Acta Biochimica Polonica

75th Anniversary of the M. Nencki Institute of Experimental Biology

Vol. 40 No. 3/1993

QUARTERLY

Minireview

Factors controlling the activity of serine- and other base-exchange enzymes and phosphatidylserine transport in mammalian cell

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Received 29 June, 1993

Regulation of base-exchange enzyme activity

In mammalian cells, phosphatidylserine is synthesized mainly by the base-exchange reaction. The base-exchange enzymes catalyse the displacement of the base moiety of various phospholipids by a free base (serine, ethanolamine or choline) [1, 2]. These enzymes are Ca^{2+}-dependent and predominantly located in the endoplasmic reticulum, although some activity has also been found in the plasma membrane and other subcellular fractions [3]. They are distinct from phospholipase D, which requires Ca^{2+} or Mg^{2+}, and are unable to form phosphatidylethanol in the presence of ethanol [4, 5]. The enzymes catalyzing incorporation of ethanolamine, choline and serine show different kinetic parameters [2]. Localization of these enzymes in the transverse plane of the endoplasmic reticulum membrane also seems to be different. In rat brain membranes, the enzymes incorporating both ethanolamine and choline are located on the cytoplasmic surface, whereas the active centre of the serine-incorporating enzyme seems to be also accessible from the extracytoplasmic surface [6, 7]. Some authors claimed that, in liver tissue, the active centres of all base-exchange enzymes are expressed on the cytoplasmic side of microsomal vesicles [8], whereas others suggested the same localization as in brain tissue [9].

The base-exchange reaction, leading to the formation of phosphatidylserine, may be a part of the methylating pathway [10] that is believed to operate in liver but not in brain. On the other hand, in brain, the incorporation of serine into phospholipids by base-exchange may be important in supplying the pool of free choline. Recent studies on cultured Chinese hamster ovary cells have shown that phosphatidylcholine is the preferred lipid substrate for serine incorporation [11]. In his studies on liver tissue, Bjerve [12] observed that both phosphatidylcholine and phosphatidylethanolamine can serve *in vitro* as substrates for the serine base-exchange. However, the enzyme partially purified from brain prefers ethanolamine lipids as substrates for the exchange with free serine [13]. The ethanolamine-serine base-exchange enzyme which was purified nearly to homogeneity by the same group could effectively use phosphatidylethanolamine and aso lectins as lipid substrates but not pure phosphatidylcholine or other lipids [14].

Although the base-exchange enzymes have been known for many years, their regulation is still an unsolved problem. Recent studies indicate that several factors may be involved in this process. One of them is the availability of Ca^{2+}. This is particularly important in the case of the serine-incorporating enzyme, due to its specific localization in endoplasmic reticulum membranes. Recent studies with various cultured cells [9, 15] have shown that the release of calcium from the endoplasmic reticulum by ionophores (A_{23187} or ionomycin) strongly lowers

phosphatidylserine synthesis. Thapsigargin and 2,5-di(t-butyl)-benzohydroquinone, inhibitors of Ca^{2+}-ATPase have a similar effect. In this case their action can be explained by preventing the pumping of Ca^{2+} into endoplasmic reticulum cisternes. From early studies [2] it is also known that optimum Ca^{2+} concentration is a function of pH (and *vice versa*) and is different for the enzymes acting with choline, ethanolamine and serine. Thus, the availability of calcium directly affects not only the V values but also K_m values of the enzymes for free bases.

Among the three base-exchange enzymes in the brain microsomal membrane, the serine-exchange activity was found to be stimulated by sphingosine [16] and amphiphilic cations such as oleylamine, W-7, chlorpromazine and didodecyldimethylamine, whereas amphiphilic anions (bis(2-ethylhexyl)hydrogen phosphate and cholesterol sulphate) were inhibitory [17]. Since amphiphilic cations and anions have no effect on the K_m value towards L-serine but only upon the V value of the reaction, it is suggested that their effect is related to interaction of the lipid substrate with the membrane-bound enzyme. Long-chain fatty acids, particularly the unsaturated ones, also exert a modulating effect on base-exchange activities [16]. Both serine- and ethanolamine-exchange activities in rat brain microsomal membranes have been found to be stimulated by oleate, linoleate and arachidonate at low concentrations (0.1 - 0.5 mM), whereas the choline-exchange activity was inhibited by these acids [16].

Evidence for regulation *in vitro* of base-exchange activities by the phosphorylation-dephosphorylation cycle was presented [18] in experiments with brain microsomes preincubated under conditions favourable for phosphorylation by cAMP-dependent protein kinase or for dephosphorylation by alkaline phosphatases. The pretreatment with cAMP and ATP resulted in an increase of all three enzyme activities, being most effective for the choline base-exchange activity, whereas exposure of microsomes to phosphatases lowered all exchange activities [18].

