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Sidedness of yeast plasma membrane vesicles and mechanisms of activation of the ATPase by detergents

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The binding of concanavalin A and of fluorescein 5′-isothiocyanate indicate similar amount of right-side-out and inside-out vesicles in plasma membrane vesicles from either glucose-starved or glucose-fermenting yeast cells. These vesicles contain low-activity and high-activity states of the ATPase, respectively. Unmasking of latent active sites can explain the limited ATPase activation (about 2-fold) produced by several detergents on both kinds of vesicles. On the other hand, lysophosphatidic acid (oleoyl) produces a 7-fold activation of the ATPase in vesicles from glucose-starved cells. This effect is accompanied by a change in K_m of the enzyme and probably reflects a direct action of the detergent on the ATPase. A similar activation and K_m change can be obtained by sonication of the vesicles, although in this case soybean phospholipids are required for maximal activity. Apparently the low-activity state of the yeast plasma membrane ATPase can be activated not only by glucose metabolism 'in vivo' (mechanism unknown) but also by some detergents and physical treatments 'in vitro'. Experiments with purified ATPase from glucose-starved cells also indicate that lysophosphatidic acid (oleoyl) specifically activates the enzyme. These results suggest a note of caution on considering the usual interpretation of the effects of detergents on membrane enzymes, which only take into account the unmasking of latent active sites.

Introduction

The activation of membrane enzymes by detergents has usually been interpreted in the light of the classical work of De Duve on the structure-linked latency of lysosomal enzymes [1]. Unmasking of latent active sites in sealed right-side-out plasma membrane vesicles, by disruption of the permeability barrier to substrate, seems to explain the activation of the animal Na^+/K^+-ATPase [2] and plant H^+-ATPase [3] by some detergents. The yeast H^+-ATPase is also activated by detergents [4] but the mechanism is unknown. Although unmasking of latent active sites may also be operative, it is important to examine whether other mechanisms are at work. Detergents could remove inhibitory molecules or directly act on membrane enzymes altering their aggregation state, conformation or lipid environment [5]. These alternative mechanisms are particularly attractive in the case of membrane enzymes which, like the yeast plasma membrane ATPase [6], may exist in different regulatory states. The enzyme from glucose-fermenting cells exists in a high-activity state while in glucose-starved cells a low-activity state is observed. The mechanism of interconversion is unknown [6]. As demonstrated for another regulated enzyme, the Ca^{2+}-ATPase of animal plasma membranes [7], the addition of some lipids and detergents may mimic the effects of physiological regulatory mechanisms such as calmodulin binding or protein kinase phosphorylation. Apparently, the constraints on the enzyme activity inherent to regulated membrane enzymes can be released by interaction with particular lipids. Additional complications in the case of the yeast ATPase are its requirement for acidic phospholipids [8] and the reluctance of yeast plasma membranes to be disrupted by detergents [4].

We have investigated the mechanism of detergent activation of the yeast plasma membrane ATPase by testing the effects of different detergents and by correlating ATPase activation with the sidedness of plasma

Abbreviations: LPAol, L-α-lysophosphatidic acid (γ-oleoyl); LPA, DL-α-lysophosphatidic acid (γ-myristoyl); LPGol, L-α-lysophosphatidylglycerol (γ-oleoyl); LPG, L-α-lysophosphatidylglycerol (γ-palmitoyl and stearoyl); LPCol, L-α-lysophosphatidylcholine (γ-oleoyl); LPC, L-α-lysophosphatidylcholine (γ-palmitoyl and stearoyl); SDS, sodium dodecylsulfate; FITC, fluorescein 5′-isothiocyanate; Con A, concanavalin A; DOC, deoxycholic acid.

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membrane vesicles. Our results indicate that unmasking can only explain part of the detergents effects and that some detergents and physical treatments (sonication) may directly activate the ATPase.

Materials and Methods

Chemicals. Con A, horseradish peroxidase, ovalbumin, FITC, LPAol, LPG, LPC, LPCol, lauric acid, deoxycholic acid, sodium octylsulfate, oleic acid, alamethicin, amphotericin B and gramicidin D were obtained from Sigma. Crude soybean phospholipids were obtained as phosphatidylcholine type II-S (Sigma) and purified as described [9]. Ovalbumin, LPA and SDS were from Serva and Triton X-100 from Merck. LPGol was synthesized by Lipid Products (U.K.). All lipid solutions were prepared in 10 mM Tris and 1 mM EDTA (pH 8.0 with HCl). Soybean phospholipids were sonicated to clarity under nitrogen in a bath Sonifier (Laboratories Supplies Co., New York).

Membranes. Yeast plasma membranes were purified from strain BWG1-7A [10] grown in YPD medium to late exponential phase (absorbance at 660 nm about 2). The cells were harvested and washed with water. Plasma membranes from non-fermenting cells were prepared by differential and sucrose gradient centrifugation as described [11]. Plasma membranes from glucose-fermenting cells were prepared by the same procedure but the cells were incubated for 10 min at room temperature with 2% glucose before homogenization [6]. Vesicle preparations were stored at −70°C in buffer I (GTED buffer: 20% glycerol, 1 mM EDTA, 1 mM dithioerythritol and 10 mM Tris adjusted to pH 7.5 with HCl).

