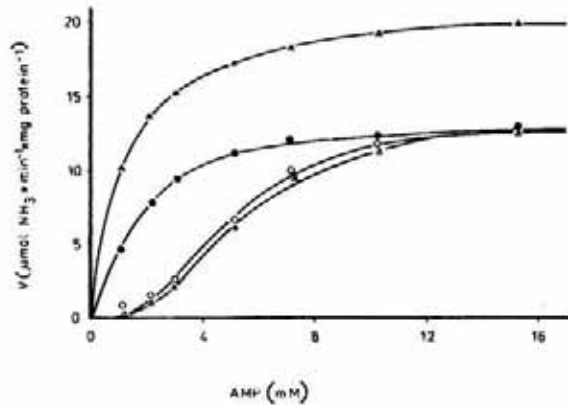


Fig. 1. Substrate saturation curves of AMP deaminase isolated from pig brain. Incubations were carried out without effectors added ( $\circ$ ), in the presence of 1 mM ATP ( $\bullet$ ), in the presence of 375 nmol of egg yolk phosphatidylcholine ( $\Delta$ ), and in the presence of 1 mM ATP plus 375 nmol of egg yolk phosphatidylcholine ( $\blacktriangle$ ). For experimental details see text

shifted the kinetics from sigmoidal to hyperbolic thereby lowering the apparent  $S_{0.5}$  from about 5 mM in the absence of ATP to about 1.5 mM in the presence of 1 mM ATP. A further shift of  $S_{0.5}$  to 0.6 mM and a 50% increase of saturation rate occurred after ATP-activated AMP deaminase had been preincubated with liposomes made of egg yolk phosphatidylcholine. In contrast to these effects of phosphatidylcholine, described also for the enzyme from rat brain [8], liposomes made of sphingomyelin exerted an inhibitory effect on pig brain AMP deaminase (Fig. 2). No effect of sphingomyelin on the value of  $S_{0.5}$  was observed. As may be seen in Fig. 2, sphingomyelin liposomes at the concentration used in this experiment (375 nmol) produced about 50% inhibition of AMP deaminase activity in both the absence and the presence of ATP throughout the whole range of substrate concentrations used. An increase of the amount of sphingomyelin to 750 nmol in the same volume caused a complete inhibition of enzyme activity (not shown).

Thus the two phospholipids investigated exhibited quite different effects on the activity of AMP deaminase. Although phosphocholine is the polar head group common to both phosphatidylcholine and sphingomyelin, the other region of each molecule displays distinctly different structural features. The hydrophobic region of phosphatidylcholine contains two hydrocarbon chains of fatty acids one of which is usually saturated and the other unsaturated. In sphingomyelin the hydrophobic region is composed of one hydrocarbon acyl chain bound in amide linkage to the primary amino group at C2 position of sphingosine. More than 60% of the fatty acids in sphingomyelin from bovine brain are saturated with a chain length

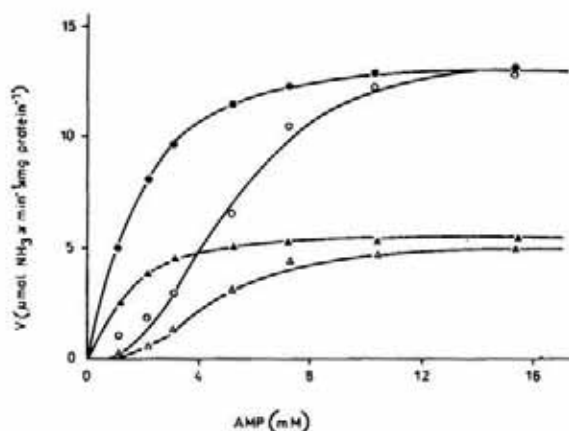


Fig. 2. The effect of sphingomyelin liposomes on AMP deaminase. Incubations were carried out without effectors added (○), in the presence of 1 mM ATP (●), in the presence of 375 nmol of bovine brain sphingomyelin (△), and in the presence of 1 mM ATP plus 375 nmol of sphingomyelin (▲). For experimental conditions see text

of 16-24 carbon atoms [3]. One might suppose that the saturated fatty acids prevailing in sphingomyelin are responsible for the inhibition of AMP deaminase. To check this possibility dipalmitoylphosphatidylcholine was used as a phospholipid containing saturated fatty acids only. The results presented in Fig. 3 show that liposomes composed of dipalmitoylphosphatidylcholine did not affect the kinetics of pig brain AMP deaminase. This is true both below (Fig. 3) and above the transition temperature of this phospholipid,