Another factor involved in the regulation of base-exchange activities is the lipid pattern of the microsomal membrane. This was demonstrated when microsomal membranes were enriched with various lipids either by the Ca^{2+}-dependent fusion mechanism [19, 20] or by a procedure involving the disruption of the membranes with detergent and their re-assembly following detergent removal [21]. As shown, for example, for liver microsomes, the incorporation of exogenous phosphatidylinositol by Ca^{2+}-dependent fusion inhibited both ethanolamine and serine base-exchange activities, leaving the choline-exchange activity practically unaffected. On the other hand, phosphatidylserine incorporated into membranes inhibited ethanolamine- and choline-exchange activities only slightly and, when inserted in high amounts, also the serine-exchange activity [22]. A weak inhibition of the serine-exchange activity by phosphatidylserine and lack of effect of phosphatidic acid on all three base-exchange activities in liver are in contrast to the data on rat brain microsomes [20] and clearly show that base-exchange is differently regulated by phospholipid composition in these two tissues. However, phosphatidylserine inserted into liver microsomal vesicles re-assembled after detergent removal had a very strong inhibitory effect on the serine base-exchange activity [23]. The explanation for this difference may be provided by the fact that, following Ca^{2+}-induced fusion, the phospholipid could enter certain domains of the membranes, whereas following re-assembly a random distribution of lipids in re-aggregated vesicles could be expected.

In liver and brain microsomes the effect of other exogenous phospholipids, i.e. phosphatidylcholine and phosphatidylethanolamine, on base-exchange enzymes was found to be strongly dependent on molecular species. Usually, unsaturated phospholipids inserted in low amounts were stimulatory, whereas saturated species seem to be inhibitory [23, 24].

Regulation of phosphatidylserine transport

Phospholipid composition of the membrane may also be important for the release of newly synthesized phospholipids from the site of their synthesis. This may concern, first of all, phosphatidylserine, the lipid which has to be transported to mitochondria for decarboxylation. Recent studies have shown that *in vitro*, in the reconstituted system containing microsomes and mitochondria, the transport of phosphatidylserine proceeds without partici-

pation of any soluble phosholipid transfer protein [25 - 27]. Moreover, evidence has been presented that newly synthesized phosphatidylserine is much more efficiently exported to mitochondria than the bulk phospholipid [27]. The import of phosphatidylserine to mitochondria and the export of phosphatidylethanolamine from mitochondria were described as collision processes [26]. Different results have been, however, obtained with phosphatidylserine liposomes used as lipid donor for mitochondrial decarboxylase. In such a case the translocation-dependent decarboxylation of phosphatidylserine was found to be enhanced by rat liver cytosol or a non-specific lipid transfer protein purified therefrom [28 - 31]. Divalent cations at millimolar concentrations and monovalent cations at much higher concentrations inhibit the protein-mediated transport of phosphatidylserine. This effect is due to a limited interaction of the protein with less negatively charged donor membranes [31]. These results and other data [32, 33] indicate that a non-specific lipid-transfer protein lowers the energy barrier of lipid-monomer dissociation by interacting with the membrane. The protein becomes a part of the transient complex that seems to be influenced by electrostatic interactions. It should be added that this protein being cationic, favours the electrostatic interaction. Whether a non-specific lipid transfer protein participates in the intracellular transport of phosphatidylserine to mitochondria *in vivo* is not clear. Regardless of this question, a stimulatory effect of ATP on phosphatidylserine transport from the endoplasmic reticulum to mitochondria in baby hamster kidney cells and in permeabilized Chinese hamster ovary cells has been reported [34, 35].

The intramitochondrial transport of phosphatidylserine represents another problem. The author's data [36] and those of Voelker [25] have shown that the active centre of the decarboxylating enzyme located in the inner mitochondrial membrane [37, 38] is exposed to the intermembrane space. Thus, phoshatidylserine introduced into the outer membrane by means of either the non-specific lipid transfer protein, collision mechanism or fusion, is then translocated to the inner membrane. Involvement of contact sites in this process has been proposed for yeast [39] and liver mitochondria [40 - 42]. Phosphatidylethanolamine, the product of decarboxylation, moves through contact sites and can be found in the outer mitochondrial membrane [42, 43]. In brain mitochondria the newly formed phosphatidylethanolamine does not leave the inner mitochondrial membrane; this indicates that decarboxylation of phosphatidylserine in liver has a different biological meaning than in brain [44]. However, a recent study on liver mitochondria with the use of fluorescent pyrenyl derivatives of phosphatidylserine and phosphatidylethanolamine suggests that decarboxylation of phosphatidylserine occurs in the inner leaflet of the outer mitochondrial membrane (Jasińska, Zborowski and Somerharju, unpublished).

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