Assay of ATPase activity. The final assay volume of 1 ml contained 30 μg plasma membrane protein and the indicated amounts of detergents or phospholipids. Buffer composition was: 5 mM $MgSO_4$, 50 mM KNO_3, 5 mM sodium azide, 0.2 mM ammonium molybdate and 50 mM 4-morpholineethanesulfonic acid (pH 5.7 with Tris). Unless otherwise indicated, the ATP concentration was 2 mM. Incubations were carried out at 30°C for 10 min and P_i production was determined as described [12].

Sonication of plasma membrane vesicles. Plasma membranes were diluted to 0.2–2 mg/ml in buffer I. Aliquots of 200–300 μl were sonicated in 1.5 ml Eppendorf tubes immersed in ice. A B-12 Branson Sonifier with microtip was employed at setting 5 (60 watts). Pulses of 15 s were followed by 1 min cooling periods.

Protein determination. The method of Bradford [13] was used, with the Bio-Rad Protein Assay Reagent and bovine γ-globulin as standard.

FITC binding. Aliquots of 6.25 μg plasma membranes in 12.5 μl sodium borate buffer (50 mM, pH 8.65) were preincubated for 5 min at room temperature in the absence (intact vesicles) or presence (opened vesicles) of 0.25 mg/ml LPC. Then 12.5 μl of 80 μM FITC in the same buffer was added and the samples further incubated for 2 h at room temperature in the dark. The labelled samples were solubilized for 10 min at room temperature with 25 μl SDS-sample buffer [14] containing 40 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride and 10 mM EDTA. Aliquots of 12 μl were separated by SDS electrophoresis [14] in the dark on 8% polyacrylamide gels. The transilluminated fluorescent bands were photographed using a Polaroid camera fitted with a Wratten No. 12 filter. The gel was then stained with Coomassie blue R-250.

Con A binding. Aliquots of 2 μg plasma membrane in 10 μl buffer I were incubated for 5 min at room temperature in the absence (intact vesicles) or presence (opened vesicles) of 1 mg/ml SDS. After dilution with 50 mM sodium bicarbonate, triplicate aliquots of 100 μl containing up to 0.2 μg plasma membrane protein were incubated for 1.5 h at room temperature in the wells of a Nunc microtitre plate. Non-bound material was discarded and the wells washed twice with blocking buffer (1.2% polyvinylpyrrolidone and 0.1% gelatin in phosphate-buffered saline) and incubated for 1.5 h with 250 μl of this buffer. The wells were washed twice with binding buffer (0.1 mM $CaCl_2$, 0.1 mM $MnCl_2$ and 20 mM sodium phosphate, pH 6.8) and incubated for 1 h with 100 μl of this buffer containing 6.25 μg/ml Con A. After washing three times with binding buffer, three times with blocking buffer and three more times with binding buffer, each well was incubated for 1 h with 100 μl of 6.6 μg/ml horseradish peroxidase in binding buffer and extensively washed as after the binding of Con A. Bound peroxidase was assayed with 200 μl of 0.5 mg/ml *O*-phenylenediamine and 0.01% H_2O_2 in 20 mM sodium phosphate (pH 6.5). After 5–10 min the peroxidase reaction was stopped with 50 μl 2 M H_2SO_4 and the absorbance of each well determined at 490 nm using a Cambridge Technology microtitre plate reader.

Electron microscopy. Specimens were prepared for electron microscopy after diluting the glycerol buffer more than 10-fold. Negative staining was performed for 30 s with 1% uranyl acetate pH 5.5 on glow-discharged carbon-collodion grids. Micrographs were taken in a Philips EM 301 electron microscope operating at 80 kV with a magnification of 25000 ×.

Purification and delipidation of ATPase. The ATPase was purified [11] from either glucose-starved or glucose-fermenting cells and delipidated [8] as described.

Results

Sidedr·ss of yeast plasma membrane vesicles

Plasma membrane vesicles from glucose-fermenting or glucose-starved cells were assayed for their sidedness by two methods which gave comparable results for both

membrane types. The first method utilizes the binding of FITC to the cytoplasmically oriented active site of the ATPase. Site-directed mutagenesis has demonstrated that Lys-474 in the active site of the ATPase [15] is the only target for FITC in yeast plasma membrane vesicles under the experimental conditions described in Methods (Portillo, F. and Serrano, R., unpublished data). Above pH 8 FITC has two negative charges and is unlikely to cross the permeability barrier of the yeast plasma membrane. As indicated in Fig. 1, the dominant polypeptide labelled under our experimental conditions was the 100 kDa ATPase band [15]. In addition, with both vesicle preparations the fluorescence of this band