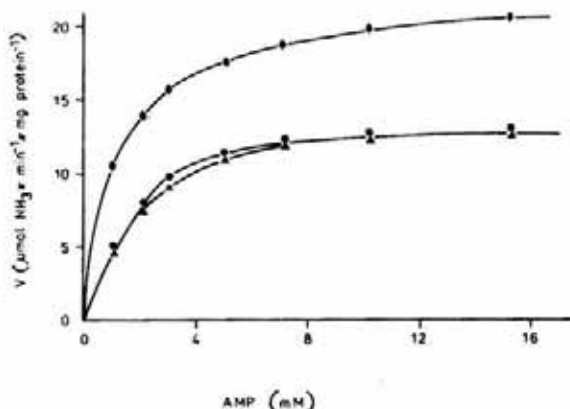


Fig. 3. The effect of dioleoyl and dipalmitoylphosphatidylcholine containing liposomes on ATP activated AMP deaminase from pig brain. Incubations were carried out in the presence of 1 mM ATP (●), in the presence of 1 mM ATP plus 375 nmol of dioleoylphosphatidylcholine (◆), and in the presence of 1 mM ATP plus 375 nmol of dipalmitoylphosphatidylcholine (▲). For experimental details see text

as shown by carrying out the experiment at the temperature of 42°C and 45°C (not shown). In contrast, dioleoylphosphatidylcholine displayed a similar effect as egg yolk phosphatidylcholine.

In the naturally occurring molecules of phosphatidylcholine the average number of *cis* double bonds is 1.1 - 1.5, but in sphingomyelin it is only 0.1 - 0.35. The results presented indicate that this structural difference between phosphatidylcholine and sphingomyelin is not the only reason for the diversity of the effects of these two phospholipids on the AMP deaminase kinetics. Perhaps more important are the differences of the interfacial region of the two phospholipid molecules. In sphingomyelin this region contains components of the amide linkage, a free hydroxyl group on C3 and a *trans* double bond between C4 and C5 of sphingosine. The interfacial region of sphingomyelin thus provides a possibility to form hydrogen bond both as a donor and as an acceptor of hydrogen. In contrast, phosphatidylcholine can act as a hydrogen acceptor only [3]. In sphingomyelin molecule the region which is a hydrogen donor resides in the relatively low dielectric constant environment. This makes the hydrogen bond of this phospholipid with proteins, cholesterol and some other compounds stronger than the corresponding bonds of glycerophospholipids [14]. The presence of hydrogen bonds results in an increased stability of sphingomyelin-containing membranes [2]. It has also been shown that liposomes containing egg yolk phosphatidylcholine are much better cholesterol donors for liver microsomes than liposomal vesicles containing sphingomyelin from bovine brain [1]. It is possible that sphingomyelin associating strongly with the molecules of AMP deaminase creates a nonactive, "latent" enzyme form.

This may be important in the regulation of adenosine production since sphingomyelin was reported to form an active complex with plasma membrane 5'-nucleotidase [15]. Smith & Cantab [16] showed that a pronounced increase of sphingomyelin content occurs during development of atherosclerosis, this compound reaching 40% of the total lipid in the intima and in advanced aortic lesions its content can be as high as 70 - 80% of the total phospholipids.

AMP deaminase is one of several enzymes susceptible to the regulatory effects exerted by phospholipids present in the membranes. The results presented here suggest the way by which changes of membrane lipid composition caused by aging or pathology might influence cellular metabolism.

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