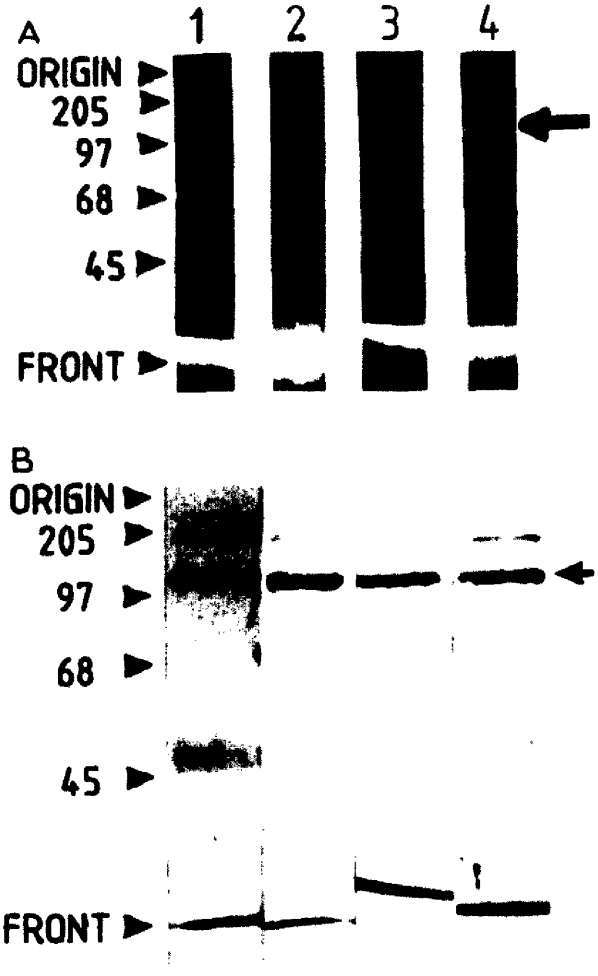

Fig. 1. Determination of the sidedness of yeast plasma membrane vesicles by the accessibility of FITC binding sites. Vesicles from either glucose-fermenting (lanes 1 and 2) or glucose-starved (lanes 3 and 4) cells were labelled with FITC after preincubation in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of the detergent LPC. Arrows at the right side indicate the position of the 100 kDa ATPase band. Arrows at the left side indicate the positions of origin, front and molecular size standards (kDa indicated). (A) Transilluminated fluorescent bands. Front contains free FITC. (B) Coomassie blue R-250 stained gel to show similar loading of the lanes. The ATPase is the major protein in the preparation.

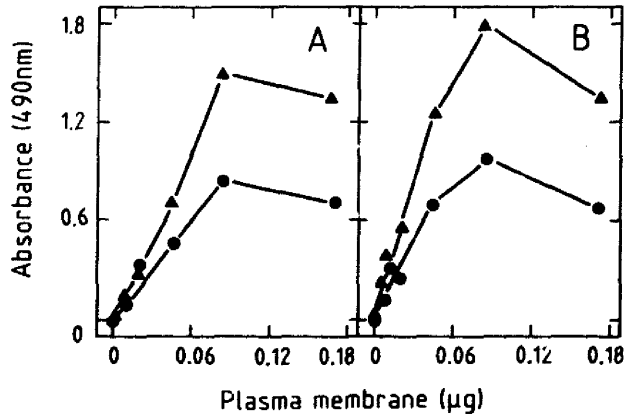

Fig. 2. Determination of sidedness of yeast plasma membrane vesicles by the accessibility of Con A binding sites. (A) Vesicles from glucose-fermenting cells. (B) Vesicles from glucose-starved cells. Circles, no SDS treatment . Triangles, vesicles treated with SDS.

was about doubled by treatment with LPC, indicating that the detergent exposed latent sites in the ATPase. These results suggest similar proportions of closed inside-out plus broken vesicles (directly accessible to FITC without detergent) and closed right-side-out vesicles (only labelled after opening with detergent). Similar results were obtained with LPC concentration from 0.25 to 1 mg/ml, suggesting that maximum accesibility was obtained. Lower LPC concentrations were less effective.

The other method for sidedness assessment is more quantitative and relies on the ability of Con A to bind to exposed mannose residues on the external face of the plasma membrane. The assay detects accessible mannose residues associated with plasma membrane vesicles bound directly to the wells of microtitre plates. As shown in Figs. 2A and B, there is reasonably linear binding of Con A up to about 80 ng of plasma membrane protein. Higher amounts of vesicles led to decreased Con A binding. This probably reflects weak binding of some vesicles when the microtitre plate is saturated. When the vesicles were pretreated with SDS they exhibited higher binding of Con A, suggesting that the detergent has exposed latent Con A binding sites. The relative binding of Con A to intact and SDS-opened vesicles suggest that the proportion of right-side out plus broken vesicles (directly accessible to Con A without detergent) is about 60% and 50% for the plasma membranes from glucose-fermenting and glucose-starved cells, respectively. Similar results were obtained with SDS concentrations from 1 to 4 mg/ml, suggesting that maximal accessibility was obtained. Lower SDS concentrations were much less effective. The identity of the shape of the Con A-binding profiles (including the point of saturation at 80 ng protein) of intact and SDS-opened vesicles indicates that, after dilution, the SDS had little effect on either the binding of lysed plasma membrane vesicles to the microtitre plates or on

the binding of Con A to the exposed carbohydrate. Control experiments indicated that the measured Con A binding, both in intact and SDS-opened vesicles, is completely inhibited by 25 mM methyl α-mannoside, suggesting that it is specific for membrane carbohydrate.

Activation of plasma membrane ATPase by detergents

The results of sidedness determinations suggested that about half of the ATPase active sites may be latent and therefore that detergents, by disrupting the permeability barrier to ATP, could activate the enzyme by a factor of about 2. In a previous investigation [4] the ATPase activity of detergent-treated vesicles was assayed in the presence of an excess of soybean phospholipids. This was required to counteract the removal of essential acidic phospholipids [8] by the high concentrations of detergent employed. Under these conditions different detergents such as Triton X-100, octyl glucoside, Zwittergent-14, LPC and DOC produced a 3–4-fold activation of the ATPase [4]. However, the interpretation of these detergent effects is complicated by the fact that, in addition to the permeabilization of the vesicles, endogenous phospholipids are exchanged with the soybean phospholipids added in the assay. It has been shown that soybean phospholipids are better activators of delipidated ATPase than yeast plasma membrane phospholipids [8].

We have tested the effect of low concentrations of detergents which could increase the permeability of the vesicles to ATP without delipidating the ATPase [16]. These experiments were performed with plasma membrane vesicles from both glucose-fermenting and glucose-starved cells, which exhibit high- and low-activity states of the ATPase, respectively [6]. As indicated in Fig. 3, mild detergents such as Triton X-100, sodium octylsulfate, lauric acid and DOC in concentrations up to 20 μg/ml had no effect on ATPase activity. On the other hand the strong anionic detergent LPAol produced maximal activation, 2-fold in the vesicles from glucose-fermenting cells and 7-fold in the vesicles from glucose-starved cells. In the first case the saturated homolog LPA was equally effective while in the second case LPA was much less effective than LPAol (3-fold versus 7-fold activation). The two regulatory states of the ATPase also differed in their response to other detergents. LPC was inactive with the low-activity state but increased the ATPase of the high-activity state 1.6-fold. On the other hand, LPG and LPCol produced similar activation with both membrane preparations (about 1.6-fold). SDS and LPGol activated 1.6-fold in vesicles from glucose-fermenting cells and 3-fold in vesicles from glucose-starved cells.

It seems that in vesicles from glucose-fermenting cells detergent activation of the ATPase (1.6–2-fold) can be explained by opening of right-side-out vesicles, with LPA and LPAol being the most effective. On the other hand, a direct activation of the ATPase by LPAol has to be invoked to explain the large activation (7-fold) produced by this detergent in vesicles from glucose-starved cells. Other detergents such as LPA, SDS and LPGol also activate the low activity state of the ATPase slightly more (3-fold) than expected from the sidedness (2-fold) and therefore they could also directly activate the enzyme, although to lower extent than LPAol. Consistent with the notion of vesicle opening followed by direct activation of the ATPase at higher LPAol concentrations is the observation that 6–8 μg of this detergent activate about 2-fold regardless of the physiological state. Further activation by higher amounts of this detergent (up to 15–20 μg) is only observed in vesicles from glucose-starved cells.

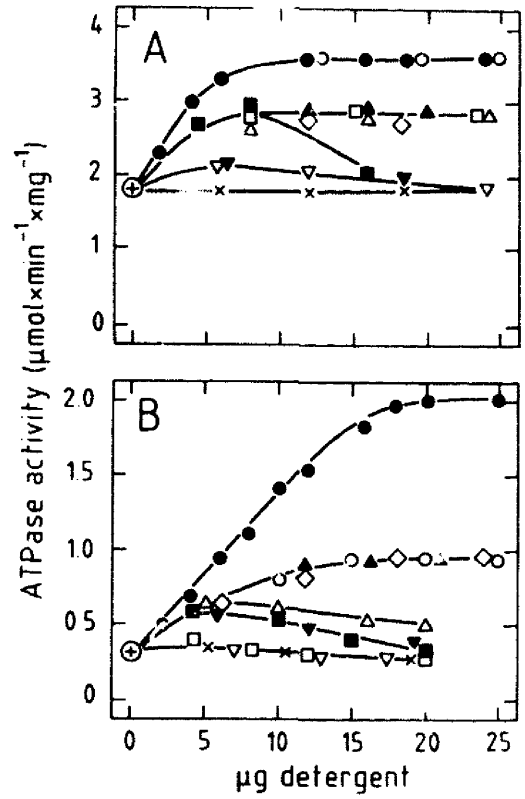

Fig. 3. Activation of the yeast plasma membrane ATPase by detergents in vesicles from glucose-fermenting (A) or glucose-starved (B) cells. Assays included 30 μg plasma membrane protein and the indicated amounts of detergents. ○, LPA; ●, LPAol; △, LPG; ▲, LPGol; □, LPC; ■, LPCol; ◇, sodium dodecylsulfate; ▼, lauric acid; ▽, deoxycholic acid; ×, Triton X-100 or sodium octylsulfate.

This interpretation was supported by the kinetic properties of the detergent-activated enzyme. Detailed kinetic studies utilizing ATP concentrations from 0.1 to 10 mM have indicated that the yeast plasma membrane ATPase exhibits slightly sigmoidal kinetics [17]. However, as indicated in Fig. 4, in the range from 0.5 to 3 mM ATP the double-reciprocal plots are approximately linear and V_{max} and apparent K_m values can be extrapolated. Although these K_m values lack theoretical

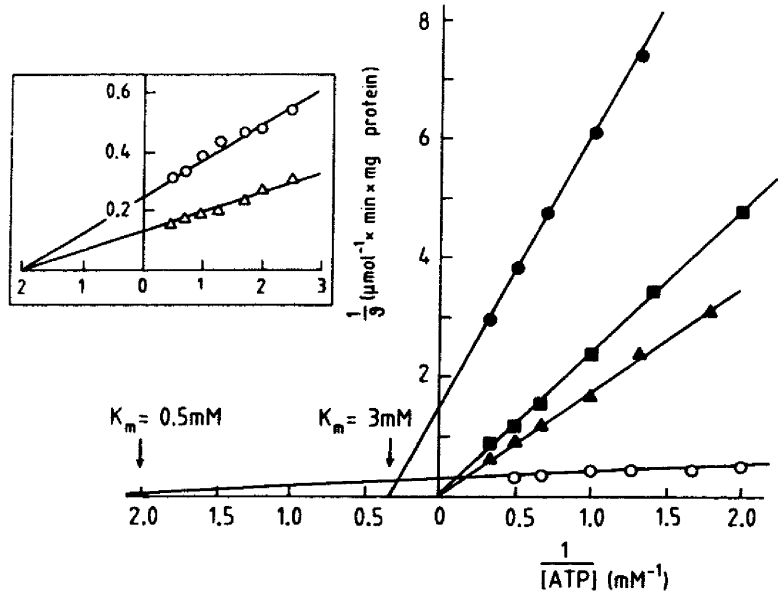

Fig. 4. Double-reciprocal plots of the effect of ATP concentration on plasma membrane ATPase activity. Open symbols, vesicles from glucose-fermenting cells. Closed symbols, vesicles from glucose-starved cells. Circles, no additions or treatments. Squares, vesicles sonicated for 45 s and assayed with 200 μg sonicated soybean phospholipids. Triangles, assay included 20 μg LPAol. The inset shows the kinetics of the glucose-activated enzyme in a different scale. The upper line of the insert corresponds to the lower one of the main graph, where it is repeated to show the kinetic differences between the activation of the ATPase by either glucose 'in vivo' or LPAol and sonication 'in vitro'. Each point represents the average of two determinations differing in less than 10%. The straight lines were fitted by the least-squares method (linear regression coefficients, $r = 0.993–0.997$). In the case of closed squares and triangles no evidence for saturation was obtained in the concentration range explored (0.5–3 mM) and therefore the K_m must be greater than 10 mM. Similar values (within 10%) for the K_m were obtained with three different membrane preparation of glucose-starved and glucose-fermenting cells.

value [17], they provide estimates of the affinity of the enzyme for ATP. As indicated in Fig. 4, LPAol not only increases V_{max} but also the apparent K_m of the ATPase in the low-activity state (from 3 mM to more than 10 mM; an accurate estimate could not be obtained for the later). This supports a direct interaction of the detergent with the enzyme which alters its kinetic properties. In the case of the high-activity state LPAol does not modify the K_m of the ATPase (see insert of Fig. 4) and therefore the increase in V_{max} can reasonably be attributed to permeabilization of the vesicles. As is apparent from Fig. 4, the physiological activation of the ATPase by glucose results in both a decrease of the apparent K_m (from 3 to 0.5 mM) and an increase of V_{max}. Clearly, the mechanism of activation of LPAol 'in vitro' is different from that of glucose 'in vivo'.

A greater susceptibility to delipidation of the low-activity state of the ATPase may explain the differential response of the two states of the enzyme to detergents. We have observed that treatment of the vesicles with high concentrations of bile salts inactivates the low-activity state much more than the high-activity state, the effect being reversed by addition of soybean phospholipids (see Table I). Therefore, LPC and LPCol activate the high-activity state by unmasking latent ATPase but fail to do the same with the low-activity state probably because in addition of unmasking they removed essential lipids from the enzyme.

Other compounds tested as activators of the yeast plasma membranes ATPase included oleic acid, amphotericin B, alamethicin and gramicidin D. All of them were without effect at low concentrations (up to 10 μg/ml) and inhibited the enzyme at higher concentrations.

TABLE I

Reactivation of purified and delipidated ATPase by lysophospholipids

ATPase was purified from either glucose-starved or glucose-fermenting cells and delipidated by cholate treatment [8]. 10 μg enzyme were incubated with either soybean phospholipids (100 μg) or the indicated lysophospholipids (10 μg) and ATPase activity determined as indicated in Methods. Results are the average of two determinations differing in less than 10%. n.d., not determined.

Lipid addition	ATPase activity (μmol·min^{-1}·mg protein^{-1})	
	enzyme from glucose-starved cells	enzyme from glucose-fermenting cells
None	0.06	0.6
Soybean phospholipids	2.0	3.4
LPAol	1.0	1.7
LPGol	0.25	n.d.
LPG	0.20	1.8

Activation of plasma membrane ATPase by sonication

A fortuitous observation unravelled another mechanism for activation of the low-activity state of the ATPase: sonication of the vesicles. As indicated in Fig. 5 (circles), sonication results in about 2-fold activation and the addition of soybean phospholipids in the assay of the sonicated vesicles results in 4-fold activation. In the case of the high-activity state (Fig. 5, triangles) sonication did not affect the activity and if soybean phospholipids were included in the assay of the sonicated vesicles only a 1.6-fold activation was observed.

As the exogenous phospholipids had no significant effect on unsonicated vesicles, these results suggest that sonication somehow facilitates the subsequent interaction of the ATPase with the exogenous phospholipids. Replacement of the endogenous phospholipids by soybean phospholipids could explain the small activation observed with vesicles from glucose-fermenting cells because the latter lipids satisfy slightly better the requirements of the yeast ATPase [8]. As depicted in Fig. 6 sonication not only reduces the size of membrane vesicles but also generates particulate structures too small to be vesicles. These structures could contain delipidated ATPase susceptible to activation by exogenous soybean phospholipids. The large activation (4-fold) observed in the case of vesicles from glucose-starved cells requires a different explanation because replacement of endogenous phospholipids by soybean phospholipids can only activate 1.4–2-fold [8]. As indicated in Fig. 4, sonication induces the same change in apparent K_m as LPAol. Therefore sonication seems also to directly modify the ATPase.

Activation of delipidated ATPase

We previously reported [8] that LPAol is a better activator of purified and delipidated ATPase than LPG. We interpreted this observation as evidence of a requirement for unsaturated fatty acyl chains in the acidic lysophospholipids. The results of the present work, suggesting that LPAol directly modifies the ATPase, and the availability of LPGol prompted a reinvestigation of the effect of lysophospholipids in purified and delipidated enzyme from either glucose-starved or glucose-starved or glucose-fermenting cells (Table I). Although delipidation was less effective in the case of enzyme from glucose-fermenting cells (see above), some general conclusions can be made. It is clear that lysophospholipids are less effective than phospholipids in terms of reactivating the delipidated enzyme. This may be related to their character of strong detergents. On the other hand, it seems that the superiority of LPAol over LPG as an activator is only observed with enzyme from glucose-starved cells and in this case LPAol is still much better than LPGol. Therefore the lack of unsaturated fatty acyl chain does not explain the difference between LPG and LPAol previously reported [8]. LPAol seems to specifically activate the purified ATPase from glucose-starved cells by a mechanism which is independent of the phospholipid requirements of the enzyme.

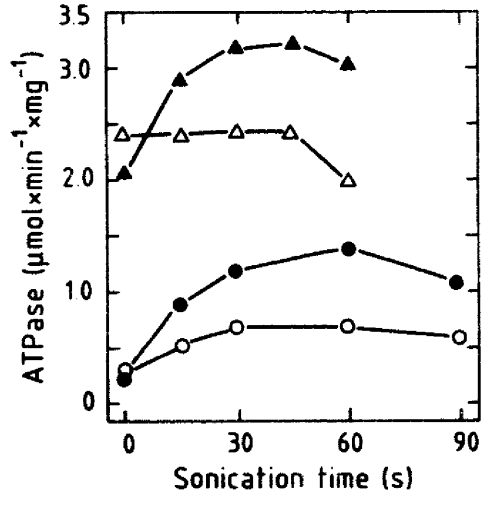

Fig. 5. Activation of the yeast plasma membrane ATPase by sonication. Triangles, vesicles from glucose-fermenting cells. Circles, vesicles from glucose-starved cells. Open symbols, normal assay conditions. Closed symbols, assays included 200 μg sonicated soybean phospholipid.

Discussion

The ability of Con A to bind to the carbohydrate present in the external face of eukaryotic plasma membranes provides a convenient method to ascertain the sidedness of vesicles. In the past either the binding of [^{14}C]Con A to vesicles [18], the effect of Con A binding on the microelectrophoretic mobility of vesicles [19] or the binding of vesicles to immobilized Con A [19] have been utilized. We have developed a simple ELISA-like method based on the binding of Con A to vesicles bound to the wells of microtitre plates. Multivalent, bound Con A is then detected by interaction with the glycoprotein horseradish peroxidase. Sidedness determination by this simple, non-radioactive method has been validated by comparison with FITC binding to the active site of the ATPase facing the cytoplasmic side of the membrane. Sidedness of plant [19] and animal [20] plasma membrane vesicles has also been estimated from the effect of trypsin on ATPase activity, with the proteinase causing inactivation of the enzyme only in inside-out vesicles. However, in the case of the yeast ATPase limited trypsin digestion may actually activate the enzyme [21] and there are concerns about trypsin permeabilizing the vesicles to ATP [19,20]. Therefore assays based on Con A binding seem to most reliable for sidedness determination and the procedure developed in the present work, with vesicles bound to microtitre plates, may provide a simpler alternative to previous methods.

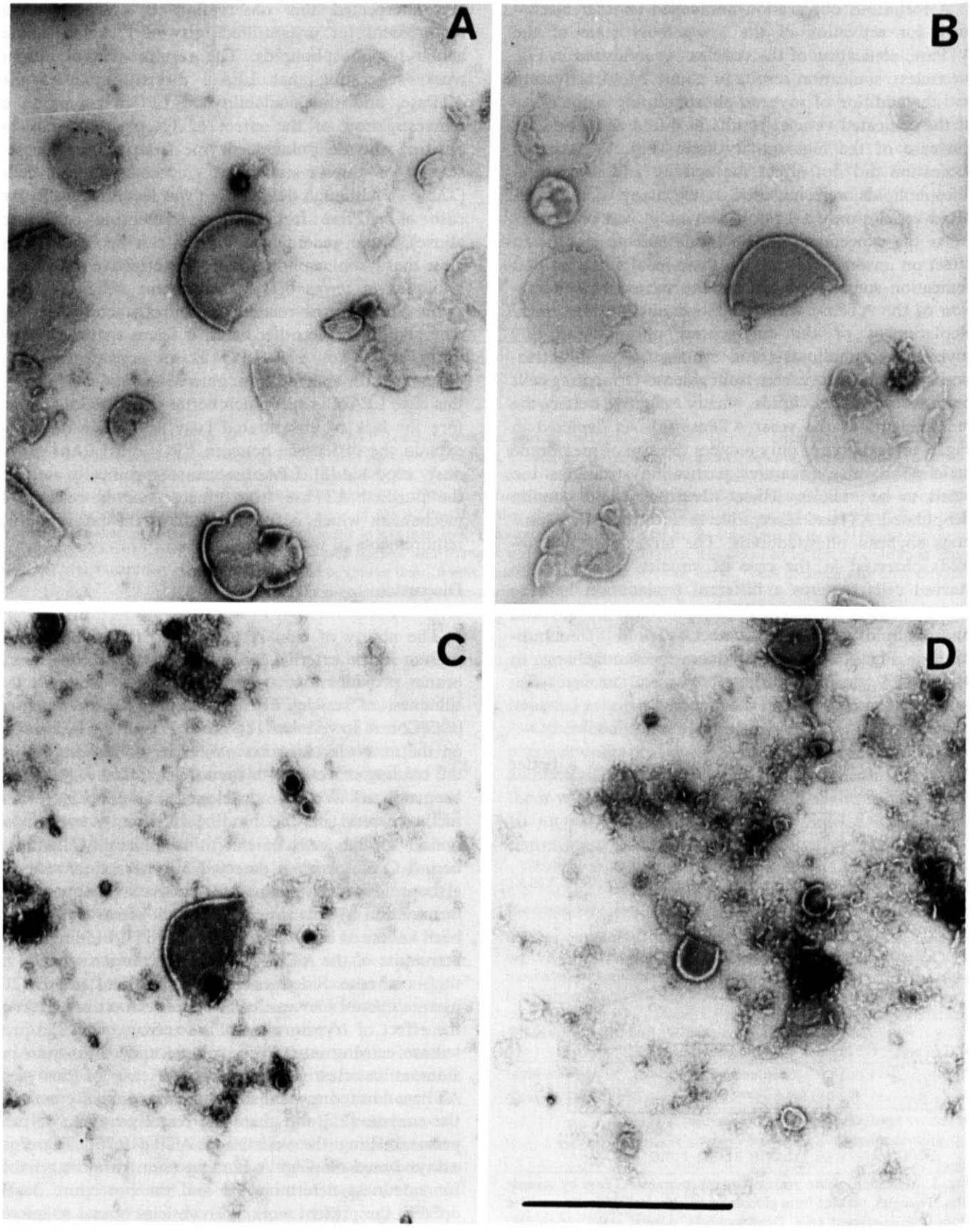

Fig. 6. Electron micrographs of plasma membrane vesicles from glucose-fermenting cells (A and C) and glucose-starved cells (B and D) without sonication (A and B) or after 45 s sonication (C and D). Bar indicates 1 μm.

Once vesicle sidedness has been reliably estimated, it is possible to determine if the activation of the yeast plasma membrane ATPase by detergents can be explain by unmasking of latent active sites in right-side-out vesicles. The first point to discuss is the specificity of the detergent effect on vesicles from either glucose-fermenting or glucose-starved cells, containing high- and low-activity states of the ATPase, respectively [6]. In the former case the maximum activation observed (about 2-fold) is compatible with unmasking because there seems to be similar proportions of inside-out and right-side-out vesicles in plasma membrane preparations. However, under the conditions of low detergent concentration in the present experiments this degree of activation is only observed with strong detergents such as SDS and lysophospholipids and their maximal effect is achieved by 6–8 μg detergent per 30 μg of plasma membrane protein. Triton X-100 and DOC are ineffective, in accordance with the reluctance of yeast plasma membranes to be solubilized by these detergents [4]. Higher concentrations of these detergents do lyse plasma membrane vesicles but then the ATPase becomes delipidated and inactivated [4]. The slightly higher efficiency of LPA and LPAol over all the other detergents may suggest a better disruption of the permeability barrier to ATP. LPAol does not modify the K_m of the ATPase for ATP and therefore it does not seem to directly modify the active site of the enzyme. Activation by sonication and soybean phospholipids is small and can be explained by exchange of endogenous phospholipids by soybean phospholipids, which are slightly better activators of the ATPase [8].

A different situation is found with vesicles exhibiting the low-activity state of the ATPase (from glucose-starved cells). Both sonication and high amounts of LPAol (15–20 μg per 30 μg protein) produced a considerable (4–7-fold) activation of the ATPase and a concomitant change in K_m (from 3 mM to more than 10 mM). Therefore, in addition of unmasking (which also appears to be achieved by 6–8 μg LPAol per 30 μg protein) or lipid exchange as described above, these treatments seem to directly modify the low-activity state of the ATPase. Other detergents such as LPA, LPGol and SDS are less effective (3-fold activation) but also seem to directly activate the ATPase to a limited extent. A similar conclusion was reached by studying the activation of purified and delipidated ATPase by different lipids. LPAol is a much better activator than LPGol or LPG, despite the fact that phosphatidylglycerol is a better activator than phosphatidic acid [8]. Apparently LPAol activates the purified ATPase by a different mechanism than other related lysophospholipids, suggesting that it is an specific modulator of the enzyme.

It is tempting to speculate that the low-activity state of the ATPase contains structural constraints which inhibit its activity and which can be released by some anionic detergents or sonication. The physiological activation of the enzyme by glucose may release these constraints by a different mechanism, such as protein kinase phosphorylation [22,23]. An important difference between the 'in vitro' activation described here and the effect of glucose is that glucose fermentation 'in vivo' increases the V_{max} and reduces the K_m of the ATPase [6] while LPAol and sonication increase V_{max} without improving the affinity (actually, the K_m is increased). Some additional factors may be missing in the 'in vitro' system. The effect of LPAol may be related to that part of the glucose-activating mechanism which affects the V_{max} of the ATPase, the K_m change being caused by a different part of the mechanism. In this respect, it is interesting that both phorbol esters [22] and acidification [24] modify the K_m of the ATPase without affecting its V_{max}. These two factors may only affect the latter part of the glucose-activating mechanism.

Recent experimental results with truncated ATPase genes lacking the C-terminal domain of the enzyme indicate that this domain is inhibitory in glucose-starved cells (Portillo, F., Larrinoa, I. and Serrano, R., unpublished data). Therefore it is possible that the postulated constraint which is released by either glucose fermentation 'in vivo' or LPAol and sonication 'in vitro' involves the interaction of this C-terminal domain with the active site of the ATPase.

LPC has recently been demonstrated to directly activate the plant plasma membrane ATPase under 'in vitro' conditions [25,26]. Apparently LPC has the same effect on the plant ATPase as LPAol on the yeast ATPase. The activity of the plant plasma membrane ATPase is regulated during physiological responses to hormones by an unknown mechanism [15]. The generation of either LPC in plants or LPAol in yeast under physiological conditions which result in activation of the ATPases deserves further investigation. Although some preliminary attempts to demonstrate the participation of lipids in the activation of the yeast ATPase by glucose have been unsuccessful [8], this negative result may only reflect limitations of the lipid preparations employed and further work is required to explore this possibility.

Regardless of the possible physiological role of the detergent effects seen in this and other studies [25,26], these results suggest a note of caution on considering the usual interpretation of the effect of detergents unmasking latent membrane bound enzymes. Proper assessment of vesicle sidedness is essential for correct interpretation of detergent effects.

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